Transient Receptor Potential Channels as Drug Targets: From the Science of Basic Research to the Art of Medicine

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Abstract—The large Trp gene family encodes transient receptor potential (TRP) proteins that form novel cation-selective ion channels. In mammals, 28 Trp channel genes have been identified. TRP proteins exhibit diverse permeation and gating properties and are involved in a plethora of physiologic functions with a strong impact on cellular sensing and signaling pathways. Indeed, mutations in human genes encoding TRP channels, the so-called “TRP channelopathies,” are responsible for a number of hereditary diseases that affect the musculoskeletal, cardiovascular, genitourinary, and nervous systems. This review gives an overview of the functional properties of mammalian TRP channels, describes their roles in acquired and hereditary diseases, and discusses their potential as drug targets for therapeutic intervention.

I. Transient Receptor Potential Channels: A Brief Introduction

In 1969, a Drosophila mutant was discovered that was defective in light sensing and exhibited only transient light-induced receptor potentials (TRPs) instead of the normal maintained response (Cosens and Manning, 1969). This finding was explained by a defect in an ion channel and triggered the discovery of the large gene family baptized “trp genes” that encode TRP channels (for the history of this discovery, see Minke, 2010; Hardie, 2011; Montell, 2011). By the latest count, the TRP channel superfamily contains 28 mammalian members (27 in humans; see Fig. 1 for the TRP “family tree”) and is subdivided into six subfamilies, all of which permeate cations (reviewed in Clapham et al., 2001; Clapham, 2003; Nilius et al., 2007b; Wu et al., 2010; Nilius and Owsianik, 2011, Gees et al., 2012).

As known thus far, all TRP channels share some structural similarities (similarities and differences are shown in Fig. 2). They contain six transmembrane spanning regions (S1–S6), they have a pore-forming loop between the fifth (S5) and sixth (S6) regions, their C termini and N termini are intracellular, and they probably function mostly as heterotetramers or homotetramers (Gaudet, 2008, 2009; Wu et al., 2010; for more detailed information, please see the International Union of Basic and Clinical Pharmacology TRP Channel Database (www.iuphar-db.org/index.jsp).

The function of TRP channels is critically regulated by a variety of interacting and/or associated proteins, some of which are described in this work. For more detailed information, interested readers are referred to the excellent database on Mammalian Transient Receptor Potential Channel-Interacting Protein (www.trpchannel.org).

TRP channels are nonselective cation channels located in the plasma membrane. Some function as Ca\(^{2+}\) entry channels. Upon activation, they generate cell depolarization that can result in activation or inactivation of voltage-dependent ion channels and modulate the driving force of ion flux through channels and transporters. TRPs can also function as intracellular ion channels, mainly as Ca\(^{2+}\) release channels, in several cell organelles such as lysosomes, endosomes, the Golgi network, the endoplasmic reticulum, and synaptic vesicles (Fig. 3) (Gees et al., 2010). A functional subset of TRP channels—vanilloid TRP channels TRPV1, TRPV2, TRPV3, and TRPV4; ankyrin TRP channel TRPA1; melastatin TRP channel TRPM8; and canonical TRP channel TRPC5—is distinguished by their sensitivity to temperature (reviewed in Szallasi et al., 2007; Voets, 2012). In combination, these channels (collectively referred to as thermoTRPs) cover a broad range of temperatures, ranging from noxiously hot to dangerously cold.

TRP channels are polymodal channels, that is, they can be activated by a plethora of physical (voltage, temperature, force, pressure, and tension) and chemical (both endogenous and exogenous) stimuli (see Nieto-Posadas et al., 2011; Jara-Oseguera and Islas, 2013). The mode of TRP channel activation by these stimuli at the molecular level is, however, still poorly understood. “Chemical” activation of TRP channels by both endogenous and exogenous compounds will be reviewed in the particular channel chapters. There is an ongoing controversy about the direct “voltage activation” (or “modulation by voltage”) of TRP channels such as TRPC5, TRPV1, TRPV3, TRPM3, TRPM8, TRPA1, and polycystic TRP channel TRPP3 (and probably others as well). As opposed to voltage-gated channels that only sense changes in the transmembrane electrical field, TRP channels are polymodal (i.e., integrators of various cellular signals), a property that renders them coincidence detectors (Ramsey et al., 2006; Nieto-Posadas et al., 2011). The role of TRP channels in temperature sensing is well established.

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However, the list of thermoTRPs is expected to shrink/change in the near future because a number of postulated temperature-sensitive TRP channels (including TRPV2, TRPV3, TRPM4, and TRPM5) are probably not functionally involved in thermosensing. The mechanisms by which TRP channels detect changes in temperature are not clear (for critical reviews, see Latorre, 2009; Baez-Nieto et al., 2011; Voets, 2012; Jara-Oseguera and Islas, 2013).

Controversy remains with regard to the function of TRP channels as direct mechanosensors, especially in mammals (see Pedersen and Nilius, 2007). Although TRPN1 (a member of the no mechanoreceptor potential C family) is clearly part of the pore-forming α-subunit of the native mechanoactivated or stretch-activated ion channels (SACs) in ciliated sensory neurons of the worm *Caenorhabditis elegans* and the Johnston organ of the fruit fly *Drosophila melanogaster* (together with the TRP channels Nanchung and Inactive) (reviewed in Wilson and Corey, 2010), the situation in mammals remains controversial. For example, the mechanosensitive transducer channel in hair cells of the organ of Corti is clearly not TRPA1 (as was originally suggested by Corey et al., 2004) but is probably the newly discovered TRP-unrelated transmembrane channel-like proteins TMC1 and TMC2 (Pan et al., 2013). Although both TRPV4 and TRPA1 have been implicated in the development and maintenance of mechanical hyperalgesia during inflammation, their roles in physiologic mechanosensing remain to be established. Importantly, none of the TRP channels suggested to function as SACs in free nerve endings and/or Merkel cell-neurite complexes can be directly activated by pressure under experimental conditions (e.g., fast-pressure clamps system) that are used to record SAC-like activity. Other putative mechanosensitive TRP channels (e.g., TRPA1, TRPC1, and TRPC6) could not be activated by membrane stretch in expression systems. It is clear that the gamut of the evidence (extensively discussed in Christiansen and Corey, 2007; Nilius and Honoré, 2012; Pedersen and Nilius, 2007; Roudaut et al., 2012; Delmas and Coste, 2013; Eijkelkamp et al., 2013) does not support a direct role for TRP channels in mechanosensing. Indeed, TRPV4 is most likely indirectly force-regulated via a mechanosensing phospholipase A2 (PLA2). Possible exceptions include TRPC1 as a sensor for light touch sensation in cutaneous low-threshold mechanosensory neurons (Garrison et al., 2012) and TRPA1 as a slowly adapting SAC in sensory neurons (discussed later in this work). A proteomic network analysis of TRP proteins.
channels may resolve some of the above-described controversies by providing novel insights into the functional impact of putative TRP channel $\beta$-subunits and associated proteins.

This review first describes the main properties of all TRP channels (Nilius and Owsianik, 2011; Zheng, 2013). We then highlight their role in hereditary channelopathies (Nilius and Owsianik, 2010b) and acquired diseases.

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**Fig. 2.** Similarities and differences between the topologic structure of selected TRP channels, representing the ankyrin (TRPA1), canonical (TRPC3), melastatin (TRPM2), and vanilloid (TRPV1) subfamilies. TRPA1 is characterized by the unusually high number of ankyrin repeats (after which it was named) at its N terminus, and it lacks the TRP box at its C terminus that is present in the TRPC, TRPV, and TRPM subfamilies. TRPC3 interacts with the scaffolding protein caveolin at its N terminus (that may directly link TRPC3 to the $\alpha$-subunit of GPCRs); on the C terminus, it has recognition sites for CaM and the IP$_3$ receptor. This binding domain is also referred to as the CaM/IP$_3$R binding site (CIRB). TRPC3 has also been shown to interact with immunophilins (endogenous cytosolic peptidyl-propyl isomerases) as part of the signalplex. TRPM2 possesses four TRPM homology regions (MHR1–MHR4) at its N terminus. At its C terminus, TRPM2 has a nucleoside diphosphate-like moiety X (NUDIX) domain that displays ADPR activity. The ankyrin repeats at the N terminus of TRPV1 are linked to the association of the channel with PI3K, ATP, and CaM. The C terminus carries CaM and PIP$_2$ recognition sites. For further details on TRPV1 regulation, also see Fig. 4. Courtesy of Dr. Rachelle Gaudet (Harvard University).

**Fig. 3.** Early endosomes (EEs) derive endocytotic vesicles (EVs) from the plasma membrane. Recycling endosomes (REs) and the late endocytotic pathway via late endosomes (LEs) to the lysosomes (LYs) are indicated. Intermediate transport vesicles (TVs) bud from the endoplasmic reticulum (ER) and trans-Golgi network (TGN). Secretory vesicles (SVs), secretory granules (SGs), and synaptic vesicles (SyVs) are derived from early endosomes. Intracellular locations of TRP channels are indicated by the channel number. Reprinted with permission from Gees et al. (2010).
Finally, we summarize the current state of the field of drug discovery and developmental efforts directed at TRP channels (see also Nilius, 2007; Nilius et al., 2007b; Moran et al., 2011; Kaneko and Szallasi, 2013).

A. Canonical Transient Receptor Potential Subfamily

The canonical TRP (TRPC) subfamily was the first trp gene family to be cloned in mammals (Wes et al., 1995; Zhu et al., 1995). It contains seven mammalian members (of note, in humans trpc2 is only a pseudogene). As a general remark, activation of these channels is not well understood. Inserted in the plasma membrane, most of the TRPCs are spontaneously active. All TRPC channels contain a conserved TRP box, EWKFAR, in their C-terminal tail and three to four N-terminal ankyrin repeats. All TRPC channels are nonslective cation channels and are permeable for Ca^{2+}. On the basis of sequence homology and similarities in function, TRPCs fall into four groups: TRPC1, TRPC2, TRPC3/TRPC6/TRPC7, and TRPC4/TRPC5 (Vazquez et al., 2004). The pharmacological characterization of these channels is hindered by the lack of subtype-specific agonists and antagonists (Bon and Beech, 2013).

In general, G protein–coupled receptors (GPCRs) play a central role in the regulation of TRPC channels. Indeed, TRPCs may be one of the most important downstream targets for GPCRs. In many instances, signals from GPCRs to TRPCs are mediated via lipid signaling (Kukkonen, 2011). For example, TRPC3, TRPC6, and TRPC7 are mostly activated by members of the Gq/11 family, which are coupled downstream to phospholipase C (PLC)–β. However, for TRPC4/TRPC5, Go_{i/o} (rather than Gq/11) family members are the dominant activators. Most of the TRPCs are also activated via a PLCγ pathway, downstream of several tyrosine kinase receptors such as the fibroblast growth factor receptor-1 and the brain-derived neurotrophic factor (BDNF). Others comprise the cytokine receptors bone morphogenetic protein receptor type II and tumor necrosis factor (TNF) receptor superfamily member 1α, which are coupled to the small body size-mothers against decapentaplegic family of proteins that transduce extracellular signals from the transforming growth factor (TGF) receptor to the nucleus (Zhang et al., 2013a). Further details are outside the scope of this review.

A hallmark of TRPC channels is their differential modulation by lipids, such as diacylglycerol (DAG), phosphatidyl inositol, lysophospholipids, oxidized phospholipids, sphingosine phosphates, and gangliosides, which either change channel activity or influence TRPC insertion in the plasma membrane. It is noteworthy that these lipids are coupled to several proteins, such as caveolins, GPCRs, and the phospholipid-binding proteins, such as the Saccharomyces cerevisiae 14-like (SEC14) and spectrin domain 1 (SESTD1) proteins (Beech, 2012). TRPCs can be coupled with the cytoskeleton via Homer scaffolds with actin or localized in channelosomes with caveolae adapted to the scaffolding protein, caveolin-1. These connections are important for focal adhesions, cell migration, and actin stress fiber formation (Ong and Ambudkar, 2011; Stuber et al., 2012). Members of the closely related TRPC3/TRPC6/TRPC7 group of channels can be activated by DAG, independent of the stimulation of protein kinase C (PKC), suggesting that DAG is the PLC-derived product that mediates the physiologic activation. By contrast, the TRPC1/TRPC4/TRPC5 group of channels, which are also activated by receptor-induced PLC, are unresponsive to DAG (Venkatachalam et al., 2003).

The activation mechanism of TRPCs via PLC stimulation is still not completely resolved. All TRPCs exhibit overlapping binding sites for, and interact to a varying extent with, calmodulin (CaM) and the inositol-1,4,5-trisphosphate (IP3) receptor [the binding site is referred to as the CaM/IP3R binding site; Fig. 2] (Trost et al., 2001; Zhu, 2005). Most of the TRPCs are inhibited by a Ca^{2+}/CaM-dependent mechanism and removal of this inhibition may lead to activation. TRPC channels have been reportedly involved in the control of smooth muscle activity (Albert et al., 2009), endothelial cell function (Morita et al., 2011), regulation of the blood-brain barrier (Brown et al., 2008), control of the cell cycle (Madsen et al., 2012), and various functions in the brain such as growth cone guidance, neurotransmitter release, synaptic plasticity [long-term depression (LTD) and long-term potentiation (LTP)], and brain development (Tai et al., 2009; Henle et al., 2011; Hutchins et al., 2011; Amaral and Pozzo-Miller, 2012; Chae et al., 2012). Furthermore, TRPCs are involved in the regulation of neurosecretion in gonadotropin-releasing hormone neurons by stimulating peptide release and activating the reproductive axis in mammals (Zhang et al., 2008a). In this context, kisspeptin, a GPCR ligand (GPR54, also known as Kiss1R), dominates the excitability of gonadotropin-releasing hormone neurons (Rønnekleiv and Kelly, 2013). This pathway seems to be generally involved in the regulation of synaptic plasticity and neurotransmitter release.

TRPC1, the first mammalian Trp gene, was cloned in 1995 and 1996 (Wes et al., 1995; Zhu et al., 1995, 1996; Zitt et al., 1996). TRPC1 was defined as a Ca^{2+}-permeable ion channel. It is widely expressed in heart, brain, lung, liver, spleen, kidney, testes, maybe all epithelial cells, smooth muscle cells, endocrine pituitary cells, and glial cells (Wang et al., 1999; Strübing et al., 2001). There are conflicting reports of whether TRPC1 homomers can form functional channels (i.e., whether TRPC1 is really a pore-forming α-subunit; Strübing et al., 2001; Storch et al., 2012). The finding that overexpressed homomeric TRPC1 channels were described as stretch activated by some (Maroto et al., 2005) but not others (Gottlieb et al., 2008; Sharif-Naeini et al., 2008) is also puzzling. By contrast, there is no doubt that TRPC1 can form functional heteromers with other TRPC channels.
TRPC1 is closely related to TRPC6 and TRPC7, with a sequence identity of approximately 75% (topologic structure shown in Fig. 2). The CaM/IP_3R binding site region of TRPC3 exhibits higher CaM affinity than seen in other TRPCs (Wedel et al., 2003; Zhu and Tang, 2004). TRPC3 can form homomorphic channels but also heteromerizes with TRPC1/TRPC4/TRPC5/TRPC6/TRPC7 in some cell types (Strübing et al., 2003; Dietrich et al., 2005a). TRPC3 is highly expressed in endothelial cells (in caveolae) (Adebiyi et al., 2011; Senadheera et al., 2012), in the brain (e.g., cerebellum, cortex, substantia nigra, and hippocampus) both in neurons and glia (Huang et al., 2007; Roedding et al., 2009; Li et al., 2010; Mitsumura et al., 2011; Zhou and Lee, 2011), in the pituitary gland, smooth muscle, and cardiac muscle cells (Watanabe et al., 2008; Gonzalez-Cobos and Trebak, 2010), as well as in sensory organs such as the cochlea (Tadros et al., 2010).

TRPC3 is constitutively active and is regulated by monoglycosylation (Dietrich et al., 2003). TRPC3 is directly activated by both DAG and DAG analogs (Hofmann et al., 1999). It is noteworthy that a very selective inhibitor for TRPC3, pyrazole compound-3 (Pyr3) was recently identified (Kiyonaka et al., 2009). PKC has an inhibitory effect on TRPC3 currents (Venkatachalam et al., 2003). TRPC3 activation is coupled to purine receptors both in endothelial and smooth muscle cells (Kamouchi et al., 1999; Reading et al., 2005). In neurons, TRPC3 is activated by BDNF and is possibly associated with the BDNF receptor Trk-B. This link appears to be essential for the guidance of nerve growth cones by BDNF (Li et al., 2005b; Amaral and Pozzo-Miller, 2007, 2012).

In the cerebellum, TRPC3 has been identified as an essential player in LTD (Kim, 2013). In T lymphocytes, TRPC3 is activated by T-cell receptor–triggered immune responses and is probably directly coupled to PLCγ1/γ2, which mediates translocation of the channel (Philipp et al., 2003; van Rossum et al., 2005). TRPC3 is inhibited by cGMP/protein kinase G (PKG); in endothelium, it provides a negative feedback mechanism for the generation of nitric oxide (NO) (Yao and Garland, 2005). In vascular smooth muscle cells, this mechanism may induce relaxation (Chen et al., 2009a). In endothelial cells, vascular cell adhesion molecule-1 (VCAM-1) expression and monocyte adhesion depend on TRPC3 (Smedlund et al., 2010). Cardiac atrial natriuretic peptide binds to the transmembrane guanylyl cyclase and stimulates a unique cGMP-independent pathway, including TRPC3 activation, which might be involved in cardiac hypertrophy (Klaiber et al., 2011). In this context, it is interesting that TRPC3 is involved in mechanosensing, probably via the mechanotransducer zyxin (Suresh Babu et al., 2012).

The genes encoding small C-terminal domain phosphatases, which are highly expressed in the heart,
contain microRNAs (e.g., miR-26) that negatively regulate TRPC3 expression (Sowa et al., 2012). The nonreceptor tyrosine kinase Src has a stimulatory effect on TRPC3 (Vazquez et al., 2004). This channel is also activated via stimulation of metabotropic glutamate receptors (mGluRs), which is dependent on phospholipase D but not PLC (Glitsch, 2010). PLCγ contains a so-called split pleckstrin-homology domain. The C-terminal half of this split pleckstrin-homology domain has been proposed to interact with an N-terminal site of TRPC3. This direct interaction may cause an increased surface expression of TRPC3 upon PLCγ activation (van Rossum et al., 2005).

Moreover, TRPC3 is involved in the control of the erythropoietin-dependent survival, proliferation, and differentiation of mammalian erythroid progenitors (Hirschler-Laszkiewicz et al., 2011). TRPC3 contributes to the constitutive Ca2+ influx in macrophages (Tano et al., 2011). TRPC3 is also involved in the immunoreceptor activation in mast cells (Cohen et al., 2009). Orexin (hypocretin), a neurotransmitter that regulates arousal, wakefulness, and appetite, is able to activate TRPC3 via orexin type 1 receptor (Peltonen et al., 2009; Louhivuori et al., 2010). Activation of mGluR1 in the cerebellum initiates slow excitatory postsynaptic potentials (EPSPs), which are mediated by TRPC3 (Hartmann et al., 2008, 2011). Finally, it was recently suggested that a TRPC3/TRPC6 complex may form (at least part of) the mechanotransduction channel in cochlear hair cells (Quick et al., 2012). Surprisingly, Trpc3 knockout mice did not show the expected alterations in brain development (Hartmann et al., 2008).

TRPC4 is a homolog of TRPC5. It contains a PDZ-binding motif in its C-terminal tail that binds PDZ domain scaffolding proteins. PLCβ1 and PLCβ2 associate with TRPC4 via this domain (Tang et al., 2000). TRPC4 can form functional homomultimers, as well as heteromultimers with TRPC1, TRPC3, TRPC5, and TRPC6 (Strübing et al., 2001, 2003). Similar to other TRPC channels, TRPC4 is widely expressed, including in endothelium, smooth muscle cells, brain (geniculate nucleus, hippocampus, dentate gyrus), the kidney, keratinocytes (in the skin and gingiva), as well as in interstitial cells of Cajal (reviewed in Pedersen et al., 2005). Homotetrameric TRPC4 and heterotetrameric TRPC1/TRPC4 channels are activated after activation of PLC-coupled receptors but the mechanisms that link receptor activation to channel gating are still controversial (Hofmann et al., 1999; Schaefer et al., 2000). TRPC4 is associated with PKG-phosphorylated vasodilator-stimulated phosphoprotein, which inhibits the channel (Wang et al., 2007). Tyrosine phosphorylation by epidermal growth factor receptor (EGFR) activation is critical for plasma membrane translocation and activation of TRPC4; this requires a direct interaction between TRPC4 and the spectrin cytoskeleton (Odell et al., 2008). The two splice variants of TRPC4 are differentially regulated. TRPC4α and the shorter TRPC4β, which lacks 84 amino acids in the cytosolic C terminus, are highly expressed in smooth muscle and endothelial cells. Both TRPC4 variants are activated by Gq/PLC-coupled receptors. Importantly, TRPC4α, but not TRPC4β, was strongly inhibited by intracellularly applied phosphatidylinositol 4,5-bisphosphate (PIP2), which binds to the C terminus of TRPC4α. This inhibitory action was dependent on the association of TRPC4α with actin cytoskeleton that binds to the PDZ domain. PIP2 breakdown is a required step in activation of TRPC4α but PIP2 depletion alone is not sufficient for channel opening, which additionally requires Ca2+ and pertussis toxin-sensitive Gαi-proteins. TRPC4α is also strongly voltage dependent (Jeon et al., 2008; Otsuguro et al., 2008). Moreover, TRPC4 channels can be directly activated by the trivalent cations La3+ and Gd3+ (Schaefer et al., 2000), independently on interference with the Ca2+ sensor.

Similar to TRPC1, TRPC4 is believed to participate in store-operated Ca2+ entry in pulmonary artery endothelial cells, leading to the formation of gaps between endothelial cells and subsequent endothelial barrier disruption. The large molecular weight immunophosphokin FK506-binding proteins FKBP51 and FKBP52 associate with the store-operated Ca2+ entry/TRPC4 channel protein complex (Kadeba et al., 2013). From the phenotype of Trpc4 knockout mice, it is deduced that this channel can be activated via muscarinic receptors and is essential for endothelium-dependent vasorelaxation (Freichsel et al., 2001) and regulation of the endothelial barrier function (Tiruppapathi et al., 2006).

TRPC5 shares approximately 64% sequence identity with TRPC4. The TRPC5 protein also contains a PDZ-binding motif in the C terminus that interacts with the Na+/H+ exchanger regulatory factor (NHERF), an adapter protein involved in signal transduction (Obukhov and Nowycky, 2004). TRPC5 forms homomultimers or heteromerizes with TRPC1, TRPC3, TRPC4, and TRPC6. TRPC1/TRPC5 is the major heteromer in the brain (Strübing et al., 2003). TRPC5 is widely expressed, especially in the brain (hippocampus, cortex, cerebellum, substantia nigra, entorhinal cortex, amygdala, the pre-Bötzinger complex, and the olfactory bulb) (De March et al., 2006; Fowler et al., 2007; Yan et al., 2009), but also in the heart, liver, lung, spleen, testis, and uterus (Philipp et al., 1998; Beech, 2007). TRPC5 shows considerable similarities to TRPC4 in its gating properties. Homotetrameric TRPC5 and heterotetrameric TRPC1/TRPC5 channels are activated via PLC-coupled receptors, but the mechanism underlying this link is not yet clear (Beech, 2007). The muscarinic receptor/Gαq11/PLCβ1 cascade seems to play a crucial role in the activation of brain TRPC5 (Yan et al., 2009). TRPC5 is modulated by lipids (e.g., sphingosine-1-phosphate) and, in general, it senses bipolar phospholipids (AL-Shawaf et al., 2011).
TRPC5 is modulated by covalent modification of reactive cysteines and functions as a redox sensor. It is activated by extracellular application of the endogenous redox protein, thioredoxin (Xu et al., 2008), by endogenous hydrogen peroxide (Yamamoto et al., 2010; Naylor et al., 2011), oxidized phospholipids (Al-Shawaf et al., 2010), and NO (Yoshida et al., 2006). As is the case for all TRP channels, currents through TRPC5 are modulated by plasma membrane insertion or retrieval of the channels. Plasma membrane insertion of TRPC5 can be promoted by epidermal growth factor (EGF). This effect requires the presence of Rho GTPase, Rac1, PI3K, and phosphatidylinositol 4-phosphate 5-kinase (Bezzerides et al., 2004). Protein kinase A (PKA) inhibits TRPC5 via Gα proteins (Sung et al., 2011). TRPC5 is also inhibited by neuroactive steroids (Majeed et al., 2011). PI2 is involved in channel regulation. Its breakdown is required for desensitization of TRPC5 current, but PI2 depletion alone is insufficient for channel desensitization (Kim et al., 2008a). Extracellular lanthanides (at low concentrations), Gd3+, lead, increased [Ca2+]i, and also protons, directly activate TRPC5 (Jung et al., 2003; Zeng et al., 2004; Semtner et al., 2007; Blair et al., 2009; Sukumar and Beech, 2010). TRPC5 is in cross-talk with voltage-activated Ca2+ channels in several tissues (Gross et al., 2009). It is noteworthy that TRPC5 is also activated by cold; this observation adds TRPC5 to the list of thermoTRPs (Zimmermann et al., 2011).

The activation pattern of TRPC5 becomes complex because of its striking Ca2+ dependence (Blair et al., 2009), and especially because of its voltage dependence. Indeed, TRPC5 I–V curves change during the activation-deactivation cycle, shifting between outwardly rectifying and doubly rectifying shapes. Depolarization activates the channel (Obukhov and Nowycky, 2008). It is noteworthy that TRPC5 is also a redox sensor: it senses oxidative stress and induces adaptation reactions. Finally, TRPC5 is activated by cell swelling, which hints to some mechanosensing properties (Gomis et al., 2008).

TRPC5 is reportedly involved in various cellular functions such as cell migration, contribution to the working memory establishment, growth cone guidance and dendrite extension, cardiac pacemaking, and mitosis. TRPC5 is also involved in erythropoietin effects, motility of gastrointestinal smooth muscle, and much more (Lee et al., 2003c; Ju and Allen, 2007; Kaczmarek, 2010; Tian et al., 2010; Liu et al., 2011b; Zhang et al., 2011b; Kaczmarek et al., 2012).

An intriguing new modulation mechanism describes the activation of TRPC5 by hypo-osmotic cell swelling via activation of Gq-coupled receptors, which, in turn, enhances the activation of TRPC5 by regulating the membrane insertion of this channel. Because Gq-coupled receptors and TRPC5 are coexpressed in several tissues (e.g., vascular system, glia cells, and neurons), the Gq-coupled receptor mechanism of TRPC5 activation may have interesting and far-reaching implications in arterial pressure sensing and mechanotransduction (Jemal et al., 2013).

From the phenotype of Trpc5 knockout mice it can be deduced that this channel is involved in fear-related behavior (reviewed in Kaczmarek, 2010). The TRPC5-null mouse shows no apparent anatomic or developmental defects. However, a reduction in responses mediated by synaptic activation of group I metabotropic glutamate and cholecystokinin (CCK)-2 receptors in amygdala neurons was present in the Trpc5 knockout animals (Riccio et al., 2009). This nucleus is involved in fear reactions, which are diminished in the knockout mouse (Riccio et al., 2009).

TRPC6 shares a sequence identity of approximately 75% with TRPC3 and TRPC7. It likely has four ankyrin repeats, which interact with MxA (a member of the dynamin protein family), and has two glycosylation sites, which control channel activity and trafficking (Dietrich and Gudermann, 2007). TRPC6 can form either homomultimers or heteromultimers with TRPC3 and TRPC7 (Dietrich et al., 2005a). TRPC6 is widely expressed, especially in smooth muscle cells, in lung, heart, brain, and kidney (glomerular podocytes), as well as in blood and immune cells. TRPC6 expression has also been reported in the adrenal gland, bone, epididymis, intestine, ovary, pancreas, and prostate (see Dietrich and Gudermann, 2007). In contrast with TRPC3, TRPC6 is (almost) inactive under unstimulated conditions, which might be attributed to the double glycosylation (Dietrich et al., 2003). TRPC6 is activated by Gauq11-coupled GPCRs and forms the essential part of the vascular α1-adrenoceptor–activated Ca2+-permeable cation channel complex in smooth muscle (Inoue et al., 2001; Jung et al., 2002b). Endotoxin lipopolysaccharide (LPS) activates TRPC6 in endothelial cells in a Toll-like receptor–dependent manner (Tauseef et al., 2012).

There is increasing evidence to suggest that TRPC6 plays important functions in smooth muscle and endothelial cells. TRPC6 is directly activated by DAG and DAG analogs (Hofmann et al., 1999; Estacion et al., 2004). PKCδ exerts an inhibitory effect on TRPC6 through direct phosphorylation (Bousquet et al., 2010). Tyrosine kinases also activate TRPC6. This is thought to play an important role in VEGF-mediated angiogenesis that requires TRPC6 activation (Hamdollah Zadeh et al., 2008; Ge et al., 2009). TRPC6 is activated by cAMP, possibly via the cAMP-P13K-PKB-MEK-extracellular signal-regulated kinase (ERK) 1/2 cascade (Shen et al., 2011a). TRPC6 plays a key role in excitatory synaptic transmission, plasticity, and neural development. It inhibits N-methyl-d-aspartate (NMDA)–induced currents probably via activation of calcineurin and functions as a negative modulator of NMDA receptors (Shen et al., 2013). TRPC6 channels can be negatively regulated by the NO/cGMP/PKG pathway (Takahashi et al., 2008; Koitabashi et al., 2013).
2010). PI3K and its antagonistic phosphatase, phosphatase and tensin homolog, are involved in the activation of TRPC6 and promote its plasma membrane insertion (Monet et al., 2012). Insertion is also promoted by Rab11, a member of the Ras superfamily of monomeric G proteins (Cayouette et al., 2010). Of note, TRPC6 couples to the Na+/Ca2+ exchanger and thus might contribute to any signaling mechanism that depends on localized cytosolic subplasmalemmal Na+ elevation (Poburko et al., 2008).

At least in endothelial cells, cAMP/PKA induces intracellular translocation of TRPC6 channels in caveolin-1-rich areas, which potentiate its activation (Fleming et al., 2007). The epoxygenase metabolite 11,12-epoxyeicosatrienoic acid potentiates the contractile responses in pulmonary arteries via TRPC6 activation (Loot and Fleming, 2011). Platelet-activating factor also activates TRPC6 in endothelial cells. On the basis of these observations, it was suggested that TRPC6 is involved in the control of the endothelial barrier. For example, activation of TRPC6 disturbs the barrier function and translocates it in caveolae of endothelial cells (Samapati et al., 2012).

As shown now for many TRP channels, TRPC6 is activated by reactive oxygen species (ROS; e.g., H2O2) (Graham et al., 2010; Ding et al., 2011). TRPC6 activity is potentiated by flufenamate and the eicosanoid 20-hydroxyeicosatetraenoic acid (Inoue et al., 2001; Jung et al., 2002b). It is also activated by thrombin and platelet-activating factor and plays an important role in platelet activation (Hassock et al., 2002; Dionisio et al., 2011, 2012). Remarkably, TRPC6 has been also identified as a “candidate” mechanosensing TRP channel that is activated by stretch and may contribute to mechanosensing in the heart (Spassova et al., 2006; Dyachenko et al., 2009).

Hyperforin (structure shown in Fig. 6), one of the main bioactive compounds that underlie the antidepressant actions of the medicinal plant St. John’s wort (Hypericum perforatum), is a potent TRPC6 activator and promotes channel expression (Leuner et al., 2007; Griffith et al., 2010; Harteneck and Gollasch, 2010).

As with all TRPCs, TRPC6 is a nonselective cation channel with Ca2+ selectivity similar to its closest homologs, TRPC3 and TRPC7. However, although TRPC6 is Ca2+ permeable, under physiologic conditions TRPC6 induces depolarization and mainly Na+ enters the cell via this channel. It is only at very negative potentials that Ca2+ entry through TRPC6 becomes significant (Estacion et al., 2006). TRPC6 is also a Zn2+-permeable channel (Gibbon et al., 2011).

With regard to its functional impact, TRPC6 has been identified as an important player in dendritic growth, neuronal survival, and synapse formation (Jia et al., 2007; Zhou et al., 2008). Strikingly, mice overexpressing TRPC6 show enhanced spine formation and improved spatial learning and memory formation in the Morris water maze test, suggesting a role for the channel in synaptic and behavioral plasticity (Zhou et al., 2008). The important role of TRPC6 in smooth muscle cells is supported by data from Trpc6 knockout mice, which unexpectedly exhibit a slightly elevated blood pressure associated with enhanced basal and agonist-induced cation entry. This effect is due to a compensatory upregulation of TRPC3 and TRPC7 (Dietrich et al., 2005b).

TRPC6 plays a unique and indispensable role in acute hypoxic pulmonary vasoconstriction (also known as the Euler–Liljestrand mechanism), a local vasoconstriction in the lung that shifts blood flow from hypoxic to normoxic areas, thereby maintaining effective gas exchange. Trpc6 knockout mice lack the hypoxic pulmonary vasoconstriction reflex and develop arterial hypoxemia during conditions of regional hypoventilation (Weissmann et al., 2006). It is noteworthy that TRPC6 is expressed in podocytes, where it is required for establishing the glomerular filtration barrier (Reiser et al., 2005). TRPC6 also plays an activating role in cell migration downstream of the CXC-type, Gq protein–coupled chemokine receptors and TRPC6 downregulation strongly impairs the chemotaxis of neutrophils (Damann et al., 2009; Lindemann et al., 2013).

TRPC7 occurs in two different splice variants. It shares an 81% and 75% overall identity with TRPC3 and TRPC6, respectively. It forms homomultimers, but also heteromultimers with TRPC1, TRPC3, and TRPC6, and is widely expressed in tissues from brain, eye, heart, intestine, kidney, lung, and pituitary gland. It is constitutively active and can be further activated by GPCRs or DAG analogs. It is negatively regulated by extracellular Ca2+ and PKC (see Numaga et al., 2007). TRPC7 is also negatively regulated by the cGMP-regulated protein kinase I pathway; that is, cGMP-regulated protein kinase I–mediated phosphorylation inhibits the channel (Yuasa et al., 2011). The functional role of TRPC7 is still not well understood and phenotypic alterations in Trpc7 knockout mice have not yet been described.

B. Vanilloid Transient Receptor Potential Subfamily

The TRPV subfamily comprises six members that, based on homology, fall into four groups: TRPV1/ TRPV2, TRPV3, TRPV4, and TRPV5/ TRPV6. This vanilloid subfamily was named after its founding member, the vanilloid (capsaicin) receptor VR1 (Szallasi and Blumberg, 1999; Nilius and Owsianik, 2011). Members of the TRPV subfamily function as tetrameric complexes with each subunit containing six N-terminal ankyrin repeats (Gaudet, 2008, 2009). All members have a TRP box in their C terminus (Fig. 2). TRPV1, TRPV2, TRPV3, and TRPV4 are modestly permeable to Ca2+, whereas TRPV5 and TRPV6 are the only highly Ca2+ selective channels in the TRP family. These latter channels are tightly regulated by [Ca2+]i.
Remarkably, TRPV1 has a dynamic pore, meaning that it shows pore dilation during stimulation (Chung et al., 2008) (Fig. 4). TRPVs seem to be also permeable for large cations (Chung et al., 2008). The physiologic significance of this dilation is unclear. TRPV channels show a complex pharmacology including very selective agonists for some TRPVs, such as capsaicin (structure shown in Fig. 5) for TRPV1, but mostly less selective activators such as 2-aminoethoxydiphenyl borate (2-APB; see Fig. 6 for structure) for TRPV3 and, to a lesser extent, also TRPV2 and TRPV1 (for a comprehensive review, see Vriens et al., 2009). Ruthenium red blocks all TRPV channels with variable potencies (IC₅₀ values within the range of 0.1–9.0 μM) (for a review, see Vennekens et al., 2008).

TRPV1 has been cloned from different species (including humans, guinea pigs, rabbits, mice, chickens, and pigs), revealing marked species-related differences in pharmacological profiles. It has long been known that birds (unlike rodents) are essentially insensitive to the pungent action of capsaicin, forming the experimental foundation of “squirrel-free” bird feed (reviewed in Szallasi and Blumberg, 1999). Rabbits are also much less sensitive (approximately 100-fold) to capsaicin than rats or humans. Remarkably, point mutations in only two key residues (547 and 550; Fig. 4) are responsible for most of these differences in capsaicin potencies (Jordt and Julius, 2002; Gavva et al., 2004).

Most recently, the first high-resolution (3.4 Å) structure of TRPV1 was described in the presence of vanillotoxin, double-knot toxin (from *Psalmopoeus cambridgei*), and resiniferatoxin (RTX) (Cao et al., 2013a; Liao et al., 2013). As expected, S1–S4 bundles are located in the periphery of the channel and are rather “rigid” (which is unusual for a putative voltage sensor). The four S5 to S6 bundles tetramerize and form the ion pore. Importantly, the TRPV1 pore features two constrictions: a funnel-like extracellular pore forms the selectivity filter and functions as an upper gate, whereas the lower gate is located in the middle of the S6 helix. Both gates must be simultaneously open to allow ion permeation. Double-knot toxin opens the extracellular pore, whereas RTX (and also capsaicin) operates the lower gate and also interacts with the S5 to S6 linker. Interestingly, the TRP box is involved in capsaicin binding and leads to opening of the lower gate in S6. Obviously, there is cooperativity between the two pores.

![Fig. 4. TRPV1 channel topology highlighting key residues and amino acids (hot spots) involved in gating function in response to different stimuli (e.g., capsaicin, protons, and heat). The hot spots represent cumulative evidence from site-directed mutagenesis experiments; they provide evidence for distinct vanilloid (capsaicin/RTX), proton, alkaline (base), heat, and allicin recognition sites. Black circles designate phosphorylation sites for PKA and PKC that are involved in channel sensitization. LPA, lysophosphatidic acid. Reprinted with permission from Szolcsányi and Sándor (2012).](image-url)
to control TRPV1 gating. The determination of the high-resolution structure of TRPV1 marks a breakthrough in TRP channel research.

TRPV1 predominantly forms homotetramers. Although heteromers with TRPV2, TRPV3, and TRPV4 have been reported, their existence remains debated (Hellwig et al., 2005). (TRPA1/TRPV1 heteromers will be discussed later as potential analgesic targets.)

Several TRPV1 splice variants have been identified. A short N-terminal splice variant was isolated from supraopticus nucleus neurons where it might be involved in osmosensing (Sharif Naeini et al., 2006). Another truncated form seems to be present in taste cells, mediating amiloride insensitive salt taste (Pingle et al., 2007; Lyall et al., 2010).

The exact tissue expression pattern of TRPV1 is still under discussion. Historically, capsaicin sensitivity was considered to be the “functional signature” of primary sensory neurons (reviewed in Szallasi and Blumberg, 1999; Szolcsányi, 2004). Indeed, expression of TRPV1 initially seemed to be restricted to sensory neurons (Szallasi and Blumberg, 1990; Caterina et al., 1997). Subsequently, a wide variety of tissues and cell types were reported to express TRPV1 (albeit at much lower levels than in sensory ganglia), including both neurons (throughout the whole neuroaxis of the rat, from the olfactory bulb through the cortex and basal ganglia to the cerebellum; see Szallasi and Di Marzo, 2000) and nonneuronal cells (in kidney, pancreas, testes, uterus, spleen, stomach, small intestine, liver, lung, bladder, skin, skeletal muscle, mast cells, macrophages, and leukocytes) (reviewed in Tominaga and Tominaga, 2005; Szallasi et al., 2007). These reports are puzzling and difficult to reconcile with the well characterized spectrum of capsaicin actions. Indeed, a recent article utilizing a TRPV1 reporter mouse (Cavanaugh et al., 2011) found only minimal TRPV1 expression in a few discrete brain regions (e.g., caudal hypothalamus).

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Outside of the central nervous system (CNS), TRPV1 expression was mainly found in arteriolar smooth muscle
cells (i.e., in tissue that are involved in thermoregulation) (Cavanaugh et al., 2011). This observation may provide an alternative mechanistic explanation for the opposing actions of TRPV1 agonists (hypothermia) and antagonists (hyperthermia) on body temperature regulation. Capsaicin and its ultrapotent analog RTX (structure shown in Fig. 5) cause “reddening” (vasodilatation) when applied to rodent or human skin (reviewed in Szallasi and Blumberg, 1999). This effect was traditionally attributed to neurogenic inflammation. Indeed, the mouse ear erythema assay was used as a surrogate marker to detect capsaicin-like bioactivity (Szallasi and Blumberg, 1989). The mechanism by which capsaicin evokes hypothermia is essentially unknown, but it was speculated that it might be a direct effect on preoptic neurons involved in body temperature regulation (Hori, 1984). Conversely, most (but not all) TRPV1 antagonists evoke a febrile reaction, implying an essential role for TRPV1 in the regulation of body temperature (see Gavva, 2008). (For a comprehensive and critical state-of-the-art review on TRPV1 and thermoregulation, see Romanovsky et al., 2009.) If capsaicin can directly constrict (and TRPV1 antagonist dilate) arteriolar smooth muscles via TRPV1 to control blood flow, this effect may have profound consequences on body temperature. Of note (as discussed later), both TRPV1 agonists and antagonists dramatically differ in their ability to influence body temperature. For example, the small molecule TRPV1 antagonist PHE377 blocks capsaicin- and proton-evoked currents in rat sensory neurons, and is active in various rodent models of inflammatory and neuropathic pain (see Trevisani and Szallasi, 2010). Yet unlike other TRPV1 antagonists, PHE377 does not elevate body temperature. To explain these unexpected findings, one may speculate that TRPV1 channels in sensory neurons and arterial smooth muscle cells differ in their ligand-recognition properties.

TRPV1 was first identified as the vanilloid receptor VR1 by its highly selective binding of capsaicin and the even more selective RTX (Szallasi and Blumberg, 1990). The binding domain of TRPV1 for capsaicin and RTX has been located to the binding pocket between TM3 and TM4 (see Fig. 4) (Gavva et al., 2004; Lee et al., 2011). Structurally related vanilloids are also potent activators of the channel. Furthermore, TRPV1 is activated by heat as well as divalent (e.g., Ni²⁺) and trivalent cations (e.g., Gd³⁺) (Tousova et al., 2005). It is noteworthy that TRPV1 is activated both by acidification (protons) and alkalization (Dhaka et al., 2009). [Na⁺]₀, negatively regulates the gating and polymodal sensitization of the TRPV1 channel (Obta et al., 2008). To note, molecular mechanisms of TRPV1 activation were recently reviewed in Jara-Oseguera et al. (2010).

Despite extensive research, the physiologic regulation of TRPV1 by “endovanilloids” is poorly understood (Di Marzo et al., 2002; Palazzo et al., 2013). Endogenous activators include the cannabinoid receptor ligands anandamide and oleamide (structures shown in Fig. 5), the eicosanoids HPETE [12-(S)-5-hydroperoxyeicosatetraenoic acid], 15-(S)-HPETE, 5-(S)-hydroxyeicosatetraenoic acid, 20-hydroxyeicosatetraenoic acid, leukotriene B4 (LTB4), N-arachidonoyldopamine (NADA), N-oleoyldopamine, and polyamines (reviewed in Morales-Lázaro et al., 2013). Negatively charged intracellular lipids can also activate TRPV1 (Lukacs et al., 2013). Generally speaking, TRPV1 activation by these compounds requires such high concentrations that are unlikely to occur under physiologic conditions. Other endogenous TRPV1 activators include ROS and reactive nitrogen species (Tang et al., 2010a; Takahashi and Mori, 2011; Linley et al., 2012) and oxidized linoleic acid (LA) metabolites such as 9-hydroxyoctadecadienoic acid and 13-hydroxyoctadecadienoic acid (Patwardhan et al., 2009; Alsalem et al., 2013). NO can downregulate the function of TRPV1 through activation of the cGMP/PKG pathway in peripheral sensory neurons (Jin et al., 2012b). It is noteworthy that oleoylethanolamide (OEA), an endogenous lipid that regulates appetite and body weight, can directly activate TRPV1 (Ahern, 2003).

TRPV1 is a shared target for a number of structurally unrelated irritant compounds in nature. For example, TRPV1 is activated by capsaicin (responsible for the piquancy of hot chili peppers), RTX (from the soap of Euphorbia resinifera), piperine (in Piper nigrum), camphor (from Cinnamomum camphora), curcumine (in Curcuma longa), and [6]-Gingerol, a major constituent of ginger (see Szallasi and Blumberg, 1999; Vriens et al., 2009; Nilius and Appendino, 2011, 2013). For dietary modulation of TRPV1 to control appetite, food supplementation by nonpungent capsinoids such as capsiate (Fig. 5) has been advocated (Ludy et al., 2012). Capsiate is, however, not selective for TRPV1: Indeed, it can also activate TRPA1 (Shintaku et al., 2012). Conversely, some TRPA1-selective compounds such as allicin (from garlic) and allyl-isothiocyanate (in mustard oil; structure shown in Fig. 7) can also activate and/or sensitize TRPV1 (Macpherson et al., 2005; Everaerts et al., 2011; Alpizar et al., 2014). Indeed, allyl-isothiocyanate increases

![Fig. 7. Representative TRPA1 agonists. Cinnamaldehyde is from cinnamon. Allyl-isothiocyanate is from mustard. 4-Hydroxynonenal is a diffusible product of lipid peroxidation. Umbellularone is from the Californian headache tree Umbellularia californica. Cannabidiol is a major nonpsychoactive constituent in cannabis. Courtesy of Dr. Giovanni Appendino.](image)
carbohydrate oxidation (Mori et al., 2011), and thereby reduces hyperglycemia after glucose challenge (Mori et al., 2013), in TRPA1-null mice, whereas these effects are absent in the TRPV1 knockout animals. Of note, certain venoms and toxins from spiders (e.g., Tarantula), jelly fish, and snakes cause pain by directly activating TRPV1 (reviewed in Kumar et al., 2014).

TRPV1 is a voltage-gated channel that is activated by depolarization. Most of the TRPV1 activators induce a leftward shift in voltage dependence, resulting in channel activation (Voets et al., 2004; Nilius et al., 2005b). It is noteworthy that TRPV1 shows a rapid and a slow desensitization. This phenomenon is a Ca\(^{2+}\)-mediated process: it is abolished in the absence of extracellular Ca\(^{2+}\) or by intracellular Ca\(^{2+}\) chelation. Ca\(^{2+}\) entry (albeit the fractional Ca\(^{2+}\) current through TRPV1 is only 5\%) results in a sufficiently high Ca\(^{2+}\) concentration to trigger desensitization (a negative feedback mechanism). Desensitization is now mainly considered to be a dephosphorylation event. Indeed, it is inhibited by inhibitors of calcineurin, a Ca\(^{2+}\)-dependent phosphatase. Dephosphorylation events include sites that have been phosphorylated by PKA, PKC, and Ca\(^{2+}\)/CaM-dependent kinase II (CaMKII) (summarized in Fig. 4). Molecular events that promote channel phosphorylation sensitize the TRPV1 channel; this is often due to a leftward shift of the voltage dependence (i.e., in a depolarizing direction at the channel protein) (Nilius et al., 2005b; Tominaga and Tominaga, 2005).

The complexity of TRPV1 channel regulation is further increased if we take into account the modulator effects viaGPCRs that, in turn, increase cAMP [e.g., prostaglandin E\(_2\) (PGE\(_2\)) receptors], DAG, and IP\(_3\) [e.g., angiotensin, bradykinin (BK), proteinase-activated receptor-2 (PAR-2), nerve growth factor (NGF) receptors], which all indirectly target PKA, PKC, and CaMKII (Fig. 12). In addition, PIP\(_2\) is changed. Although PIP\(_2\) was initially considered as a tonic inhibitor of TRPV1 (Prescott and Julius, 2003), it is now accepted that PIP\(_2\) is required for channel activation, whereas PIP\(_2\) depletion conversely causes desensitization (Stein et al., 2006; Rohacs, 2007; Klein et al., 2008). PIP\(_2\) binds to a proximal C-terminal region of the TRPV1 protein (Ufret-Vincenty et al., 2011). The enhancement of TRPV1 activity by PIP\(_2\) may require the presence of Pirt, a phosphoinositide-binding protein (Kim et al., 2008a).

Indeed, in Pirt-deficient dorsal root ganglion (DRG) neurons both noxious heat- and capsaicin-evoked currents are significantly attenuated. Furthermore, Pirt-null mice exhibit deficits in behavioral responses to histamine, a pruritogenic compound that activates TRPV1\(^+\) sensory afferents (Patel et al., 2011).

However, this view has been challenged because Pirt does not alter the phosphoinositide sensitivity of TRPV1 in HEK293 cells. In addition, there is no fluorescence resonance energy transfer signal between TRPV1 and Pirt, and dissociated DRG neurons from Pirt knockout mice have an apparent affinity for PIP\(_2\) indistinguishable from the wild types (Ufret-Vincenty et al., 2011). The previous view on PIP\(_2\) as a negative regulator of TRPV1 recently again received attention. Purified TRPV1 reconstituted into artificial liposomes is fully functional in the absence of phosphoinositides, thus PIP\(_2\) has likely no obligatory role in channel activation, and, even more striking, introduction of PIP\(_2\) inhibits the channel (Cao et al., 2013a).

A more dynamic regulation TRPV1 activity is mediated by various scaffolding proteins. For example, TRPV1 activity is enhanced by interaction of TRPV1 with the A-kinase anchoring protein (AKAP) 79/150. AKAP79/150 forms a multiprotein complex with TRPV1/PKA/PKC (Zhang et al., 2008d; Efendiev et al., 2013). β-Arrestin-2, another scaffolding protein, promotes TRPV1 desensitization (Por et al., 2012). TRPV1 is variably N-glycosylated (glycosylation sites are shown in Fig. 4), and glycosylation is a key determinant of capsaicin regulation of TRPV1 desensitization and permeability (Veldhuis et al., 2012). In addition, TRPV1 is functionally coupled to the sensory neuron-specific Mas-related G protein–coupled receptor (Mrgpr)-X1, which is important for noiception (Solinski et al., 2012). Mrgpr-X1 sensitizes TRPV1 to heat and protons in a PKC-dependent manner. Direct Mrgpr-X1–mediated TRPV1 activation is independent of Mrgpr-X1–induced Ca\(^{2+}\) release and PKC activity or other TRPV1 affecting enzymes, such as lipooxygenase and PI3K. Mutations in the DAG or PIP\(_2\) binding sites in TRPV1 decreased Mrgpr-X1–induced TRPV1 activation. The functional interaction between Mrgpr-X1 and TRPV1 results in a PKC-dependent TRPV1 sensitization and DAG/PIP\(_2\)-mediated activation (Solinski et al., 2012).

Surface expression is an efficient regulator of TRPV1 activity. One important player that promotes surface expression of TRPV1 is NGF, which activates a signaling pathway in which PI3K plays the crucial role and a downstream Src kinase phosphorylates the channel (Zhang et al., 2005). Cyclin-dependent kinase (CDK)-5 positively regulates TRPV1 surface localization. TRPV1-containing vesicles bind to the forkhead-associated domain of the kinesin-3 family member 13B (KIF13B) and are thus delivered to the cell surface. Overexpression of CDK5 (or its activator, p35) promoted, whereas the inhibition of CDK5 activity prevented, the KIF13B–TRPV1 association, indicating that CDK5 promotes TRPV1 surface expression (Xing et al., 2012). Surface expression of TRPV1 is also regulated by the ubiquitin ligase MYCBP2 (Holland et al., 2011).

Insertion of TRPV1 into the plasma membrane is an important regulatory mechanism. Growth factors such as NGF and insulin-like growth factor (IGF)-1 activate PI3K and/or CDK5, which, in turn, mediate TRPV1 plasma membrane insertion signaling (Zhang et al., 2005; Lilja et al., 2007; Canetta et al., 2011; Xing et al., 2012). TRPV1-containing vesicles bind to the forkhead-associated
domain of the KIP13B, which mediates the delivery to the cell surface (Xing et al., 2012). Intracellular trafficking of TRPV1 also depends on microtubules (Goswami et al., 2006).

It is noteworthy that TRPV1 is the major channel for the detection and integration of nociceptive chemical and thermal stimuli in sensory nerve fibers (thin-myelinated Aβ-fibers and unmyelinated C fibers) innervating most of our organs (see Szallasi et al., 2007; Julius, 2013). This concept is supported by the Trpv1 knockout mouse, which shows markedly reduced thermal hyperalgesia after inflammation and injury (Caterina et al., 2000; Davis et al., 2000). As discussed later, this makes TRPV1 an obvious target for novel analgesic compounds.

TRPV2 shows a 50% sequence similarity to TRPV1. TRPV2 mostly forms homomers, but heteromers with TRPV1, TRPV3, and TRPV4 have also been reported (Hellwig et al., 2005; Cheng et al., 2007b). TRPV2 is probably the least understood member of the TRPV subfamily. Cloned as a "capsaicin receptor homolog with a high threshold for noxious heat," TRPV2 is expressed in a subset of DRG neurons (Caterina et al., 1999). In addition, it is present in certain hypothalamic brain nuclei and the magnocellular neurons of the paraventricular and supraoptic nucleus (Nedungadi et al., 2012). In the brain, TRPV2 was also detected in astrocytes (Shibasaki et al., 2013). The function of TRPV2 in the CNS is essentially unknown. Furthermore, TRPV2 is expressed in the heart, gastrointestinal tract, lung (alveolar cells), pancreas, retina, and both smooth and skeletal muscle cells (Muraki et al., 2003; Beech et al., 2004). TRPV2 has also been described as an intracellular ion channel residing in the membranes of early endosomes, where it may act as a Ca\(^{2+}\) release channel (Saito et al., 2007).

TRPV2 is activated by extremely noxious heat (>53°C) (Caterina et al., 1999). Growth factors such as IGF-1 (and also insulin) transfer the channel to the plasma membrane, where it seems to be constitutively active (Kanzaki et al., 1999; Iwata et al., 2002; Hisanaga et al., 2009). The key enzyme for plasma membrane insertion for many TRP channels is PI3K, which probably activates TRPV2 without changes in plasma membrane insertion (Penna et al., 2006). Other reports, however, describe an increased TRPV2 trafficking to the plasma membrane (Nagasawa et al., 2007). In pancreatic β cells, plasma membrane insertion of TRPV2 is regulated by PI3K after insulin treatment and this mechanism accelerates the exocytotic response during the glucose-induced insulin secretion (Aoyagi et al., 2010). TRPV2 associates with recombinase gene activator, which probably plays a role in the maturation of the channel and promotes surface expression (Stokes et al., 2005). The antiaging Klotho also promotes plasma membrane insertion (Lin and Sun, 2012). Moreover, TRPV2 was found to be directly phosphorylated by PKA. Of note, TRPV2 forms a complex with the AKAP-like protein ACBD3, which appears to link TRPV2 to PKA (Stokes et al., 2004). Depletion of PIP\(_2\) causes Ca\(^{2+}\)-dependent desensitization of the channel (Mercado et al., 2010).

Although it is highly expressed in the brain, the functional role of TRPV2 is unclear. Reportedly, TRPV2 promotes growth cone guidance (Shibasaki et al., 2010) and negatively controls glioma cell survival and proliferation in an ERK-dependent manner (Nabissi et al., 2010). TRPV2 is believed to be a major player in macrophage migration (Nagasawa et al., 2007). In macrophages, it is localized abundantly in the podosomes, which are adhesion structures required for cell migration. TRPV2 functions as the Ca\(^{2+}\) entry channel in these structures (Nagasawa and Kojima, 2012). TRPV2 mediates the gastric adaptive relaxation in the stomach (Mihara et al., 2013). It is required for the LPS-induced cytokine production in macrophages (Yamashiro et al., 2010). TRPV2 also plays a role in mast cell degranulation (Zhang et al., 2012a). It was widely assumed that TRPV2 would be responsible for (at least part of) the residual heat sensitivity in Trpv1 knockout mice. However, the effect of Trpv2 knockout in mice on thermal responsiveness has not yet been established and preliminary findings actually indicate that TRPV2 has no obvious role in heat sensing. Increasing evidence links TRPV2 to the innate immune system. Indeed, zymosan-, IgG-, and complement-mediated particle binding and phagocytosis are impaired in mice lacking the TRPV2 receptor (Link et al., 2010).

TRPV3 shares a 43% sequence similarity with TRPV1. It is still controversial whether TRPV3 can form heteromers with TRPV1, TRPV2, or TRPV4 (Cheng et al., 2007b). Expression of TRPV3 was reported both in neurons and non-neuronal tissues, including the tongue, testis, skin keratinocytes, and the cells surrounding the hair follicles (Peier et al., 2002; Xu et al., 2002; Moqrich et al., 2005; Borbíró et al., 2011).
TRPV3 is activated by innocuous warm temperatures above 30°C–33°C. Unlike TRPV1, TRPV3 is sensitized by repeated stimulations with temperature (Xu et al., 2002). Furthermore, sensitization of TRPV3 to warm temperatures is influenced by different endogenous proinflammatory agents such as BK, histamine, ATP (binding to ankyrin repeats), PKC, and PGF2α (Huang et al., 2008; Mandadi et al., 2009; Phelps et al., 2010). TRPV3 is activated by protons and is highly expressed in keratinocytes. Interestingly, α-hydroxyl acids from natural sources, which function as proton donors when applied to the skin and are used in the cosmetic industry, activate TRPV3 (Cao et al., 2012a,b). 2-APB (already mentioned as a TRPV2 agonist) also activates TRPV3 most likely by binding to two cytoplasmic sites, one of which is close to the TRP box (Hu et al., 2009b).

TRPV3 is activated by an array of natural compounds, including camphor, thymol, carvacol (see Fig. 6 for structure), carveol, borneol, cresols, eugenol (structure shown in Fig. 6), and Boswellia resin, which are all sensitizers for temperature activation of the channel (Vriens et al., 2009). These natural compounds are, however, not selective for TRPV3. Indeed, camphor also activates TRPV1 (Xu et al., 2005) and TRPM8 channels (Selescu et al., 2013). In contrast with other TRP channels, PIP2 hydrolysis (and/or pharmacological inhibition of phosphatidylinositol-4 kinase to block PIP2 synthesis) potentiates TRPV3. In excised patches, TRPV3 is potentiated by PIP2 depletion. PIP2 directly interacts with a specific protein motif and reduces TRPV3 channel open probability (Doerner et al., 2011; for review, see Nilius et al., 2014).

As anticipated from its high expression in keratinocytes, TRPV3 is required for several skin functions, including skin barrier formation and hair morphogenesis. In the skin, TRPV3 forms a signaling complex with TGF-α and EGFR. Activation of EGFR increases TRPV3 channel activity to induce an increased release of TGF-α (Cheng et al., 2010a).

The role of TRPV3 in heat sensing is supported by the phenotype of the Trpv3 knockout mouse, which shows strong deficits in responses to innocuous and noxious heat but not in other sensory modalities (Moqrich et al., 2005). Heating of keratinocytes causes ATP release, which, in turn, activates sensory nerves fibers; this mechanism is defective in Trpv3 knockout mice (Mandadi et al., 2009). It came as a surprise that TRPV3 is functionally connected to the M-type potassium channel K,7,2 in keratinocytes. M-channel activators dramatically enhance the release of ATP induced by TRPV3 agonists (Reilly et al., 2013). TRPV3 is also involved in the hippocampal LTD; therefore, it was suggested to play a role in synaptic plasticity (Brown et al., 2013; for recent comprehensive reviews on TRPV3, see Nilius and Biró, 2013; Nilius et al., 2014).

TRPV4 has a proline-rich region and six ankyrin repeat domains (ARDs) in its cytosolic N-terminal part.
Because TRPV4 is activated in many different tissues and is involved in very different cellular processes, it might become an important, but difficult, drug target (see Vincent and Duncton, 2011). Because Trpv4 knockout mice only show only very minor deficits in temperature sensing, TRPV4 is possibly not a functional thermoTRP (Lee et al., 2005). TRPV4 is important for the maintenance of skin barrier function (Denda et al., 2007). It is noteworthy that TRPV4 is highly expressed in chondrocytes (Muramatsu et al., 2007); indeed, it is important for the normal development of the bone growth plates. In the skin, TRPV4 activation strengthens the epidermal tight junction (which includes occludin, claudin-4, and tight junction regulatory proteins), and is thus important for the integrity of the skin barrier (Akazawa et al., 2013). Deduced from the Trpv4 knockout phenotype, TRPV4 might function as a mechanosensor and chemosensor, such as sensing for osmoreceptive neurons of the circumventricular organ and for sensing luminal tonicity in cholangiocyte cilia, which are required for bile formation. TRPV4 also plays an important role in the bladder (mostly in urothelial cells), where it is involved in sensing of the intravesicular pressure and regulation of the detrusor function (for reviews, see Gevaert et al., 2007; Everaerts et al., 2008). Moreover, TRPV4 is activated in endothelial cells with the consequences of activation of endothelial IK(Ca) channels, vasodilation, and reduction of the myogenic tone that underpins tissue blood flow autoregulation (Bagher et al., 2012). Finally, TRPV4 is involved in the glucocorticoid regulation of the synaptic input to neuroendocrine cells in the hypothalamic paraventricular nucleus (Boychuk et al., 2013).

TRPV5 exhibits a low homology to TRPV1 to TRPV4 (<30%) but shares 74% identity with TRPV6. TRPV5 can form homomers but also heteromers with TRPV6. TRPV5 expression, which is influenced by estrogen, parathormone (PTH), 1,25 dihydroxyvitamin D₃, and dietary Ca²⁺ intake, is high in the placenta, kidney (predominantly in the distal convolute and connecting tubules), and bone, but also in pancreatic β cells and the inner ear (Hoenderop et al., 2005).

TRPV5 and TRPV6 are the only highly Ca²⁺ selective TRP channels. It is only in the absence of extracellular Ca²⁺ that they transport monovalent cations. TRPV5 is a constitutively active channel. Repeated stimulation of the channel results in current decay and this process seems to be Mg²⁺ and Ca²⁺ dependent. [Ca²⁺]ᵢ acts as an important feedback inhibitor for Ca²⁺ entry via TRPV5. Both intracellular and extracellular acidic pH block this channel (see Vennekens et al., 2008). TRPV5 is effectively regulated by plasma membrane insertion and retrieval, which is controlled by many interacting proteins such as CalM, S100A10-annexin II, the PKC substrate 80K-H, Calbindin 28K, and the sodium-hydrogen exchange regulatory cofactor 2 (NHERF2). NHERF2 stabilizes TRPV5 when inserted to the plasma membrane. Interaction of TRPV5 with the Ca²⁺-binding proteins S100A10 and annexin II is required for plasma membrane insertion. The antiaging hormone Klotho regulates TRPV5 activity by modifying its glycosylation status and increases the plasma membrane-bound fraction of the channel (Chang et al., 2005). An important regulatory pathway involves the tissue serine protease, kallikrein. Kallikrein activates the BK receptor-2, which, in turn, activates TRPV5 DAG-dependently via PLCγ and PKC (de Groot et al., 2008; Boros et al., 2009). Phosphorylation of the Ca²⁺ reabsorption channels increases TRPV5 membrane insertion and delays retrieval. Binding to calbindin D₂₈K functionally results in local buffering of entering Ca²⁺ and inhibits channel inactivation (for review, see Hoenderop et al., 2005).

TRPV5 is effectively regulated by WNK4, a protein serine/threonine kinase, which also promotes plasma membrane insertion of the channel (Jiang et al., 2008b). TRPV5 is also coupled with the extracellular Ca²⁺-sensing receptor. Activation of the Ca²⁺-sensing receptor stimulates TRPV5, but not TRPV6 (Topala et al., 2009). Interaction of TRPV5 with 80K-H, a substrate for PKC, inhibits Ca²⁺ entry and increases the TRPV5 sensitivity to intracellular Ca²⁺, thereby accelerating the feedback inhibition of the channel.

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The parathyroid hormone PTH activates the cAMP-PKA signaling cascade, which rapidly phosphorylates TRPV5, thereby activating the channel (de Groot et al., 2009). Rab11a, which is colocalized with TRPV5 (and also TRPV6) in vesicular structures underlying the apical plasma membrane, also promotes channel insertion in the plasma membrane (for reviews, see de Groot et al., 2008; Boros et al., 2009).

The main function of TRPV5 is Ca\(^{2+}\) reabsorption in the kidney. Trpv5 knockout mice show impaired Ca\(^{2+}\) reabsorption in the distal convoluted and connecting tubules, resulting in calcuiuria and polyuria with significantly more acidic urine than that seen in wild-type mice (Hoenderop et al., 2003).

TRPV6 is the closest relative of TRPV5. It is a highly Ca\(^{2+}\)-selective TRP channel that is also permeable to Ba\(^{2+}\), Sr\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), and Cd\(^{2+}\), as well as for La\(^{3+}\) and Gd\(^{3+}\). It is abundantly expressed in the intestine, placenta, uterus, pancreas, epididymal epithelium, brain, and stomach (Hoenderop et al., 2005). TRPV6 is localized to the brush-border membrane of intestinal enterocytes and functions there as the main Ca\(^{2+}\) entry channel. TRPV6 is also expressed in kidney, where it is mainly found in cortical and medullary collecting ducts. TRPV6 is a constitutively open ion channel and shows a striking negative feedback inhibition by Ca\(^{2+}\) entry (e.g., an increase in [Ca\(^{2+}\)]) (Nilius et al., 2002). Expression of TRPV6 is upregulated by vitamin D (Balesaria et al., 2009). Both PIP\(_2\) and cytoplasmic ATP are important for maintaining TRPV6 activity. Mg\(^{2+}\)ATP is the substrate for the lipid kinase type III, phosphatidylinositol 4-kinases, which allows the resynthesis of PIP\(_2\). Ca\(^{2+}\)-dependent inactivation of TRPV6 (and also TRPV5) is mediated by a Ca\(^{2+}\)-dependent...
activation of a PLC, inducing PIP₂ depletion (Zakharian et al., 2011). TRPV6 can also bind to CaM. Overexpression of CaM reduces currents through TRPV6 and accelerates inactivation (Derler et al., 2006). TRPV6 also interacts with S100A10 and annexin II, which promotes plasma membrane insertion. The PDZ proteins NHERF2 and NHERF4 form scaffolds for TRPV6 and stabilize plasma membrane insertion.

Fig. 10. Examples of selective TRPA1 antagonists.

Fig. 11. Representative examples of compounds that block TRPM8 (AMTB, JNJ41876666, PBMC, and AMG9678), TRPV4 (HC-067047), and TRPC3 (Pyr3) channels.
required for Ca\textsuperscript{2+} buffering close to the channel and TRPV6 binds to calbindin-D(9k); this interaction is regulated by Mg\textsuperscript{2+} of TRPM1 via DAG, synaptic efficiency of the rod-bipolar cells is regulated by mGluR6, its receptor, mGluR6, activates TRPM1. Glutamate is released from the adjacent rod photoreceptor cells. Binding to mGluR6 closes the channel. After activation, TRPM1 desensitizes and the light response decays (Morgans et al., 2009; Koike et al., 2010). The functional role of TRPM1 in the retina is to provide the ON response of bipolar cells. It is also expressed in the pupillary reflex and plays a role in skin pigmentation (i.e., in the function of melanocytes). In neonatal human epidermal melanocytes, TRPM1 expression correlates with melanin content, suggesting that TRPM1 is critical to normal melanocyte pigmentation and thus represents a potential target for pigmentation disorders (Oancea et al., 2009; Hughes et al., 2012). TRPM1 regulates pigmentation by controlling melanin synthesis in skin melanocytes (Devi et al., 2009, 2013).

TRPM2 is the main channel for intestinal trans-epithelial Ca\textsuperscript{2+} absorption (Hoenderop et al., 2005). 

**TRPM6**

TRP6 knockout mice show decreased Ca\textsuperscript{2+} absorption and, subsequently, decreased serum Ca\textsuperscript{2+} levels (Bianco et al., 2007). More strikingly, TRPV6 contributes to intestinal calcium transport only under conditions when the dietary calcium supply is limited. In such situations, TRPV6 regulates bone formation and mineralization (Lieben et al., 2010). TRPV6 controls the extracellular Ca\textsuperscript{2+} concentration toward the distal segments of the epididymal duct, which is essential for survival of spermatozoa. Loss of functional channels causes severely impaired fertility due to a reduction in motility and fertilization capacity of sperms (Weissgerber et al., 2010). TRPV6 is a key element in Ca\textsuperscript{2+}/1,25-dihydroxyvitamin D3-induced keratinocyte development (Lehen'kyi et al., 2007).

**C. Melastatin Transient Receptor Potential Subfamily**

The melastatin TRP (TRPM) family comprises eight members. All have a 25-amino acid TRP box, the functional impact of which is still uncertain. The N terminus of all eight members is much longer than in other TRP channels; it contains a TRPM homology region shared by all TRPM proteins (Fig. 2). TRPM channels lack the N-terminal ankyrin repeats. Members of the TRPM family, on the basis of sequence homology, fall into three subgroups: TRPM1/TRPM3, TRPM4/TRPM5, and TRPM6/TRPM7 with TRPM2 and TRPM8 representing structurally distinct channels. TRPM channels exhibit highly variable permeability to Ca\textsuperscript{2+} and Mg\textsuperscript{2+}, ranging from Ca\textsuperscript{2+}-impermeable (TRPM4 and TRPM5) to highly Ca\textsuperscript{2+} and Mg\textsuperscript{2+} permeable (TRPM6 and TRPM7) (for review, see Nilius et al., 2007b).

TRPM1 is the least studied member of the melastatin family. It was first identified as a tumor suppressor protein expressed at high levels in melanocytes but at very low levels in metastatic melanoma cells (i.e., it shows an inverse correlation with melanoma progression) (Duncan et al., 1998). Microphthalmia-associated transcription factor (MITF), a melanocytic marker used in diagnostic surgical pathology, is a major regulator of TRPM1 expression (Miller et al., 2004). MITF is also required for miR-211 expression, suggesting that the tumor suppressor activities of MITF and/or TRPM1 may at least partially be due to miR-211 (Mazar et al., 2010; Margue et al., 2013).

At least five different TRPM1 isoforms have been reported, which makes the functional analysis difficult. These isoforms are expressed in melanocytes/melanoma cells, as well as in the brain and retina (Oancea et al., 2009). Initially, expression data revealed only very small currents through TRPM1. Therefore, TRPM1 was first thought to be an ion channel whose function was critical to normal melanocyte pigmentation. If so, TRPM1 may be a potential target for pigmentation disorders (Oancea et al., 2009). TRPM1 can be weakly activated by the steroid, pregnenolone sulfate. By contrast, TRPM1 is inhibited by Zn\textsuperscript{2+} (Lambert et al., 2011).

In the retina, TRPM1 is expressed on ON bipolar cells, which invert the signal of light responses to hyperpolarizing to depolarizing before passing them on to ganglion cells. TRPM1 colocalizes with mGluR6, a G\textsubscript{a} and G\textsubscript{b} protein. Light responses are generated when TRPM1 is activated. Removal of glutamate from its receptor, mGluR6, activates TRPM1. Glutamate is released from the adjacent rod photoreceptor cells. Binding to mGluR6 closes the channel. After activation, TRPM1 desensitizes and the light response decays (Morgans et al., 2009; Koike et al., 2010). The synaptic efficiency of the rod-bipolar cells is regulated via modulation of a block by Mg\textsuperscript{2+} of TRPM1 via DAG, PIP\textsubscript{2}, and PKC\textgreek{a} (Rampino and Nawy, 2011). TRPM1 associates with nyctalopin, a small leucine-rich repeat proteoglycan. Its absence causes a complete inhibition of TRPM1 (Bojang and Gregg, 2012). Nyctalopin has been shown to interact with TRPM1, and the expression of TRPM1 on the dendritic tips of the bipolar cells is dependent on nyctalopin expression. TRPM1 is also expressed on a subset of cells in the ganglion cell layer, including melanopsin-expressing photosensitive retinal ganglion cells (Hughes et al., 2012).

The functional role of TRPM1 in the retina is to provide the ON response of bipolar cells. It is also involved in the pupillary reflex and plays a role in skin pigmentation (i.e., in the function of melanocytes). In neonatal human epidermal melanocytes, TRPM1 expression correlates with melanin content, suggesting that TRPM1 is critical to normal melanocyte pigmentation and thus represents a potential target for pigmentation disorders (Oancea et al., 2009; Hughes et al., 2012). TRPM1 regulates pigmentation by controlling melanin synthesis in skin melanocytes (Devi et al., 2009, 2013).

TRPM2 is a Ca\textsuperscript{2+}-permeable cation channel characterized by a functional nudix hydrolase domain that is highly homologous to the ADP-pyrophosphatase NUDT9 that cleaves ADP-ribose (ADPR) into ribose-5 phosphate and AMP. TRPM2 forms functional tetrameromers (see Eisfeld and Lückhoff, 2007). A C-terminal CC domain plays an important role in the TRPM2 channel assembly.
TRPM2 is highly expressed in the brain (both in neurons and glial cells, especially in the substantia nigra and hippocampus), in monocytes, as well as in spleen, heart, lung, eye, vascular endothelium, thymocytes, pancreatic β cells, neutrophils, and leukocytes (reviewed in Eisfeld and Lückhoff, 2007; Sumoza-Toledo and Penner, 2011). In pancreatic β cells, TRPM2 has been shown to function not only as a plasma membrane channel but also as an intracellular channel mediating release of Ca^{2+} from intracellular lysosomal stores (Lange et al., 2009).

TRPM2 is directly activated by both NAD and ADPR: this depends on hydrolyzation after binding with the NUDT9 motif. It is noteworthy that TRPM2 is a redox-sensitive channel. Products of oxidative stress (e.g., H_{2}O_{2}) activate TRPM2. In contrast, inhibitors of oxidative stress (e.g., glutathione, catalase, dimethylthiourea, antioxidants, and mannitol) close the channel. Sirtuins (NAD+-dependent deacetylases) synthesize nicotinamide and ADPR, which, in turn, activate TRPM2 and promote cell death (Grubisha et al., 2006). Oxidative DNA damage activates poly(ADP-ribose)polymerase-1 (PARP-1); the poly(ADPR) then is rapidly degraded to ADPR by poly(ADPR)glycohydrolase. PARP-1 and poly(ADPR)glycohydrolase can, therefore, indirectly activate TRPM2, leading to Ca^{2+} entry. This may initiate a cell death signaling pathway (Blenn et al., 2011). Activation of TRPM2 is sensitized by an increase of [Ca^{2+}]_{i} (Du et al., 2009b). Heat (>35°C) potentiates channel activation (Togashi et al., 2006). In contrast with other TRP channels, 2-APB inhibits TRPM2.

TRPM2 is now defined as a sensor for intracellular oxidation. TRPM2 is implicated in cell death by oxidative stress (Takahashi et al., 2011a; Wyrsh et al., 2012). Furthermore, TRPM2 is believed to be a major player in LPS-dependent cytokine production, which is inhibited by TRPM2 downregulation. Therefore, TRPM2 is a main player in monocyte functions (Wehrhahn et al., 2010; Kashio et al., 2012). It also plays a critical role in chemotaxis in dendritic cells (Sumoza-Toledo et al., 2011). In pancreatic β cells, TRPM2 activation by cyclic ADP-β is involved in insulin secretion (Togashi et al., 2006). TRPM2 channels residing in lysosomes act as ADPR-activated calcium release channels, which are critically linked to β cell death induced by oxidative stress (Lange et al., 2009). TRPM2 is also involved in NMDA receptor–dependent LTD (Xie et al., 2011). TRPM2 has been implicated in neuropathic pain (Haraguchi et al., 2012). Finally, megakaryocytes are stimulated via ADPR-dependent TRPM2 activation (Naziroğlu, 2011a,b).

A splice variant of TRPM2 has been reported as a cell-shrinking or hypertonicity-induced cation channel, which is functionally involved in apoptosis and cell proliferation (Numata et al., 2012).

TRPM3 is closely related to TRPM1 and is the TRP channel with the most splice variants (Oberwinkler et al., 2005). TRPM3 expression is most prominent in brain, kidney (human), and pituitary cells. Expression was also demonstrated in liver, pancreatic β cells, ovaries, testes, spinal cord, pulmonary endothelium, sensory (trigeminal and DRG) neurons, neuroblastoma cells, iris, and retinal pigmented cells (Lee et al., 2003a). TRPM3 shows some basal activity that increases with decreasing intracellular Mg^{2+} concentrations (Oberwinkler et al., 2005). This channel is rapidly and reversibly activated by extracellular pregnenolone sulfate, a neuroactive steroid (Wagner et al., 2008). By contrast, progesterone inhibits the channel (Majeed et al., 2012). Other TRPM3-activating stimuli (depending on the splice variants) are D-erythro-sphingosine and nifedipine. TRPM3 is activated by heat and functions as a voltage-dependent channel that is activated by depolarization (Vriens et al., 2011). Some splice variants are inhibited by extracellular Na^{+}. The natural compounds, naringenin (structure shown in Fig. 6) and hesperetin (which belong to the citrus fruit flavanones), and the deoxybenzoin ononetin are potent blockers of TRPM3 (Straub et al., 2013a,b).

TRPM3 is thought to play important roles in pancreatic islets, oligodendrocytes, and vascular smooth muscle. Importantly, it provides an entry pathway for Zn^{2+}, which might be especially important for pancreatic β cells (Wagner et al., 2010). Indeed, TRPM3 might be involved in insulin release. In insulinoma cells, pregnenolone sulfate induces, via TRPM3, an enhanced expression of the zinc finger transcription factor, Egr, which drives Pdx-1, a major regulator of insulin gene transcription (Mayer et al., 2011). Data from Trpm3 knockout mice clearly show that TRPM3 plays a role in heat perception and mediates pain responses (Vriens et al., 2011). Finally, TRPM3 is involved in glutamnergic signaling and synaptic plasticity in cerebellar Purkinje cells (Zamudio-Bulcock et al., 2011).

A novel permeation pathway was recently proposed for TRPM3 (Vriens et al., 2014). In addition to the canonical S5/S6 permeation pore, a secondary, independent permeation pathway may exist in the S1–S4 module (related to the “omega-pore” in voltage-activated ion channels). This secondary TRPM3 pore may be gated by combined application of endogenous neurosteroids and exogenous chemicals such as clomipramine. This alternative pathway is still functional even if the canonical pore is blocked, and this enables massive Na^{+} influx at negative voltages and enhances excitation of sensory neurons, worsening the TRPM3-depndent pain response.

TRPM4 shares a 40% sequence homology with TRPM5. Both channels are activated by Ca^{2+}; this is interesting because these channels are Ca^{2+} impermeable and they function as monovalent cation permeating/depolarizing ion channels. TRPM4 contains a number of binding sites...
for CaM in the C-terminal end, and for ATP in the N-terminal end. The channel also has phosphorylation sites for PKC. All of these domains modulate the Ca$^{2+}$ sensitivity of the channel (Nilius et al., 2005a). Two different splice variants, TRPM4a and TRPM4b, have been identified but only TRPM4b is a functional channel, whereas TRPM4a acts as a negative regulator (Nilius and Vennekens, 2006; Gees et al., 2012).

Expression of TRPM4 is ubiquitous, with high expression levels found in kidney, heart [abundant in sinoatrial (SA) node and pacemaker tissue], placenta, brain [cerebellum, hippocampus, striatum], and pancreas. Significant TRPM4 expression was also reported in the human gastrointestinal tract, prostate, colon, bladder (mostly in detrusor muscle), testes, lymphocytes, spleen, lung, pituitary, skeletal muscle, adipose tissue, and bone (reviewed in Ullrich et al., 2005; Nilius and Vennekens, 2006).

TRPM4 is directly activated by an increase in [Ca$^{2+}$], which also leads to desensitization of the channel. Desensitization is due to a Ca$^{2+}$-induced breakdown of PIP$_2$ (Nilius et al., 2006). TRPM4 is a voltage-dependent channel, activated by depolarization. The channel is inhibited by intracellular ATP and several other nucleotides, as well as by polyamines (Nilius and Vennekens, 2006). PKC, CaM, and decavanadate all activate TRPM4 probably via an increase of its sensitivity to elevated [Ca$^{2+}$]. ROS also activate TRPM4, as does the pyrazole derivative, BTP2 [N-[4-[3,5-bis(trifluoromethyl)1H-pyrazol-1-yl]phenyl]-4-methyl-1,2,3-thiadiazole-5-carboxamide]. The channel protein may associate with the sulfonylurea receptor 1 (Sur1) which renders it sensitive to the K$_{ATP}$ channel blocker glibenclamide (Woo et al., 2013).

In nonexcitable cells, activation of TRPM4 acts as a break on Ca$^{2+}$ entry following receptor-mediated Ca$^{2+}$ signals by reducing the driving force for Ca$^{2+}$ entry through store-operated channels. This mechanism underlies the increased Ca$^{2+}$ signal that follows IgE-dependent activation of mast cells. In Trpm4 knockout mice, mast cell activation leads to enhanced allergic response (Vennekens et al., 2007). Via the same mechanism, TRPM4 is involved in nuclear factor of activated T cells (NFAT) signaling, cytokine production, and motility in T lymphocytes (Weber et al., 2010).

In magnocellular cells of the supraoptic nucleus and the paraventricular nucleus of the hypothalamus, TRPM4 plays a role in magnocellular cells of the supraoptic nucleus and the paraventricular nucleus of the hypothalamus in the generation of fast after depolarization, which determines the distinct firing properties of vasopressin-releasing neurons in this region (Teruyama et al., 2011). These neurons also express TRPV1 and TRPV4, with TRPV1 being essential for their spontaneous activity (Sudbury and Bourque, 2013). In general, TRPM4 seems to act as a pacemaking channel in several neuronal networks. It also generates spontaneous depolarization in excitable cells following action potentials. TRPM4 has also been implicated in cerebral blood flow regulation and causes smooth muscle contractions. In cerebral arteries, it is involved in myogenic constrictions. Its activation generates transient inward currents that modulate smooth muscle as well as cardiac muscle contractions (Guinamard et al., 2010). This channel negatively regulates catecholamine secretion from chromaffine cells. Indeed, Trpm4-deficient mice develop hypertension due to an increased release of catecholamines (Mathar et al., 2010). In pancreatic α cells, an increase in [Ca$^{2+}$], causes glucagon secretion. Depolarizing currents generated by TRPM4 constitute an important component in the control of this response (Marigo et al., 2009). The physiologic role of TRPM4 in the pancreas is, however, still unclear.

TRPM5 and TRPM4 are closely related channels with 40% sequence homology. TRPM5 is a monovalent-selective cation channel with negligible Ca$^{2+}$ permeability. TRPM5 expression was shown mainly in the large intestine, pancreatic β cells, duodenum, stomach, lung, testis, brain, and the vomeronasal organ (Fonfría et al., 2006b; Colsoul et al., 2010). It is noteworthy that TRPM5 is expressed in taste receptor cells and other chemo-sensory cells of the taste signaling cascade in the vomeronasal organ, olfactory epithelium, brainstem, enterocrine cells in stomach, duodenum, large intestine, respiratory tract, and pancreatic β cells (see Nilius and Appendino, 2013). Furthermore, TRPM5 expression was reported in the testis, where it is believed to be involved in spermatogenesis (Li and Zhou, 2012).

In solitary chemosensory cells, TRPM5 mainly functions as a regulator of the release of several endocrine messengers, including the two incretin hormones, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide, as well as ghrelin, CCK, and the opioid peptides β-endorphin, Met-encephalin, and uroguanylin (Kinnamon, 2012). Some TRPM5-containing taste receptor cells are coupled (as in the gastric groove) to neuronal nitric-oxide synthase (nNOS), suggesting paracrine interactions (Eberle et al., 2013). TRPM5 in taste receptor cells of the tongue is important for the transduction of sweet, amino acid, and bitter stimuli that bind to GPCRs that are coupled to PLCβ. This, in turn, causes Ca$^{2+}$ release from the endoplasmic reticulum via IP$_3$ and resultant IP3R activation (Chaudhari and Roper, 2010; Nilius and Appendino, 2013). TRPM5 is directly activated by intracellular Ca$^{2+}$, but has a higher affinity for [Ca$^{2+}$]. Ca$^{2+}$ also desensitizes TRPM5 currents (i.e., it functions as a negative feedback mechanism for channel activation). Desensitization by Ca$^{2+}$ is attributed to PIP$_2$ depletion (Nilius et al., 2007a). Similar to TRPM4, TRPM5 is activated by heat. Heat activation results in a leftward shift of the voltage dependence of activation (Nilius et al., 2005b). TRPM5 is directly activated by arachidonic acid, and indirectly by monoglyceride lipase, cyclooxygenase.
TRPM5 acts as a positive regulator of glucose-induced insulin release. Glucose generates bursts of action potentials that subsequently trigger a Ca\(^{2+}\) transient. The depolarization during the interburst interval which determines the frequency of the Ca\(^{2+}\) transients (and, therefore, the efficiency of insulin release) is sensitively controlled by the depolarizing Ca\(^{2+}\)-activated currents through TRPM5. In addition, there seems to be a role for TRPM5 in glucose-induced insulin secretion beyond membrane depolarization (reviewed in Colson et al., 2011, 2013).

TRPM6 contains a functional serine/threonine protein kinase in the C-terminal end of the protein, which resembles members of the α-kinase family, a family with no sequence homology to any other conventional protein kinases (Drennan and Ryazanov, 2004). TRPM6 functions as a homotetramer, but it can also form a functional heterotetramer with TRPM7. TRPM6 expression is found predominantly in epithelial cells of the kidney (distal convoluted tubule) and small intestine, the colon, the mammary gland, and the testes. TRPM6 forms an Mg\(^{2+}\)-permeable channel. The expression of TRPM6 is regulated by multiple factors, including dietary Mg\(^{2+}\), magnesiotropic hormones, and drugs. TRPM6 is constitutively active. Low pH potentiates the activation of TRPM6. Intracellular Na\(^+\)- and Mg\(^{2+}\)-ATP decrease currents through TRPM6. ATP regulates TRPM6 channel activity via its α-kinase domain independently of the α-kinase activity. TRPM6 is activated by low 2-APB concentrations (whereas TRPM7 is inhibited) and currents through TRPM6/TRPM7 heteromers are only slightly enhanced (Dimke et al., 2011). EGF is a magnesiotropic hormone that increases TRPM6 activity. It upregulates TRPM6 expression via activation of an ERK/activator protein-1-dependent pathway. Expression of the channel is also upregulated by the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway, and rapamycin (an immunosuppressant drug that blocks mTOR) reduces TRPM6-mediated Mg\(^{2+}\) transport. The receptor for activated C-kinase 1 was identified as the first associated protein of the TRPM6 α-kinase domain. The intracellular Mg\(^{2+}\) concentration plays a feed-forward role in controlling TRPM6-mediated Mg\(^{2+}\) influx, preventing Mg\(^{2+}\) overload during epithelial Mg\(^{2+}\) transport (Cao et al., 2008). TRPM6 is downregulated by oxidative stress, which is attenuated by methionine sulfoxide reductase B1, an antioxidant enzyme coexpressed with TRPM6 (van der Wijst et al., 2009; Cao et al., 2010). In summary, TRPM6 is an essential for intestinal Mg\(^{2+}\) absorption and renal Mg\(^{2+}\) reabsorption; thus, it plays a critical role in Mg\(^{2+}\) homeostasis (Ferrè et al., 2011).

TRPM7 is 50% homologous to TRPM6. It also contains a C-terminal α-kinase domain (Fig. 13). TRPM7 can form both homomers and heteromultimers with TRPM6. TRPM7 is ubiquitously expressed. High expression levels were found in human heart, pituitary gland, bone, and adipose tissue (Fonfria et al., 2006b). TRPM7 can also function as an intracellular channel. For example, in cholinergic synaptic vesicles, TRPM7 regulates neurotransmitter release. TRPM7 forms a complex with synapsin I and synaptotagmin I, and it directly binds to snapin and controls the EPSP amplitude in a pH-dependent fashion (Krapivinsky et al., 2006).

TRPM7 is permeable to divalent cations: it conducts Mg\(^{2+}\) (but also protons). Similar to TRPM6, when permeated predominantly by monovalent cations, the current-voltage relationship of the channel exhibits pronounced outward rectification that results from voltage-dependent block by extracellular divalent cations. TRPM7 is also a channel permeable for trace metals such as Zn\(^{2+}\), Co\(^{2+}\), and Mn\(^{2+}\). TRPM7 is tightly regulated by [Mg\(^{2+}\)], and also by intracellular Mg-ATP, which causes inhibition. Inward currents through TRPM7 are activated by low pH, which decreases block by Ca\(^{2+}\) and Mg\(^{2+}\) of monovalent currents through the channel. Hypoxia and ROS production induce activation of the channel in brain neuronal cells with concomitant Ca\(^{2+}\) overload and Mg\(^{2+}\) deregulation (Zhang et al., 2011a). NGF and its receptor, TrkA, reduce this hypoxic overexpression of TRPM7 via a PI3K pathway (Jiang et al., 2008a). In this respect, it is interesting that many exogenous compounds (e.g., ginsenoside and carvacrol) block TRPM7 (Parnas et al., 2009). 5-Lipoxygenase inhibitors are also potent blockers of the channel. TRPM7 is believed to be a mechanosensor channel. It is activated by stretch. TRPM7 is thought to be involved in volume regulation. Increased shear stress induces a translocation of TRPM7 to the plasma membrane and increases currents through the channel (Oancea et al., 2006; Numata et al., 2007).

Most important, TRPM7 is critical for regulating cellular growth and embryonic development. In vivo, global disruption of TRPM7 in mice results in embryonic lethality before embryonic day 7 (Jin et al., 2008, 2012a). In vitro, pluripotent stem cells and neural stem cells with TRPM7 disruption show a defect in the formation of the stem cell monolayer that is required for organogenesis. The TRPM7 channel kinase is required for embryogenesis (Jin et al., 2012a; for a review, see Paravicini et al., 2012). During early embryogenesis, TRPM7 is critical for heart development (myocardial proliferation), although this mechanism seems to be dispensable after birth (Sah et al., 2013). In adult tissues, TRPM7 may play a role as a pacemaker channel in Cajal cells and thus affect intestinal motility (Kim et al., 2011a). The channel is also present in human atrial myocytes, where its expression is upregulated during atrial fibrillation (Zhang et al., 2012c).
TRPM7 increases NOS expression in endothelial cells and thereby facilitates NO production in an ERK pathway-dependent manner. Therefore, TRPM7 was postulated to be an important player in vasomotor control (Inoue and Xiong, 2009). High-calcium microdomains ("calcium flickers"), which are essential for activation of cell migration, are generated by TRPM7 activation. The channel interacts at the posterior appendix in many migrating cells with the calcium-activated potassium channel, KCa3.1. TRPM7 effects on migration play a role in angiogenesis (Wei et al., 2009a) and in osteoclastogenesis (Yang et al., 2013) and regulate the function of vascular endothelial cells (Baldoli and Maier, 2012; Baldoli et al., 2013).

TRPM8 shares an approximately 42% sequence identity with TRPM2, its closest relative. TRPM8 functions as a homotetramer. It is highly expressed in pain- and cold-sensitive C fibers, as well as in the bladder, prostate, hippocampus, skin, brown adipose tissue (BAT), vascular smooth muscle cells, macrophages, and sperm (reviewed in Voets et al., 2007). TRPM8 is activated by cooling (<22°C) and pharmacological agents [e.g., menthol (structure shown in Fig. 6), linalool, geraniol, and eucalyptol] and chemicals [e.g., icilin and carboxamides] that evoke a cooling sensation. Extracellular protons suppress the activity of TRPM8 probably via surface charge screening and an intracellular pH drop. TRPM8 is inhibited by an increase in extracellular Ca2+, because of a surface charge screening (Mahieu et al., 2010).

Structurally diverse agents function as TRPM8 antagonists, including the first-generation TRPV1 blockers BCTC [N-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carboxamide; structure shown in Fig. 9], and capsazepine, and the generic TRP channel blocker ruthenium red (reviewed in Nilius et al., 2007b; DeFalco et al., 2011). Interestingly, the "endoanilloid" anandamide and NADA inhibit TRPM8 activation by both icilin and menthol. Thus, anandamide and NADA might represent the first endogenous TRPM8 inhibitors (De Petrocellis et al., 2007). Arachidonic acid also inhibits TRPM8. This inhibition is mediated by a cytosolic PLA2 (Bavencoffe et al., 2011). By contrast, TRPM8 seems to be activated via iPLA2-dependent synthesis of lysophosphatidates. TRPM8 is downregulated by PKC, probably via an indirect pathway. Ca2+ activates PKC, which, in turn, activates protein phosphatase 1, causing dephosphorylation and desensitization of TRPM8 (Premkumar et al., 2005). PIP2 is critically involved in the activation of TRPM8 by both cold and menthol. It is likely that positively charged residues in the highly conserved proximal C-terminal TRP domain of TRPM8 act as interaction sites for PIP2. Depletion of PIP2 desensitizes the channel via a Ca2+-dependent activation of PLCδ (Rohacs, 2007). Ca2+-CaM reverses the activating effect of PIP2, switching channel gating to a low open probability state with long closed times. Similar to TRPA1, TRPM8 forms stable complexes with intracellular polyphosphates and their enzymatic breakdown inhibits the channel. The G protein subunit Goq binds to TRPM8. Activation of a Goq-coupled receptor directly inhibits TRPM8 (Zhang et al., 2012b). TRPM8 is also inhibited by the Goq protein/adenylate cyclase/cAMP/PKA signaling cascade (Bavencoffe et al., 2010).

The role of TRPM8 as a cold sensor in the somatosensory system is well established. It is also involved in somatosensation and nociception (Dhaka et al., 2006). TRPM8 channels play a role in the regulation of body temperature. Agonists of this "cold temperature sensor" cause a transient increase in body temperature by stimulating BAT thermogenesis; this could constitute a promising way to treat obesity (Gavva et al., 2012). Furthermore, as discussed later, TRPM8 is required for lacrimation and contributes to the regulation of basal tear flow (Parra et al., 2010). Unexpectedly, TRPM8 is involved in the regulation of tonic GABAergic inhibition in the hippocampus (Zhang et al., 2008e).

D. Ankyrin Transient Receptor Potential Subfamily

TRPA1 is the only mammalian member of this family. Its name derives from the unusually high number (14–19) of ankyrin repeats in the N terminus (Fig. 2). TRPA1 further contains an N-terminal Ca2+-binding EF hand domain and has at least 11 (of 31) reactive cysteines for covalent modification, but lacks the typical 25-amino acid TRP domain (Nilius and Owssianik, 2011; Nilius et al., 2012).

In Drosophila, TRPA1 plays a crucial role in the temperature control of the circadian rhythm: loss of TRPA1 alters the expression of the circadian clock protein period (per) in pacemaker neurons (Lee and Montell, 2013). In mammals, TRPA1 is predominantly expressed in nociceptive neurons (DRG as well as trigeminal and nodose ganglia) and the organ of Corti, and is also highly expressed in the dental pulp. However, the channel is also widely expressed in brain, heart, small intestine, lung (both in fibroblasts and epithelial cells), skeletal muscle, skin (keratinocytes), bladder, prostate, vascular endothelial cells, and pancreas (for a review see Nilius et al., 2012). It is also expressed on taste cells, where it associates with the bitter taste receptor TAS2R60 (Knaapila et al., 2012).

TRPA1 is a voltage-dependent, Ca2+-permeable cation channel with a relatively high fractional Ca2+ current. The voltage dependence of TRPA1 has been linked to a conserved leucin residue in the putative pore helix (Wan et al., 2013). Gating of the channel induces a dilation of the pore (Chen et al., 2009b) which also increases its Ca2+- permeability (Karashima et al., 2010). TRPA1 was initially identified as a noxious cold sensor and a mechanosensor (Story et al., 2003). Indeed, at least in some species, TRPA1 can be directly gated by cold temperatures (Karashima et al., 2009; Wang et al., 2013a). Cold activation of TRPA1 occurs in rodents, but
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not in humans or monkeys because of a single amino acid variation in TM5 (Chen et al., 2013a). In contrast, heat suppresses agonist-dependent TRPA1 activation (Brierley et al., 2011; Wang et al., 2012a). TRPA1 may also function as a mechanosensor and contributes to a complex that mediates transient, mechanically activated currents in C fibers (Vilceanu and Stucky, 2010). The major activation mode of TRPA1 is, however, by covalent modification of N-terminal cysteines or lysines by electrophilic compounds (Bandell et al., 2004), which makes TRPA1 an ideal chemosensor. Indeed, TRPA1 is activated by a plethora of pungent and irritant compounds, including allyl-isothiocyanate (the pungent compound in mustard oil and wasabi; see Fig. 7 for structure), allicin (from garlic), cinnamaldehyde (from cinnamon); structure shown in Fig. 7), thymol (from thyme), carvacrol (from oregano), and other electrophilic compounds in our food, as well as by industrial isocyanates, tear gases, acrolein (an irritant in vehicle exhaust fumes and tear gas), and hypochlorite (see Bessac and Jordt, 2008; Bautista et al., 2013; Radresa et al., 2013). Most recently, bacterial endotoxins have been added to the long list of TRPA1 activators (Meseguer et al., 2014).

Furthermore, TRPA1 is activated by endogenous substances such as 4-hydroxynonenal (Fig. 7), H2O2, 15-deoxy-D12,14-Prostaglandin J2, and electrophilic lipid acids such as nitrooleic acid, which are released after inflammation, oxidative stress, or tissue damage (for details, see Nilius et al., 2012; Radresa et al., 2013). In another gating mode, TRPA1 can be activated by nonelectrophilic compounds that do not induce covalent modification. Among such compounds are Δ9-tetrahydrocannabinol (the psychoactive compound in marijuana), two nonpsychoactive cannabinoids (cannabidiol and cannabichromene), as well as BK, menthol, nicotine, anesthetics (lidocaine, propofol, general anesthetics isoflurane, desflurane), four different 1,4-dihydroxypyrindines (nifedipine, nimodipine, nicardipine, and nitrendipine), the structurally related L-type calcium channel agonist BayK8644 [methyl-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]-1,4-dihydroxypyrindine-3-carboxylate], polyunsaturated fatty acids (PUFAs), amphipathic molecules such as trinitrophenol, chlorpromazine, pinacidil (a KATP channel opener), nonpungent capsinoids, and many more. For some of these compounds, a bimodal effect was reported, changing from activation at low concentrations to block at high concentrations (Alpizar et al., 2013). TRPA1 can be activated by gasotransmitters such as NO, hydrogen sulfide (H2S), CO, CO2, and ozone. It is interesting that TRPA1 is also activated by both increased O2 concentration and hypoxia (Takahashi et al., 2011b). TRPA1 is directly activated by an increase in [Ca2+]i by intracellular Zn2+ and intracellular alkalization. Elevated [Ca2+]i also causes a striking feedback inhibition that may depend on Ca2+ entry. Thus, elevation of [Ca2+]i includes an initial potentiation/activation and a subsequent inactivation after Ca2+ entry through the channel (Wang et al., 2008b). PIP2 is a TRPA1 modulator; however, the net effect of PIP2 is unclear since both activating and inhibiting properties were described (Karashima et al., 2008).

TRPA1 is expressed in skin melanocytes, where it is activated by long-wavelength ultraviolet radiation (UVR, type A), the main component of sunlight UVR (Bellono et al., 2013). TRPA1 activation is required for the UVR-induced early increase in cellular melanin content (‘suntan’); that is, TRPA1 is an essential component of the extracutaneous phototransduction pathway in human melanocytes (Bellono and Oancea, 2013).

TRPA1 is upregulated under inflammatory conditions (reviewed in Bautista et al., 2013). Artemin, a neuronal survival and differentiation factor of the glial cell line–derived neurotrophic factor family, suppresses TRPA1 expression (Yoshida et al., 2011), but increases TRPM8 expression (Lippoldt et al., 2013). Trafficking and plasma membrane insertion of TRPA1 is regulated by the ubiquitin hydrolase and the tumor suppressor protein CYCL, which binds to TRPA1 to increase the cellular pool of the functional protein (Stokes et al., 2006). TRPA1 is functionally coupled with PAR-2. Both proteins are colocalized in DRG. Stimulation of PAR-2 activates TRPA1 (Dai et al., 2007; Terada et al., 2013). As detailed later, there is evidence that TRPV1 and TRPA1 are not only coexpressed but also functionally coupled in DRG neurons, where they may also form functional heterotetramers.

A major function of TRPA1 is its role in chemical nociception. It initiates pain behavior and avoidance when the organism is exposed to irritant TRPA1 agonists (see Fang et al., 2010). TRPA1 also plays an important role in the respiratory system in sensing harmful airborne irritants (Bessac and Jordt, 2008; Facchinetti and Patacchini, 2010). TRPA1 activation leads to the release of the vasoactive peptides substance P (SP) and calcitonin gene-related peptide (CGRP) (Bautista et al., 2013). CGRP release leads to vasodilation, whereas SP increases vascular permeability during inflammation. In addition to its role as a chemosensor, thermosensor, and (possibly) mechanosensor, TRPA1 plays an important role in the vascular bed by causing endothelium-dependent smooth muscle cell hyperpolarization and vasodilatation that requires the activity of small and intermediate conductance Ca2+-activated K+ channels (Earley, 2012).

TRPA1 also has an influence on gastrointestinal motility (reviewed in Blackshaw et al., 2010, 2013). TRPA1 agonists delay in vivo gastric emptying through serotonergic pathways. TRPA1 appears to be functionally coupled with the L-type voltage-dependent Ca2+ channel in pancreatic β cells and mediates insulin secretion. In epidermal keratinocytes, TRPA1 affects the expression of proinflammatory cytokines, including...
interleukin (IL)-1a and IL-1b (Bautista et al., 2013). In the CNS, TRPA1 modulates glycnergic transmission in synapses of the medullary dorsal horn neurons (Nilius, 2012). In the presynaptic terminals of magnocellular neurosecretory cells that produce vasopressin in the supraoptic nucleus, TRPA1 activation enhances glutamate release and plays a role in the hippocampus (for a review, see Nilius, 2012; Vennekens et al., 2012).

**E. Mucolipin Transient Receptor Potential Subfamily**

The mucolipin TRP subfamily (TRPML) contains three members that are mostly expressed in intracellular vesicles of the endolysosome system. The name is derived from mucolipin, a protein responsible for the neurodegenerative disease mucolipidosis type IV (ML-IV). TRPML1 and TRPML3 are relatively small proteins consisting of fewer than 600 amino acid residues. They contain targeting motifs to the endoplasmic reticulum and lysosomes/endosomes in the N termini and C termini and are characterized by a large loop with multiple N-glycosylation sites between the two first transmembrane segments that are located in the lumen of the organelles. TRPML functions are difficult to study because of their localization in cell organelles (Fig. 3). They play a crucial role in the trafficking and differentiation of early endosomes to multivesicular bodies, late endosomes, lysosomes, and recycling endosomes (Dong et al., 2010). Similar to TRPML channels in the endolysosomal pathway, two-pore channels (TPCs), namely TPC1, TPC2, and TPC3, are found in intracellular organelles, in particular in endosomes and lysosomes, and interact with TRPMLs.

TRPML1 is a Ca\(^{2+}\), Fe\(^{2+}\), and Zn\(^{2+}\)-permeable non-selective cation channel that is impermeable for protons and shows a strong, inwardly rectifying IV curve (see Colletti and Kiselyov, 2011). TRPML1 homomultimerizes as well as heteromultimerizes with TRPML2 and TRPML3 (Dong et al., 2010).

TRPML1 is ubiquitously expressed, with the highest expression found in the brain, heart, kidney, liver, and spleen. It is an intracellular channel that is mainly located in late endosomal and lysosomal compartments (Dong et al., 2010; Gees et al., 2010). Targeting of TRPML1 to lysosome (i.e., trafficking from the trans-Golgi network) requires dileucine motifs in both the N-terminal and C-terminal cytosolic tails that directly interact with the clathrin adaptor proteins AP1 and AP3. Another sorting motif is the stretch of polar and hydrophobic amino acids that bind to \(\alpha\)-1,3-mannosyltransferase in a Ca\(^{2+}\)-dependent manner (Abe and Puertollano, 2011).

TRPML proteins are functionally difficult to access because of their localization in intracellular organelles. TRPML1 was first studied in the varitint-waddler (Va-) like mutant TRPML1 channel (V432P) that exhibits plasma membrane localization. This mutant TRPML1 channel is constitutively active and displays a small potentiation by acidic pH (Dong et al., 2009). Under resting conditions, the basal activity of TRPML1 (and probably all TRPML channels) is most likely very low. Stimulation of channel activity occurs by variations in luminal pH or synthesis of specific phosphoinositides (e.g., PIP\(_2\)) that bind to a polybasic domain in the N terminus. Activation of TRPML1 mediates release of Ca\(^{2+}\) and (maybe also Fe\(^{2+}\)) from the lumen of endosomes and lysosomes. TRPML1 regulates the activity of other membrane proteins such as TPCs.

Efflux of luminal Ca\(^{2+}\) promotes the recruitment of specific effectors, such as \(\alpha\)-1,3-mannosyltransferase, that interact with endosomal proteins that are implicated in trafficking of endosomes/lysosomes [e.g., proteins of the endosomal sorting complex required for the transport machinery, Alix and the product of the tumor susceptibility gene 101 (TSG10)]. TRPML1 also associates with members of the lysosome-associated protein transmembrane family, which is important for the organelle remodeling (Spooner et al., 2013). TRPML1 might be also coupled to transducer of regulated CREB activity-1, which supports a role in organelle fusion (Venkatachalam et al., 2013). After Ca\(^{2+}\) release, TRPML1 tethers to the soluble \(N\)-ethylmaleimide-sensitive factor attachment receptor protein receptor, a complex required for the final steps of endolysosomal fusion. TRPML1 also regulates membrane retrieval from hybrid organelles, thus allowing organelle reformation (Shen et al., 2011b). TRPML1 activation is inhibited by PKA.

In general, TRPML1 is important for endosome/lysosome function, the fusion of amphisomes (autophagic vacuoles) with lysosomes, endosome differentiation (biogenesis), and trafficking (Fig. 3; see Colletti and Kiselyov, 2011). TRPML1 also plays a role in membrane trafficking. Abnormal lipid accumulation resulting in enlarged late endosomes occurs under conditions when TRPML1 is downregulated. TRPML1 is required for the formation of lysosomes from autolysosomes, and for the Ca\(^{2+}\)-dependent lysosomal exocytosis (Fig. 3). TRPML1 is not involved (as was previously assumed) in the \(H^+\) homeostasis of endolysosomes. However, as a Fe\(^{2+}\)-release channel, it is involved in Fe\(^{2+}\) homeostasis (Dong et al., 2008). A different function of TRPML1 is implicated in the regulation of gastric acid secretion from apical-membrane trafficking in parietal cells (Chandra et al., 2011).

TRPML2 was identified together with TRPML3. It interacts with TRPML1 and TRPML3 (see Flores and Garcia-Anoveros, 2011). TRPML2 forms inwardly rectifying channels and is permeable to Ca\(^{2+}\), Na\(^{+}\), and Fe\(^{2+}\). It is present in lysosomes, late endosomes, recycling endosomes, and, at a lower level, in the plasma membrane. The location of TRPML2 in recycling endosomes is different from that of TRPML1. TRPML2 is widely expressed in immune cells as well as the heart, kidney, liver, spleen, thymus, and lymphocytes.

TRPML2 shows a low basal activity and is slightly potentiated by acidic pH (Dong et al., 2010). It colocalizes
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F. Polycystic Transient Receptor Potential Subfamily

The nomenclature of polycystin subfamily of TRP channels is somewhat confusing, especially because the old terminology appears to be firmly entrenched in the literature. The TRPP family only contains three members: 1) TRPP1 was previously known as TRPP2, PKD2, or PC2, a name derived from the second gene, which causes autosomal dominant polycystic kidney disease (ADPKD); 2) TRPP2 was previously known as TRPP3, polycystin-like, PKD-like 2, and PKD2-like 1; and 3) TRPP3 was previously referred to as TRPP5 or PKD2-like 2 (Wu et al., 2010). [Please note that this new nomenclature introduced by Clapham and colleagues in their recent Pharmacological Reviews article (Wu et al., 2010) differs from the still official HUGO Gene Nomenclature.] The members possess a coiled-coil structure in their C terminus, form polymodal multiprotein/ion channels complexes, and have a Ca\(^{2+}\) binding EF hand motif in the C terminus.

TRPP1 is a Ca\(^{2+}\)-permeable, nonselective cation channel. TRPP1 is widely, if not ubiquitously, expressed. It is abundant in the kidney (both motile and nonmotile cilia), in bone, lacrimal glands, retina, heart, and especially in vascular smooth muscle cells (Delmas, 2005; Patel and Honoré, 2010). It contains an N-terminal endoplasmic reticulum retention signal that locks the channel in intracellular compartments. The plasma membrane location of TRPP1 is maintained via a C-terminal coiled-coil domain. TRPP1 interacts with PKD1 (also known as polycystin 1), a large 11 transmembrane segment plasma membrane adhesion protein. Probably one PKD1 forms a complex with three TRPP1 molecules. A coiled-coil domain in the C terminus of TRPP1 is critical for the formation of this complex (Yu et al., 2009c). Phosphofurin acidic cluster sorting proteins PACS-1 and PACS-2, which interact with an acidic amino acid cluster within the C-terminal domain of the TRPP1, regulate its distribution between the endoplasmic reticulum and cell surface (Kötting et al., 2005). Of note, both TRPP2 and TRPP3 lack this PACS domain.

The C-terminal region of TRPP1 also binds to the endoplasmic reticulum stress-inducible proteins Herp and protein kinase C substrate 80K-H complex (PKCSH/80K-H), which are involved in carbohydrate processing, folding, and translocation of newly synthesized glycoproteins. Both proteins stimulate retention in the endoplasmic reticulum and protect the channel from endoplasmic reticulum–associated protein degradation (Chapin and Caplan, 2010). Surface localization of TRPP2 is regulated by phosphorylation via glycogen synthase kinase 3 at a site in its N-terminal domain (Streets et al., 2006).

For activation, TRPP1 requires the activity of PLC and PI3K. Formation of PIP\(_2\) suppresses EGF-induced currents and acts as a negative regulator of TRPP1 (Ma et al., 2005). TRPP1 also interacts with the mammalian diaphanous-related formin 1, a downstream effector of Ras homolog gene family, member A. Importantly, this interaction confers voltage dependence to the channel. At negative potentials, the TRPP1 channel activity is specifically blocked by mammalian diaphanous-related formin 1 that switches in a Ras homolog gene family, member A–induced manner from an autoinhibited state at negative

with major histocompatibility complex (MHC) protein class I, glycosylphosphatidylinositol-anchored proteins, and ADP-ribosylation factor-6; TRPML2 contributes to the regulation of sorting of glycosylphosphatidylinositol-anchored proteins. TRPML2 is involved in the regulation of the trafficking between recycling endosomes and the cell surface. This function requires ADP-ribosylation factor-6 but is clathrin independent (Karacsonyi et al., 2007). Similar to TRPML1, it plays a role in lysosomal debris. TRPML1/TRPML2 heteromultimers also play a role in membrane trafficking. Moreover, TRPML2 is involved in regulation of Fe\(^{2+}\) metabolism, especially during maturation of B and T lymphocytes (Dong et al., 2010).

TRPML3 has been cloned from the Vn mouse, which shows deafness and pigmentation defects due to a mutation in the Trpml3 gene (i.e., an A419P substitution in the linker region between S4 and S5). TRPML3 is found in the cochlea, eye, kidney, lung, skin (melanocytes), spleen, and thymus, as well as in somatosensory neurons (Di Palma et al., 2002; Samie et al., 2009; reviewed in Noben-Trauth, 2011). TRPML3 is found in early and late endosomes, early lysosomes, and also in the plasma membrane. TRPML3 accumulates in the plasma membrane if endocytosis is inhibited, and is highly expressed in autophagosomes when autophagy is induced (Kim et al., 2009).

TRPML3 is a Ca\(^{2+}\)-permeable channel that is H\(^+\) impermeable. Its activity is regulated by extracytosolic Na\(^+\). In vitro, Na\(^+\)-free medium is required for channel activation. The channel slowly inactivates after such activation and subsequent readministration of Na\(^+\) dramatically increases the current. TRPML3 is also regulated by Ca\(^{2+}\) and the luminal pH.

TRPML3 is a prominent regulator of endocytosis (see Zeevi et al., 2009), membrane trafficking, and autophagy, likely by controlling the Ca\(^{2+}\) in the vicinity of cellular organelles. Overexpression of TRPML3 causes enlarged endosomes and leads to an increase in autophagy. Downregulation causes enhanced endocytosis (Kim et al., 2009). In the Vn mouse, the TRPML3 mutant results in a constitutively active channel that causes increased Ca\(^{2+}\) influx and cell death, followed by a variegated pigmentation due to melanocyte dysfunction and a loss of hair cells in the cochlea and vestibulum, which induces deafness (Di Palma et al., 2002). Finally, TRPML3 is expressed in the tongue, where it may function as a salt sensor in taste buds (Moyer et al., 2009).
TRPP1 functions as a Ca$^{2+}$ entry channel in the plasma membrane, and a Ca$^{2+}$-activated intracellular calcium release channel in the endoplasmic reticulum. It is Ca$^{2+}$ and pH dependent. A reduction in the cytosolic pH inhibits the channel (Koulen et al., 2002). As a Ca$^{2+}$ release channel in the endoplasmic reticulum, TRPP1 interacts with the IP$_3$R and probably also with stromal interacting molecule-1. Initially, TRPP1 was described as a mechanosensitive channel in the primary cilia of renal epithelial cells and vascular endothelial cells. It regulates responses to shear stress in blood vessels and in the tubular system of the kidney. This function requires interaction with PKD1 and/or TRPV4 and results in a flow-induced increase in [Ca$^{2+}$]$_i$ (Patel and Honoré, 2010). In addition to TRPV4, TRPP1 may associate with TRPC1 and TRPC4. The adhesion protein PKD1 is supposed to form a flow-sensitive ion channel complex in the primary cilium of both epithelial and endothelial cells. Flow sensing via TRPP1 in primary cilia is required for establishing the left-right asymmetry in the body manifested in the heart, lungs, and gut (Yoshiba et al., 2012).

It was recently shown that TRPP1 inhibits SACs (similar to Piezo1). TRPP1 interacts with filamin A. This cross-linking protein is critical for SAC regulation (Sharif-Naeini et al., 2009; Nilius and Honore, 2013; Peyronnet et al., 2013). TRPP1 is involved in angiogenesis via modulation of VEGF and type-1 interferons (IFNs), including IFNa/β, and plays a role in lithium-induced neurogenesis (Italia et al., 2011; Zheng et al., 2011).

TRPP2 has 71% sequence homology with TRPP1. It is a Ca$^{2+}$-permeable nonselective cation channel. It is expressed in the brain (e.g., hypothalamus), heart, retina, skeletal muscle, spleen, tongue (circumvallate, foliate, and fungiform papillae), and testis. It seems to be highly expressed in embryonic tissues (Wu et al., 1998; Ishimaru et al., 2006). TRPP2 is coexpressed (and associates) with PKD1-like 2.

TRPP2 was first described as a channel activated by both low extracellular pH and citric acid (Ishimaru et al., 2006). Activation by external pH requires PKD1-like 3. In the absence of PKD1-like 3, TRPP2 shows constitutive basal activity and is inhibited by extracellular citric acid (or low pH) and is potentiated by alkalinization. It is activated by Ca$^{2+}$ and cell swelling and shows clear voltage dependence (e.g., depolarization) (Shimizu et al., 2009).

TRPP2 probably functions as a sour taste receptor. It also acts as a pH sensor in the cerebrospinal fluid, expressed in specific neurons aligning the spinal canal (Huang et al., 2006). TRPP2 seems also to be essential for the development of the kidney and retina (Nomura et al., 1998).

It is noteworthy that TRPP2 has now been identified as component (together with PKD1-like 1) of a Ca$^{2+}$-permeable cation channel in primary cilia. It is activated by the hedgehog pathway proteins GLI2 and smoothened (SMO). This channel is thought to be an important regulator of the Ca$^{2+}$ concentration in a subcellular compartment (DeCaen et al., 2013; Delling et al., 2013), with little or no contribution to the global intracellular Ca$^{2+}$ concentration in primary cilia. It is intriguing to speculate that TRPP2 may play an important role in ciliopathies, in particular in neurodevelopmental disorders (for an exhaustive review, see Valente et al., 2014).

TRPP3 shares 59% sequence homology with TRPP1. It forms Ca$^{2+}$-permeable nonselective cation channels and is widely expressed in fetal tissues (Guo et al., 2000). The activation mechanism of TRPP3 remains to be determined.

II. Transient Receptor Potential Channels: Hereditary Diseases (Transient Receptor Potential Channelopathies)

In the past few years, several hereditary diseases caused by defects in genes encoding TRP channel proteins have been described (Fig. 14; for comprehensive reviews, see Abramowitz and Birnbaumer, 2007, 2009; Nilius, 2007; Nilius and Owsianik, 2010b; Everett, 2011); collectively, these diseases are referred to as TRP channelopathies. TRP channels also play a role in cancer development and, thus, may represent novel anti-cancer drug targets. The evidence for hereditary TRP involvement in cancer is, however, weak and inconclusive (see Santoni and Farfariello, 2011). Here we describe the hereditary TRP channelopathies and mention a few representative examples of pathogenic TRP dysfunctions. Interested readers are also referred to the Online Mendelian Inheritance in Man (OMIM) database, a frequently updated database on genetic diseases (www.ncbi.nlm.nih.gov/OMIM).

A. Canonical Transient Receptor Potential Channelopathies

TRPC channel dysfunction has been linked to many acquired diseases, such as cardiovascular, pulmonary, skin diseases, and inflammation (Abramowitz and Birnbaumer, 2009), but there are only very few examples of hereditary channelopathies. TRPC1 is thought to be involved in Gorlin syndrome, also known as basal cell nevus syndrome (OMIM 109400). It is a rare, autosomal dominant disorder with complete penetrance and variable expressivity. The syndrome is characterized by odontogenic keratocysts of the mandible and postnatal tumors including multiple basal cell carcinomas (BCCs). Mutations in the tumor suppressor gene PTCH1 (a member of the patched gene family and a receptor for sonic hedgehog) and in the TRPC1 gene seem to be responsible for the development of the tumors (Sauer et al., 2011). A novel spliced isoform of TRPC1 with...
TRPC3 might be indirectly involved in the pathogenesis of autosomal dominant spinocerebellar ataxia type 14, caused by mutations in PKCγ, which negatively regulates the channel. Mutant PKCγ in cerebellar Purkinje cells of patients with spinocerebellar ataxia type 14 could differentially impair TRPC3 function and disrupt synapse pruning, synaptic plasticity, and synaptic transmission (Shuvaev et al., 2011). Interestingly, a gain-of-function mutant in TRPC3 causes cerebellar ataxia in mice, the so-called *Moonwalker* (Mwk) mouse (Becker et al., 2009). However, a genetic screen for TRPC3 mutations in patients with late-onset cerebellar ataxia does not support a contribution of TRPC3 mutants to this disease (Becker et al., 2011). TRPC3 might also be indirectly involved in Williams–Beuren syndrome, a neurodevelopmental disorder associated with distinctive “elfin” facial appearance, unusually cheerful personality, heart or blood vessel problems (e.g., supravalvular aortic stenosis), and hypercalcemia. The main genetic defect lies in the transcription factor III gene (encoding TFII-I), which normally suppresses cell surface accumulation of TRPC3; consequently, TFII-I mutations cause a TRPC3 gain-of-function phenotype (Letavernier et al., 2012).

Excessive Ca\(^{2+}\) influx mediates many cytotoxic processes, including those associated with autoimmune inflammatory diseases such as acute pancreatitis, and Sjögren syndrome (also known as sicca syndrome), a systemic autoimmune disease in which immune cells attack and destroy the exocrine glands that produce tears and saliva. The hallmark symptom of the disorder is a generalized dryness, typically involving dry mouth (xerostomia) and dry eyes (xerophthalmia). It has been hypothesized that a gain of function of TRPC3 may be involved in the pathogenesis of these diseases, and the TRPC3-selective inhibitor Pyr3 might be beneficial in these patients (Kim et al., 2011c).

TRPC6 is involved in glomerular renal diseases. It is required for normal function of podocytes, highly specialized epithelial cells that line the urinary surface of the glomerular capillary tuft. Dysfunction or death of podocytes impairs glomerular permeability and filtration. This channel is mutated in cases of focal segmental glomerulosclerosis (FSGS), type 2 (OMIM 603965) (Henique and Tharaux, 2012).

FSGS is functionally characterized by proteinuria and progressive decline of renal function. At least 14 mutations in the N terminus (n = 7) and C terminus (n = 6) of the *Trpc6* gene are linked to FSGS type 2 (for a recent update, see Hofstra et al., 2013). All mutations show a gain-of-function phenotype. TRPC6 in the podocytes associates with the slit protein nephrin that is coupled to the nephrin-interacting adapter protein CD2AP and to podocin (reviewed in Möller et al., 2009). This complex forms the glomerular filter (Reiser et al., 2005). Downstream targets of TRPC6 are still under investigation. Activation of NFAT in TRPC6 mutants is blocked by inhibitors of calcineurin, CaMKII, and PI3K, independently of Src, Yes, or Fyn. Therefore, it is very likely that the calcineurin-NFAT pathway plays an important role in mediation of FSGS. The immunosuppressive agent FK-506 may also inhibit TRPC6 (for a review, see Schlöndorff et al., 2009; Wang et al., 2010; El Hindi and Reiser, 2011). As shown in the rat, knockdown by nephrin and/or TRPC6 small interfering RNA (siRNA) substantially reduces the protein levels of nephrin and/or TRPC6 and counteracts the permeation defect (Hauser et al., 2010).

TRPC6 is also involved in the steroid-resistant nephrotic syndrome (OMIM 600995). In Turkish children, three mutations and an intronic nucleotide substitution were described causing the sporadic form of this disease (Mir et al., 2012). A SNP in the promoter region of TRPC6 and a missense variant in exon 4 of TRPC6 might be putative causal gene variants for infantile hypertrophic pyloric stenosis (OMIM 179010) (Everett et al., 2009). Mutations in TRPC6 may also contribute to idiopathic pulmonary arterial hypertension (IPAH) (Yu et al., 2004), in which SNPs were described in a cohort of patients. The abnormal TRPC6 transcription is linked to nuclear factor-κB, an inflammatory and carcinogenic transcription factor (Yu et al., 2009b).

**B. Vanilloid Transient Receptor Potential Channelopathies**

Somewhat unexpectedly, TRPV1, the best characterized TRP channel, is not yet linked to any hereditary disease (Fig. 12). However, it was suggested that polymorphism in the TRPV1 gene may play a role in the development and maintenance of chronic pain: indeed, genetic variants of human TRPV1 with M315I and I585V channel mutations were found in Caucasian patients with an increased individual susceptibility to neuropathic pain (Armero et al., 2012). SNPs in TRPV1 were also identified in a Spanish population with increased genetic susceptibility to migraine (Carreño et al., 2012). SNPs in TRPV1 were also identified in a Spanish population with increased genetic susceptibility to migraine (Carreño et al., 2012). Interestingly, the M315I variant of the TRPV1 gene also conferred increased susceptibility (odds ratio of 1.6) to type 1 diabetes in Ashkenazi Jews (Sadeh et al., 2013).

Of note, the Miller–Dieker lissencephaly syndrome, an autosomal dominant congenital disorder characterized by a developmental defect in the brain caused by incomplete neuronal migration, is due to a chromosome 17p13.3 deletion syndrome that includes deletion of TRPV1 (Laurito et al., 2011).
An I585V variant of TRPV1 (that shows decreased channel activity) reportedly might confer decreased genetic risk for painful knee osteoarthritis (OA) (Valdes et al., 2011). The same genetic variant of TRPV1, I585V, is also protective against childhood asthma (Cantero-Recasens et al., 2010). A selective risk association of the missense TRPV1 SNP, associated with the primary progressive disease type, was recently discovered in patients with multiple sclerosis (MS) (Paltser et al., 2013). This is all the more interesting because TRPV1 seems to control the severity and progression of experimental autoimmune encephalomyelitis in mice and may indicate that TRPV1 is a critical disease modifier in experimental autoimmune encephalomyelitis and a novel target for MS therapy (Paltser et al., 2013). Indeed, TRPV1 activators (e.g., arvanil) provide symptomatic relief in a rat model of MS (Cabranes et al., 2005).

TRPV2 has been linked to Duchenne muscular dystrophy, cancer, and diabetes (Nilius et al., 2007b; Harisseh et al., 2013). However, thus far, no TRPV2 channelopathy has been detected.

TRPV3 is expressed in keratinocytes and in cells surrounding hair follicles. In mice, two gain-of-function mutations at a single site cause an autosomal dominant hairless phenotype with dermatitis (Asakawa et al., 2006). In humans, three gain-of-function mutations (G573S, G573C, W692G), one of which is identical to the mouse mutation, cause Olmsted syndrome (also Fig. 12. Simplified, schematic representation of the complex regulation of the vanilloid (capsaicin) receptor TRPV1. The upper part of the figure shows the modular structure of TRPV1. The N-terminal intracellular region starts with six ankyrin repeats (dark brown segment) and contains phosphorylation sites for different protein kinases (pale yellow dots; Ser117 for PKCα and PKA, Thr145 and Thr371 for PKA, and Tyr200 for Src kinase). It is followed by the six transmembrane domains (TM1–TM6) with a pore-forming region between TM5 and TM6. Mutational studies revealed the importance of the third and fourth TM domains in vanilloid binding (for more details, see Fig. 4). The lower part summarizes the downstream sensitization of TRPV1 by different mediators released at the site of injury or inflammation. For details and abbreviations, see section I.B. Reprinted with permission from Lázár et al. (2009).
known as “mutilating palmoplantar keratoderma with periorificial keratotic plaques” or “polykeratosis of Touraine”), a rare disorder characterized by the combination of periorificial keratotic plaques, bilateral palmoplantar keratodermas (horn-like skin growth), alopecia, and severe itching (Lai-Cheong et al., 2012; Lin et al., 2012; reviewed in Nilius and Biró, 2013). A new TRPV3 mutant, G573A, was recently identified in patient with Olmsted syndrome that can also cause multiple immune dysfunctions, such as hyper-IgE, elevated follicular T cells, and persistent eosinophilia (Danso-Abeam et al., 2013). Supporting the role of TRPV3 as an important channel in skin disorders, rosacea, a frequent chronic inflammatory skin disease that causes redness and pimples in the face and forehead, shows upregulation of TRPV3 (Sulk et al., 2012).

TRPV4 causes at least nine different channelopathies. The first to be described was the autosomal dominant brachyomelia type 3 (OMIM 113500), a relatively mild skeletal dysplasia characterized by short stature, platyspondyly (flattened vertebral bodies), reduced intervertebral spaces, scoliosis, or kyphosis (Rock et al., 2008). Triggered by this surprising finding, a number of new, TRPV4-caused skeletal dysplasias were discovered, all of which show (to various degrees) short stature, platyspondyly, defects in bone ossification, and joint abnormalities (see Nilius and Owsianik, 2010a; Nilius and Voets, 2013). These diseases are probably due to a combination of functional defects and differentiation abnormalities in chondrocytes of the bone growth plate.

The spondyloepimetaphyseal dysplasia of Maroteaux (pseudo-Morquio type 2) (OMIM 184095) shows the described pattern but manifestations are limited to the musculoskeletal system (Nishimura et al., 2010). Another dysplasia is the spondylometaphyseal dysplasia Kozlowski type (OMIM 184252): these patients exhibit short stature, mostly due to shortening of the trunk. Defects are observed in the distal metaphysis of the femur, the femoral neck and trochanteric area. Sometimes, lordosis and slight scoliosis with short fingers and deviations in finger joints, and involves mainly irregularities in the articular surfaces (Lamandé et al., 2011).

Thus far, more than 50 TRPV4 mutations (which scatter over the whole protein) have been identified, causing the six skeletal diseases (Fig. 15). Most of the mutations are gain-of-function mutations, and the degree of channel overactivity seems to determine the severity of the disease (Loukin et al., 2010a,b) (for reviews, see Nilius and Owsianik, 2010a; Nishimura et al., 2012; Nilius and Voets, 2013). A second big surprise was the discovery of the role that TRPV4 plays in autosomal dominant distal neuropathies; these involve mainly motoric defects in the distal limbs but also in the respiratory system and the vocal cord, and are sometimes associated with sensory defects (for a review, see McEntagart, 2012). The main symptom is muscle atrophy caused by degeneration of the motoneurons in the spinal ventral horn. Most of spinal muscle atrophies are phenotypically similar and all lead to muscle weakness and wasting. Furthermore, they are often combined with multiple pulmonary and orthopedic symptoms. Congenital distal spinal muscle atrophy (OMIM 600175) is a nonprogressive lower motor neuron disorder, restricted to the lower part of the body. It is sometimes associated with arthrogryposis (joint contractures), bilateral talipes equinovarus (commonly known as clubfoot), and flexion contractures of the knees and hips. Sometimes, lordosis and slight scoliosis with restricted joint movement are also observed. Congenital distal spinal muscle atrophy is a pure motor neuron disease and shows no sensory defects (Zimoń et al., 2010). Scapuloperoneal spinal muscle atrophy (OMIM 181405) is a syndrome characterized by scapuloperoneal atrophy, laryngeal palsy, scapular winging, muscle wasting in the lower limbs, vocal cord paralysis, absence of tendon reflexes, and sometimes scoliosis and light sensory defects (DeLong and Siddique, 1992; Auer-Grumbach et al., 2010; Deng et al., 2010).

Another TRPV4 channelopathy is the autosomal dominant hereditary motor sensory neuropathy type IIc (Charcot-Marie-Tooth neuropathy type 2; OMIM 606071), which is characterized by a variable degree of muscle weakness in the limbs, vocal cords, and intercostal muscles, and by asymptomatic sensory loss. It starts in infancy or childhood. The life expectancy of these patients is shortened because of respiratory failure. Facial asymmetry, tongue fasciculations, and third and sixth cranial nerve palsies have also been reported (Auer-Grumbach et al., 2010; Deng et al., 2010; Landouré et al., 2010; Rossor et al., 2012). Many patients with Charcot-Marie-Tooth neuropathy type 2...
(but also scapuloperoneal spinal muscle atrophy) have skeletal symptoms such as pes cavus, hammertoes, lumbar hyperlordosis, and scoliosis (Fig. 16), as well as bladder urgency, incontinence, tremor, and dysphagia. The TRPV4 mutations in these patients are gain of function, associated with neurotoxicity (Fig. 16). There is clearly some overlap between neuronal/axonal defects and skeletal symptoms. Thus far, 21 mutations in TRPV4 have been described that can cause neuropathies (Fig. 15). These mutations are mainly localized in the convex surface of the ARD of the TRPV4 N terminus (Fig. 15). Mutations causing skeletal dysplasia, although scattering through the whole length of the channel protein, seem to be more frequently located in the concave surface of the ARD. There are three hot spots for disease-causing mutations (Fig. 15): 1) the ARD domain; 2) the transmembrane region S3–S5; and 3) a C-terminal region were the channel associates with several members of the cytoskeleton, such as tubulin, actin, and microtubule-associated protein 7 (Inada et al., 2012).

Indeed, the discovery that a large number of mutations in the same gene (that are often located in the same domain of the channel) can cause nine different diseases creates a challenging puzzle (see Nilius and Voets, 2013). The reasons underlying the phenotypic variability (i.e., why these mutant channels result in these different diseases) remain elusive (Nilius and Voets, 2013). Mutations causing neuropathies probably have a lower penetrance than mutations causing skeletal dysplasias; this may explain why skeletal diseases often show no neurologic symptoms. Because of the high expression of TRPV4 in the inner ear and the urothelium, it is not surprising that some patients have also hearing problems and/or bladder symptoms such as overactive bladder and incontinence (reviewed in Verma et al., 2010).

The TRPV4 variant P19S was suggested to predispose the carriers to acquired chronic obstructive pulmonary disease (COPD) because of a reduced airway clearance due to decreased cilia activity, which is supposed to be a TRPV4-dependent mechanism (Li et al., 2011b). The same mutation/polymorphism also causes hyponatremia (Tian et al., 2009).

TRPV5 and TRPV6 function as Ca^{2+} (re)absorption channels. No TRPV5 or TRPV6 channelopathy has yet been described. The TRPV5 gene exhibits an unusually high frequency of four nonsynonymous SNPs among African Americans, all of which increase Ca^{2+} reabsorption and cause hypercalciuria (Hughes et al., 2008; Suzuki et al., 2008). Upregulation of both channels causes hypocalciuria (Yang et al., 2010). Interestingly, an ancestral Trpv6 haplotype that consist of three nonsynonymous polymorphisms results in three mutations in the protein with a gain-of-function phenotype. The frequency of the ancestral Trpv6 haplotype (carriers are still in the modern population) is significantly higher in Ca^{2+} stone formers compared with nonstone-forming individuals. Thus, TRPV6 might play a role in calcium stone formation in certain forms of absorptive hypercalciuria (Hughes et al., 2008; Suzuki et al., 2008).

C. Melastatin Transient Receptor Potential Channelopathies

TRPM1 was previously considered to be a tumor suppressor protein in melanoma cells. The Trpm1 gene codes two transcripts: TRPM1 channel protein in its exons and miR-211 in one of its introns. The loss of TRPM1 channel protein is an excellent marker of melanoma aggressiveness, whereas the expression of miR-211 is linked to the tumor suppressor function of TRPM1 (Mazar et al., 2010; Guo et al., 2012; Margue et al., 2013).

TRPM1 is linked to the autosomal recessive congenital stationary night blindness type 1C (OMIM 613216), a clinically and genetically heterogeneous group of retinal disorders characterized by nonprogressive impaired night vision and decreased visual acuity. It is caused by ON bipolar cell dysfunction: TRPM1 channels, gated by the mGluR6 signaling cascade, are necessary for the depolarizing light response of these cells (Audo et al., 2009; Nakamura et al., 2010). In Appaloosa horses, the coat spotting pattern is caused by a loss-of-function mutation in TRPM1. In these horses, TRPM1 dysfunction is due a retrovirus insertion that disrupts the transcription of the TRPM1 gene by premature polyadenylation (Bellone et al., 2013). Congenital stationary night blindness type 1C results from the loss-of-function of rod and cone ON bipolar cells in the mammalian retina. Patients also display myopia, reduced central vision, and nystagmus. Unlike the Appaloosa horses, none of the patients shows abnormal skin pigmentation (Li et al., 2009).

TRPM2 and TRPM7 (Fig. 13), two chanzymes, have long been thought to cause Guamanian amyotrophic lateral sclerosis (ALS-G) and Parkinsonism-dementia complex of Guam (PD-G), or Parkinsonism-dementia complex, two related neurodegenerative disorders that are endemic in the Western Pacific, including Guam (Plato et al., 2002). ALS-G and PD-G have a multifactorial
etiology, including soil and drinking water that is low in Ca\(^{2+}\) and Mg\(^{2+}\) but high in Fe\(^{2+}\), Mn\(^{2+}\), and Al\(^{3+}\), as well as the presence of the putative neurotoxin, L-β-N-methylamino-L-alanine, derived from the cycad plant (traditionally, bats that feast on cycad plants are considered a delicacy by the natives).

Mutations in the \(Trpm2\) and \(Trpm7\) genes have been implicated in the pathogenesis of these diseases. TRPM2 and TRPM7 are thought to initiate neuronal cell death by sensing oxidative stress (TRPM2 is a redox sensor); indeed, TRPM2 and TRPM7 are crucial for cell viability in neurodegenerative diseases (for a review, see Benarroch, 2008; Szydlowska and Tymianski, 2010). A subset of patients with ALS-G and PD-G are heterozygotes for a missense mutation in the \(Trpm2\) and \(Trpm7\) genes, which cause fast inactivation and increased sensitivity to inhibitory Mg\(^{2+}\) (Hermosura et al., 2005, 2008; Hermosura and Garruto, 2007). It seems that ion influx through both channels is physiologically important, and disruption of this influx may, under certain conditions, contribute to disease states (Hermosura et al., 2008). Because the ALS-G and PD-G environments are deficient in Ca\(^{2+}\) and Mg\(^{2+}\), an increased sensitivity of TRPM7 to inhibition by Mg\(^{2+}\) could even worsen the Mg\(^{2+}\) homeostasis in a Mg\(^{2+}\)-deficient environment, leading to a reduced intracellular Mg\(^{2+}\) concentration (Schmitz et al., 2003). However, a recent linkage analysis did not reveal any evidence in support of the linkage to the \(Trpm7\) locus, indicating that TRPM7 is probably not associated with ALS-G/PD-G (Hara et al., 2010).

TRPM2 might be involved in bipolar disorder type I (BD-I), characterized by one or more manic (or mixed) episodes, usually followed by major depressive episodes. One of the putative susceptibility loci of BD-I is associated with the chromosomal region that encodes TRPM2 (Liu et al., 2001; Xu et al., 2006, 2009a). SNPs in the promoter region of \(Trpm2\) are also significantly associated with BD-I. It was postulated that the TRPM2 polymorphism may contribute to the risk for DB (Xu et al., 2009a), especially if it coexists with a SNP within the iPLA2β gene (Xu et al., 2013). (The \(iPLA2β\) gene encodes an enzyme that is involved in oxidative stress.)

TRPM3 has been recently discussed as a part of the genetic background that may contribute to the comorbidity between autism and Duchenne muscular dystrophy. Indeed, in some patients, a deletion involving exons 1–9 of TRPM3 has been described (Pagnamenta et al., 2011). TRPM3 might be also involved in the pathogenesis of Kabuki syndrome (OMIM 147920), a congenital mental retardation syndrome characterized distinct facial appearance (the name comes from the facial resemblance of the patients to the stage makeup used in traditional Japanese theater), heart defects, urinary tract anomalies, hearing loss, hypotonia, short stature, joint laxity, and unusual dermatoglyphic patterns (Kuniba et al., 2009).

TRPM4 mutations cause progressive familial heart block type I (OMIM 113900), which is a progressive cardiac bundle branch disease affecting the His-Purkinje system that exhibits autosomal dominant inheritance.
TRPM4 mutants show a gain-of-function phenotype probably because of increased surface expression, leading to depolarization-induced defects in the conduction and electrical gridlock (Kruse et al., 2009; Liu et al., 2010a). Mutations were also found in patients with atrioventricular block. No mutations were found in other patients with sinus node dysfunction or long QT syndrome (Stallmeyer et al., 2012).

It is unclear whether TRPM4 plays a role in alteration of the arterial myogenic response (Bayliss effect) associated with stroke and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (Folgering et al., 2008). A subset of patients with Brugada syndrome who had a bifascicular block and/or a complete right bundle branch block was recently reported. In these patients, four TRPM4 mutants were described, resulting in either decreased expression (P779R and K914X) or increased expression (T873I and L1075P) of TRPM4 channels (Liu et al., 2013c).

TRPM6 is involved in Mg$^{2+}$ homeostasis (see Montell, 2003; Cao et al., 2008; Ferrè et al., 2011; Dimke et al., 2011, 2013). More than 35 mutations in the Trpm6 gene have been associated with the disease hypomagnesaemia with secondary hypocalemia (HSH)-1, also known as HOMG1 (OMIM 602014) (Schlingmann et al., 2005, 2007). This form of hypomagnesaemia has to be separated from other forms, such as hypomagnesaemia 2 (HOMG2) caused by misrouting of the FXYD2 Na$^+$/K$^+$-ATPase γ-subunit, renal hypomagnesaemia-3 (HOMG3) associated with hypercalciuria and nephrocalcinosis and caused by mutation in the claudin gene CLDN16 gene, renal hypomagnesaemia-4 (HOMG4) caused by mutation in the EGF gene (impaired of the basolateral sorting of pro-EGF), renal hypomagnesaemia-5 (HOMG5) (associated with hypercalciuria, nephrocalcinosis, and severe ocular involvement) caused by mutation in the claudin gene CLDN19 gene, and renal hypomagnesaemia-6 (HOMG6) caused by mutation in the divalent metal cation transporter CNNM2 gene (Dimke et al., 2013).

HSH1 (HOMG1) is a familial hypomagnesaemia with secondary hypocalemia, a rare autosomal recessive disorder characterized by very low serum Mg$^{2+}$ levels. Hypocalcemia is a consequence of parathyroid failure and PTH resistance that results from severe Mg$^{2+}$ deficiency. The disease manifests during the first months of life with generalized convulsions or signs of increased neuromuscular excitability such as muscle spasms or tetany. Untreated, the disease may be fatal or lead to severe neurologic damage and mental retardation. Successful and causal treatment comprises mainly administration of Mg$^{2+}$, usually intravenously, followed by life-long high-dose oral treatment. This treatment is effective because most of Mg$^{2+}$ reabsorption in the nephron occurs via a paracellular pathway in the thick ascending limb of the loop of Henle (by contrast, TRPM6 resides in the distal convoluted tubule, the most distal part of the nephron).

The primary defect in HSH is impaired intestinal Mg$^{2+}$ reabsorption that causes a decrease in serum Mg$^{2+}$ levels; this, in turn, seems to lower PTH output by the parathyroid gland. A decrease of both PTH and serum Ca$^{2+}$ levels (secondary hypocalcemia) results in CNS symptoms. Most Trpm6 mutations are loss of function due to truncations of the channel protein by insertion of stop codons. Single point mutations, frame shifts, exon splicing, deletions, and mutations affecting alternative splicing were also described (for reviews, see Woudenberg-Vrenken et al., 2009; Dimke et al., 2011, 2013).

TRPM8 has not yet been associated with any distinct channelopathy. However, the familial amyloidotic polyneuropathies (or familial amyloidotic neuropathies, neuropathic heredofamilial amyloidosis, familial amyloid polyneuropathy) are a rare group of autosomal dominant neuropathies of autonomic and peripheral nerves that might have some genetic relation to TRPM8 (Gasperini and Small, 2012).

D. Ankyrin Transient Receptor Potential Channelopathies

TRPA1 is associated with a pain-causing channelopathy, the autosomal dominant familial episodic pain syndrome. Patients have episodes of debilitating upper body pain, triggered by fasting and physical stress. Moreover, they show enhanced cutaneous flare response and secondary hyperalgesia to punctate stimuli. One point mutation in S4 causes a shift in gating properties of the channel with dramatic increase in inward currents and activation at normal resting potentials. Specific TRPA1 antagonists inhibit the abnormal in vitro response of the mutant TRPA1 channel, implying new therapy for this syndrome (Kremeyer et al., 2010; Wood, 2010).

An SNP causing a point mutation in the TRPA1 N terminus was recently discovered in patients with acute pain and paradoxical heat sensation (Binder et al., 2011). In vitro, this mutant TRPA1 displays higher expression at both cold and heat. However, the mutant is not activated by cold but by heat, probably because of a loss of interaction with associated proteins (May et al., 2012).

E. Mucolipin Transient Receptor Potential Channelopathies

TRPML1 mutations cause ML-IV (OMIM 252650), an autosomal recessive neurodegenerative lysosomal storage disorder characterized by psychomotor retardation and ophthalmologic abnormalities, including corneal opacity, retinal degeneration, and strabismus. In the brain, agenesis of the corpus callosum was described. Blood Fe$^{2+}$ deficiency and achlorhydria also characterize the disease. Over 80% of patients with
ML-IV are Ashkenazi Jews (Slaugenhaupt, 2002; Zeevi et al., 2009; Bach et al., 2010). Over 21, mostly loss-of-function, mutations in the Trpm11 gene have been identified in patients with ML-IV. Channel defects result in impairment of organelle Ca\(^{2+}\) release, which is required for the correct fusion/fission events and causes malfunctions in late endosome–lysosome formation (Lloyd-Evans and Platt, 2011; for a recent review, see Wakabayashi et al., 2011).

Lysosomal storage diseases are caused by the inability of the cells to process the material captured during endocytosis. In ML-IV, lysosomal hydrolases are normal, in contrast with most storage diseases. The disorder results from a defect in transport along the endosomal/lysosomal pathway, affecting membrane sorting and fusion of both endosomes and autophagosomes with lysosomes (for a review, see Dong et al., 2010). Defects in these latter steps of endocytosis and autophagy cause intracellular accumulation of lysosomal substrates. Patients with ML-IV display accumulation of large vacuolar intracellular organelles that are built up because of the defective late endosome/lysosome/phagosome fusion/fission events. They contain amphiphilic lipids (phospholipids, sphingolipids, gangliosides, mucopolysaccharides, lipofuscin, etc.) and other materials from cell organelle debris. A defective function of lysosomes also impairs autophagosome functions (i.e., clearance of the cell interior from toxic proteins and damaged cell organelles) (Vergara-Jauregui and Puertollano, 2008; Micsenyi et al., 2009). Another pathophysiological mechanism is related to the function of TRPML1 as a Fe\(^{2+}\) and Zn\(^{2+}\) channel. Absence of the lysosomal Fe\(^{2+}/\)Zn\(^{2+}\) leak would result in lysosomes overloaded with these heavy metals, leading to the loss of lysosomal function (Kiselyov et al., 2011).

TRPML1-mediated lysosomal Ca\(^{2+}\) release is also dramatically reduced in another lipid-storage disease, the Niemann–Pick type C disease (OMIM 257220), although the main disease-causing effect is due to mutations in the lysosomal two-pore TPC1 channel. Sphingomyelinins normally undergo sphingomyelinase-mediated hydrolysis in the lysosomes of normal cells, but accumulate distinctively in lysosomes of Niemann–Pick type C cells. TRPML1 channel activity is inhibited by sphingomyelins. Thus, abnormal accumulation of luminal lipids causes secondary lysosome storage by blocking TRPML1- and Ca\(^{2+}\)-dependent lysosomal trafficking (Tang et al., 2010a; Shen et al., 2012). TRPML2 and TRPML3 have not yet been reported to cause any human disease, although a TRPML3 mutation in Va mice causes deafness and altered fur pigmentation (Di Palma et al., 2002).

**F. Polycystic Transient Receptor Potential Channelopathies**

TRPP1 mutations cause the ADPKD (OMIM 613095), a disease that manifests in progressive development of large epithelium-lined cysts in the kidney, liver, pancreas, seminal tract, and arachnoid membrane. In the kidney, cysts can be formed in any segment of the nephron and are associated with an increase in the number of cells in the circumference of already dilated renal tubules. These cysts are filled with fluid that is probably secreted by the cyst epithelium. Well-developed cysts occupy much of the mass of the abnormally enlarged kidneys, and thereby compress and destroy normal renal tissue and impair kidney function. ADPKD also causes cardiovascular abnormalities, such as forming of coronary artery aneurysms and intracranial “berry” aneurysms, leading to vessel rupture, internal bleeding, chronic subdural hematomas, and defects of the vessel wall (see Dietrich et al., 2006).

TRPP1 mutations are also related to structural defects in the heart (e.g., defective septum formation) (Wu et al., 2000). PKD1, a glycoprotein with a large N-terminal extracellular region, associates with TRPP1 to form functional channels. Mutations in PKD1 (85% of cases; TRPP1 is only responsible for the remaining 15%) are the major case of ADPKD. More than 400 mutations have been described in PKD1/TRPP1 in patients with ADPKD (Audrézet et al., 2012). As discussed above, TRPP1 associates with PKD1, which is required for its function as a putative flow sensor via the primary cilium (for a review, see Patel and Honoré, 2010). TRPP1 is involved in governing cellular processes via the Janus kinase/signal transducers and activators of transcription, p53, mTOR, NFAT/AP-1 (activator protein-1), cAMP/PKA, cAMP-dependent ERK, CDK, or Wnt signaling pathways, which all may be partially involved in the pathogenesis (for review, see Takiar and Caplan, 2011).

Other functional defects may arise from dissociation of mutant TRPP1 from its submembranous cytoskeletal interaction partners (Chen et al., 2008b). The PKD1/TRPP1 complex regulates the translocation of the helix-loop-helix protein Id2, a crucial regulator of cell proliferation and differentiation, into the nucleus. An enhanced nuclear localization of Id2 in renal epithelial cells from patients with AKPKD constitutes a mechanism for the hyperproliferative phenotype causing cyst formation (Benezra, 2005; Li et al., 2005a). In general, the whole pathogenesis of ADPKD is not well understood (for an excellent review, see Chapin and Caplan, 2010). Because ADPKD is a ciliopathy, an association of TRPP1 with other ciliopathies (e.g., Joubert syndrome, a rare genetic disorder that affects the cerebellum, an area of the brain that controls balance and coordination) was postulated (Garcia-Gonzalo et al., 2011). Another example of TRPP1 involvement is Meckel syndrome (also known as Meckel–Gruber syndrome, or dysencephalia splanchynocystica), which is a rare, lethal, ciliopathic genetic disorder characterized by renal cystic dysplasia, CNS malformations, polydactyly, hepatic developmental defects, and pulmonary hypoplasia (Mason et al., 2011). TRP1 mutations are also involved in the pathogenesis of the situs inversus (i.e., deformation of the left-right body
axis), which is due to a primary ciliary dyskinesia (Bataille et al., 2011; Yoshiba et al., 2012). No diseases have been reported thus far for TRPP2 and TRPP3.

III. Transient Receptor Potential Channels: Acquired Diseases

As discussed above, the link between germ-line TRP gene mutations and human hereditary diseases (the so-called TRP channelopathies) is firmly established (Figs. 14, 15, and 16). Indeed, two TRP channel subfamilies (polycystin and mucolipin) were named after the human diseases (polycystic kidney disease and mucolipidosis, respectively) with which they are associated. Reversing the altered functions of these mutant TRP channels by therapeutic intervention is expected to provide clinical benefits in these genetic disorders. From a drug developmental point of view, diseases caused by gain-of-function mutations in TRP channels could be more amenable to therapeutic targeting since overactivation of channels could be inhibited by small molecule antagonists. By contrast, diseases caused by loss-of-function mutations, particularly truncation types, are difficult to target with small molecules, and a less validated approach such as gene therapy may be required to restore the normal TRP channel function.

In contrast with TRP channelopathies, the role of TRP channels in the development, maintenance, and shaping of acquired disease states is only beginning to be understood. Generally speaking, disease implications arising from animal studies (including phenotypic analysis of knockout mice) need to be carefully and critically evaluated because they do not always mirror human diseases, which tend to involve complicated environmental and genetic factors. In other words, the preclinical model may not properly represent the disease. For example, the TRPV1 antagonist ABT-102 [(R)-(5-tert-butyl-2,3-dihydro-1H-inden-1-yl)-3-(1H-indazol-4-yl)-urea; structure shown in Fig. 9] blocked pain behavior in a rodent model of OA (Honore et al., 2009). However, in a randomized controlled trial, it did not relieve hip arthritic pain in client-owned dogs (Malek et al., 2012). Of note, the phase II clinical trial with the AstraZeneca TRPV1 antagonist AZD1386 (Fig. 9) in patients with OA was prematurely terminated at an interim analysis for lack of analgesic efficacy (Svensson et al., 2010; Miller et al., 2014).

Another confounding factor that complicates the extrapolation of animal studies to patients is species-related differences. For instance, neurogenic inflammation triggered by the release of proinflammatory neuropeptides from primary sensory neurons plays a pivotal role in the ovalbumin model of asthma (see Lundberg, 1995). Indeed, antagonists blocking neurokinin (NK) 1 and 2 receptors ameliorate the symptoms of experimental asthma in the rat or guinea pig (Barnes, 2001), and NK1 receptor (NK1R) blockade even causes weight loss in asthmatic, ovalbumin-obese mice (Ramalho et al., 2013). These antagonists, however, showed little (if any) clinical benefit in patients with asthma (Butler and Heaney, 2007).
is in agreement with the lack of convincing evidence for a clinically meaningful neurogenic inflammatory response in human airways distal to the nasal mucosa (Barnes, 2001).

TRP channels themselves show striking (and, from a drug developmental point of view, quite worrisome) species-related differences in their activation mechanisms. For example, cold activates rodent, but not human or monkey, TRPA1 (Chen et al., 2013a). Furthermore, hTRPA1 acts as a sensor for tissue acidosis, whereas protons, conversely, inhibit rTRPA1 (de la Roche et al., 2013). This might have given false negative results when testing TRPA1 antagonists as potential analgesic agents in rodent models of cancer-induced bone pain, in which the acidic microenvironment is believed to be an important contributor to pain (Lozano-Ondoua et al., 2013).

Alternative splice variants were reported to influence TRP channel activities (in some instances in a tissue-specific manner). For example, the coexpression of TRPA1a and TRPA1b increases current density in response to agonists (Zhou et al., 2013). By contrast, the TRPV1 splice variant VR.5’sv functions as a dominant negative modulator of TRPV1 activity (Eilers et al., 2007). The expression of VR.5’ is reduced during experimental cystitis and it was speculated that this contributes to bladder hyperalgesia (Charrua et al., 2008).

Although increasing numbers of TRP channels are reported to be involved in pathophysiology of diseases, some diseases are endowed with multiple TRP targets. Pain has been one of the mainstreams of TRP research (recently reviewed in Patapoutian et al., 2009; Brederson et al., 2013; Julius, 2013) and multiple TRP channels including TRPV1, TRPV3, TRPM2, TRPM3, TRPM8, and TRPA1 are on the list of potential analgesic targets (Figs. 17, 18, 19, 20, and 21), although, as discussed below, the level of validity differs among them. Respiratory diseases that entail various pathophysiological alternations in airway smooth muscle, immune system, airway vasculature, and vagal sensory nerve are also rich with TRP targets (reviewed in Abbott-Banner et al., 2013).

In addition, multiple TRP channels are implicated in skin (Valdes-Rodriguez et al., 2013) and bladder (Charrua et al., 2010; Avelino et al., 2013) disorders as well as cancer (Prevarskaya et al., 2010; Liberati et al., 2013). Taking these multifaceted and sometimes overlapping roles of TRP channels in a disease into consideration, targeting a single TRP channel might provide an insufficient effect because it may address only limited aspects of the disease. Thus, one may argue that a “dirty” compound that acts on multiple TRP channels may be superior to a selective compound in exerting therapeutic effects.

A. The Role of Transient Receptor Potential Channels in Nociception, Pain, and Itch

1. Basic Concepts. Nociceptors were first described by Charles Scott Sherrington more than a century ago. A nociceptor (from the Latin term nocere, to hurt) is defined as a “pain cell” that is capable of sensing noxious physical (chemical, thermal, and mechanical) stimuli in the periphery and transmitting the pain signal to the CNS. These stimuli produce a sensation of intense pain leading to a protective avoidance reaction and facilitating survival. In a simplistic manner, the nociceptive pathway can be subdivided into three phases: transduction, propagation, and transmission of the signal to the spinal cord. Traditionally, drug discovery efforts largely covered pain targets involved...
inflammation pathways. However, the discovery of the TRPV1 receptor (Caterina et al., 1997), followed by the cloning of temperature-sensitive TRPV1-related channels (thermoTRPs) and the elucidation of their function, brought a great degree of interest in exploring the transduction phase (first step of nociception) in an effort to block pain at its source (reviewed in Patapoutian et al., 2009; Moran et al., 2011; Brederson et al., 2013; Julius, 2013).

In mammals, primary sensory (nociceptive) neurons form an anatomic connection between potentially...
harmful external and internal agents and the CNS. Of note, many non-neuronal cells (e.g., urothelial cells and keratinocytes) also express pain-sensing TRP channels, in particular TRPV1 (Denda et al., 2001; Birder et al., 2002; Southall et al., 2003; Wilder-Smith et al., 2007), TRPV3 (Facer et al., 2007), and TRPV4 (Chung et al., 2003; Gevaert et al., 2007). It has been also suggested (reviewed in Moran et al., 2011) that these cells may also function as unconventional (non-neuronal) pain sensors and communicate with polymodal nociceptive C fibers (Southall et al., 2003; Birder, 2005). Indeed, keratinocytes respond to capsaicin with eicosanoid, particularly LTB4 release (Jain et al., 2011), and mouse DRG neurons coexpress TRPV1 with the leukotriene B4 receptor 1 BLT1 (Andoh and Kuraishi, 2005). Moreover, keratinocyte-specific TRPV3 transgenic mice show augmented PGE2 release in response to heat (Huang et al., 2008). Epidermal TRPV4 was recently reported to orchestrate sunburn pain [and mediate ultraviolet B (UVB)–induced tissue damage], presumably by upregulating the expression of the endogenous algogenic substance, endothelin-1 (Moore et al., 2013). TRPV1-positive sensory afferents express endothelin-1 receptors; indeed, endothelin-1 both stimulates nociceptors and sensitizes them to painful stimuli (Plant et al., 2007) presumably by activating TRPA1 channels (Liang et al., 2010). Thus, it is easy to visualize a positive feedback loop between keratinocytes and sensory nerve endings that promotes neuronal sensitization.

Primary sensory neurons are bipolar cells with somata in DRG and trigeminal ganglia. The central axons of these neurons enter the CNS, where they form synapses with second-order neurons in the dorsal horn of the spinal cord (DRG neurons) or the spinal nucleus of the trigeminal tract (trigeminal ganglion neurons). Many neurons innervating the viscera are located in the nodose ganglia. Their peripheral fibers travel with the vagus nerve, whereas their central axons project to the area postrema (reviewed in Holzer, 1991; Szallasi and Blumberg, 1999). Most primary sensory neurons possess unmyelinated axons (C fibers) and are capsaicin sensitive (Holzer, 1991). A small subset of neurons with thin-myelinated axons (Aδ fibers) also expresses TRPV1 receptors. Interestingly, it has been shown that Aδ fibers that do not normally express TRPV1 do so under inflammatory conditions or after injury (Rashid et al., 2003). This abnormal, TRPV1-positive Aδ fiber population has been suggested to contribute to neuropathic pain in patients with diabetic polyneuropathy (Rashid et al., 2003; Bishnoi and Premkumar, 2013). Indeed, desensitization by TRPV1 agonists (e.g., capsaicin) relieves chronic pain in these patients (reviewed in Knotkova et al., 2008; Szallasi and Sheta, 2012) despite...
the degeneration of C fibers (Lauria et al., 2006). Most recently, TRPV1-expressing Aδ fibers have been implicated in the pathogenesis of breakthrough pain and other types of pain exacerbation conditions such as postsurgical hyperalgesia (Mitchell et al., 2014).

Given the important role of TRPV1 in pain (and the side effects of small molecule TRPV1 antagonists), novel strategies to inhibit this channel are particularly interesting. A novel analgesic strategy is to antagonize the interaction between TRPV1 and AKAP79, a scaffolding protein essential for positioning of serine-threonine A kinases adjacent to target phosphorylation sites (Btesh et al., 2013). Small peptides that prevent this interaction have been identified and shown to be analgesic in mouse models of inflammatory hyperalgesia, highlighting the potential therapeutic value of interfering with the interaction between TRPV1 and AKAP79 (Fischer et al., 2013).

TRP channels play a central role in thermal nociception (Moran et al., 2011; Julius, 2013) and as well as in the detection of noxious chemicals (Nilius et al., 2007b; Kumar et al., 2013). This is interesting biology but, per se, it would not make these channels potential targets for analgesic drugs. Importantly, TRPV1 is also activated and/or sensitized by agents in “inflammatory soup” (Figs. 12 and 21), ranging from tissue acidosis (protons) through cytokines, NGF, BK, 12-HPETE, and 15-HPETE and other arachidonic acid metabolites (reviewed in Caterina and Julius, 2001; Pingle et al., 2007; Szallasi et al., 2007). These agents act in concert to lower the heat activation threshold of TRPV1 (Di Marzo et al., 2002; Szallasi et al., 2007; Lázár et al., 2009). The fractional Ca\(^{2+}\) current via TRPV1, however, depends on the mode of channel activation. For example, it is significantly smaller for proton activation compared with capsaicin (Samways et al., 2008). This is important because [Ca\(^{2+}\)]\(_i\) is thought to play a pivotal role in the phenomenon of capsaicin desensitization (see Szallasi and Blumberg, 1999).

As reviewed elsewhere, TRPV1 is a promising target to relieve inflammatory pain (see Szallasi et al., 2007; Lázár et al., 2009; Szolcsányi and Sándor, 2012; Brederson et al., 2013; Szolcsányi and Pintér, 2013). Indeed, both genetic deletion (Caterina et al., 2000; Davis et al., 2000) and pharmacological blockade of TRPV1 ameliorate heat hyperalgesia in rodent models of inflammatory pain (reviewed in Gunthorpe and Szallasi, 2008; Gunthorpe and Chizh, 2009). Of relevance is the finding that TRPV1 expression is increased in reflux esophagitis [or gastroesophageal reflux disease (GERD)], in which “heartburn” is due to exposure to regurgitated acidic gastric contents (Matthews et al., 2004; Bhat and Bielefeldt, 2006). Yet, disappointing, in randomized clinical trials, the TRPV1 antagonist AZD1386 (see Fig. 9 for structure) provided no symptomatic pain relief in patients with GERD (Krarup et al., 2011, 2013).

TRPV1 is also elevated in inflammatory bowel disease (IBD) and irritable bowel syndrome (also known as colon irritabile), a fairly common condition of unknown etiology characterized by frequent bowel movements and tenesmus (painful straining at stool) (Yiangou et al., 2001; Chan et al., 2003). Because there is no effective medical therapy, irritable bowel syndrome is frustrating for both patients and their physicians. Therefore, it is an
exciting possibility that per os TRPV1 antagonists may provide symptomatic relief. Indeed, there is anecdotal evidence that eating hot spicy food exacerbates symptoms in patients with irritable bowel syndrome (see Szallasi and Blumberg, 1999).

**a. Differential transient receptor potential channel expression defines functional sensory neuron subtypes: Implications for drug development.** Primary sensory neurons are heterogenous in several aspects, including their anatomy, neurochemistry, and function. For example, these neurons differ in the myelin sheet that protects their axons (myelinated Aβ-, thin-myelinated Aδ-, and unmyelinated C fibers), they use different mediators (e.g., peptidergic and nonpeptidergic), and they convey different somatosensory information to the CNS (e.g., touch, pain, itch, and temperature). One way to subclassify primary sensory neurons is by the TRP channels that they express. A major population of neurons with C fibers, as well as a minor subset of Aδ neurons, coexpresses TRPV1 with the related channels TRPV3 and TRPV4 and also with TRPA1 (Figs. 17, 20, and 21) (Kobayashi et al., 2005). TRPV1, TRPV3, and TRPV4 are heat-activated channels, so their presence on the same neurons is not unexpected. It is more difficult to explain why these heat-responsive receptors are coexpressed with the "cold receptor" TRPA1. Of note, TRPA1 was reported to be heat activated in some studies (Hoffmann et al., 2013). Adding to the complexity, TRPA1 seems to be present on both peptidergic and nonpeptidergic neurons (Hjerling-Leffler et al., 2007).

A second major subset of primary sensory neurons, encompassing both A fiber and C fiber neurons, is characterized by their TRPM8 expression (Figs. 17 and 21) (Kobayashi et al., 2005). The minimal overlap between TRPV1 and TRPM8 expression suggests that TRPV1-positive neurons and TRPM8-expressing neurons are fundamentally different, although TRPA1 appears to be present on both TRPV1- and TRPM8-expressing populations (Kobayashi et al., 2005). In keeping with this concept, TRPV1-like immunoreactivity is elevated, whereas TRPM8 is, by contrast, reduced in injured human brachial plexus nerves (Facer et al., 2007). On the basis of these findings, it has been suggested that TRPV1 may be a more relevant therapeutic target than other thermoTRPs for pain related to posttraumatic neuropathy (Facer et al., 2007). Intriguingly, and in contrast with expression in the DRG, TRPM8 is coexpressed with TRPV1 in vagal sensory neurons innervating the mouse lung (Nassenstein et al., 2008).

As discussed above, TRPV1 can form functional heteromultimers with other TRP channels. Therefore, antagonists that do not distinguish between thermoTRPs may have a therapeutic value by targeting TRP heteromultimers. The shared TRP domain in these channels may represent a target for such inhibitors (García-Sanz et al., 2007). Of note, BCTC (Fig. 9), originally described as a TRPV1 antagonist, also functions as a potent inhibitor of TRPV4 and TRPM8 channels (Weil et al., 2005). Clearly, studies using BCTC as a selective TRPV1 blocker need to be carefully reevaluated (the same applies to capsazepine, another "dirty" TRPV1 inhibitor, widely used in the past to dissect TRPV1-mediated responses).

Maybe because they are ubiquitously expressed, the presence of TRPC channels in sensory neurons has attracted little attention (Elg et al., 2007). In adult mice, all seven members of the TRPC family (TRPC1–TRPC7) were detected in DRG neurons with TRPC1, TRPC3, and TRPC6 being the most abundant. Of note, TRPC3 was exclusively expressed by nonpeptidergic, TRPV1-negative neurons. TRPC3 was recently identified as a key molecular target for the excitatory effect of the IgG immune complex in rat DRG neurons (Qu et al., 2012). As discussed later, TRPC3 (in concert with TRPA1) may also be a target for nonhistaminergic itch

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Fig. 20. Schematic diagram showing the involvement of TRPA1 channels in nociception, neurogenic inflammation, and mechanical hyperalgesia. The magnified insert on the top illustrates the hypothetical role on TRPA1 in the development and maintenance of central sensitization. According to this model, endogenous TRPA1 agonists such as hepoxilin A3 (HXA3), 5,6-epoxyeicosatrienoic acid (5,6-EET), and ROS facilitate NMDA responses and thus amplify pain signaling in the spinal cord. Spinal TRPA1 activation also facilitates a process in which innocuous mechanical stimuli (from piezo2-expressing low-threshold afferents) are aberrantly transmitted to lamina 1 projection neurons and thus become painful. This mechanism is believed to play a role in mechanical allodynia. Reproduced with permission from Koivisto et al. (2014).
in sensory afferents (Than et al., 2013). Furthermore, it was suggested that TRPC1 and TRPC6 may cooperate with TRPV4 in mediating mechanical hyperalgesia (Alessandri-Haber et al., 2009). Indeed, in rat DRG neurons, the expression of TRPC4 is increased after nerve injury (Wu et al., 2008), possibly as a regenerative reaction promoting neurite growth. Furthermore, knockdown by short hairpin RNA (shRNA) of TRPC1 reduces mechanosensitivity of mouse DRG neurons (Staaf et al., 2009). Finally, rats with transposon-mediated TRPC4-knockout mutation showed ameliorated visceral pain reaction in response to colonic mustard oil exposure (Westlund et al., 2014).

Of note, thermoTRPs are also colocalized with other receptors involved in pain transmission. In an innovative study, capsaicin has been used to deliver sodium channel blockers into neurons expressing TRPV1 (Binshtok et al., 2007). QX-314 \[N-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium bromide\] is quaternary derivative of lidocaine that is ineffective when administered alone because it is not capable of crossing the membrane. However, when coadministered with capsaicin, QX-314 enters the sensory neuron through the open TRPV1 pore and gains access to its binding site on the sodium channel (Binshtok et al., 2007). This elegant approach affords selective targeting of TRPV1-expressing sensory neurons (Binshtok et al., 2007, 2009). This approach was recently further refined by identifying such TRPV1 agonists (e.g., Cap-ET) that recognize and selectively target already sensitized TRPV1 channels in inflamed tissues and augment the uptake of QX-314 (Li et al., 2011a). It is hoped that this innovative approach may tame hyperactive TRPV1 in diseased tissues and spare normal nociception. Of note, in combination with bupivacaine, QX-314 can be also used to selectively block TRPA1-expressing neurons (Brenneis et al., 2014). Indeed, as discussed later, QX-314 was used to silence neuronal pathways conducting itch (Roberson et al., 2013).

**b. Disease-related changes in transient receptor potential channel expression.** TRP channels not only show bidirectional changes during disease states (upregulation or downregulation), but can be also expressed in cells that do not normally express such channels (reviewed in Szallasi et al., 2007). These observations have important practical implications for drug development.
For example, animal experiments suggest that TRPM8 may be a relevant target to ameliorate cold hyperalgesia that develops after nerve injury (Katsura et al., 2006; Xing et al., 2007; Ji et al., 2008). In support of this hypothesis, mRNA encoding TRPM8 is increased in the rat DRG after chronic constriction injury (CCI) (Frederick et al., 2007). However, in humans (unlike in rodents), TRPM8 appears to be downregulated after nerve injury (Facer et al., 2007) and in painful dental pulp (Alvarado et al., 2007). Indeed, no evidence for the involvement of TRPM8 in cold allodynia has been found in patients with neuropathic pain (Namer et al., 2008). This is another worrisome example of the species-related differences in TRP channel biology that hinder extrapolation of animal experiments to patients.

By contrast, TRPA1 appears to be upregulated in human DRG after nerve injury (Anand et al., 2008). In rats, antisense knockdown of TRPA1 alleviates cold hyperalgesia after spinal nerve ligation (Katsura et al., 2006). However, the relevance of these observations is unclear since cold allodynia appears to be independent of TRPA1 in patients with neuropathic pain (Namer et al., 2008).

In rodents, the expression of TRPV4 is increased at both the mRNA and protein levels after mechanical nerve injury, induced by long-term compression of DRG (Zhang et al., 2008f). As detailed below, TRPV4 has also been linked to chemotherapy (e.g., taxol or vincristine)–induced neuropathy (Alessandri-Haber et al., 2004, 2008). When given intrathecally, TRPV4 oligodeoxynucleotide antisense reverses mechanical allodynia induced by long-term compression of DRG (Zhang et al., 2008f) and ameliorates mechanical hyperalgesia in animal models of neuropathy of various etiologies such as diabetes, alcoholism, and chemotherapy (Alessandri-Haber et al., 2008). TRPV4 appears to be also involved in inflammatory pain, as implied by the reduced response to inflammatory soup in Trpv4–/– mice (Chen et al., 2007). This finding is consistent with the role of TRPV4 as an osmosensor and the hypotonic nature of the inflammatory soup. The relevance of these observations is, however, questioned by the apparent lack of painful phenotype within the broad spectrum of disease-associated TRPV4 channelopathies, including both gain-of-function and loss-of-function TRPV4 mutations (Nilius and Voets, 2013).

TRPV1 shows bidirectional expression changes in various disease states (see Szallasi, 1996; Szallasi et al., 2007). TRPV1 expression is elevated after spinal cord injury (Wu et al., 2013). During inflammation and in bone cancer, TRPV1 levels also substantially increase (Niiyama et al., 2007). Conversely, TRPV1 expression is downregulated in neuropathic pain secondary to injury (Lauria et al., 2006) or after RTX treatment (Szallasi et al., 1999). In a murine model of diabetic neuropathy, the early thermal hyperalgesia was associated with increased TRPV1 expression, whereas the late thermal hypoalgesia paralleled the loss of TRPV1-like immunoreactivity (Pabbidi et al., 2008). The molecular drivers of these changes are unknown but it has been hypothesized that the downregulation of TRPV1 expression in diabetic skin is related to the diminished NGF levels (Facer et al., 2007). As reviewed elsewhere (Knotkova et al., 2008), diabetic neuropathy is a traditional indication for capsaicin-containing topical preparations. However, the clinical experience with capsaicin is conflicting, with some studies reporting significant pain relief, whereas others have been unable to replicate these results (see Knotkova et al., 2008). Of note, in addition to its interaction with TRPV1 receptors, capsaicin also exerts a direct effect on mitochondria (reviewed in Szallasi and Blumberg, 1999), which may play an important role in the development and maintenance of local analgesia (desensitization or, more correctly, defunctionalization) in the treated area (Fig. 22). How capsaicin is applied to the skin may have a significant impact on this mitochondrial effect. In the skin of patients with diabetic neuropathy, TRPV1 expressing epidermal nerve fibers are markedly reduced (Bley, 2013), accompanied by decreased TRPV3 expression in keratinocytes (Facer et al., 2007). This is in agreement with the loss of TRPV1-like immunoreactivity during the late stage of experimental diabetic neuropathy (Pabbidi et al., 2008). If so, TRPV1 blockade may have a therapeutic value in early diabetic polyneuropathy (when TRPV1 is enhanced) but not in established diabetic polyneuropathy (when TRPV1 is downregulated).

Although long-term morphine administration is strictly speaking not a disease, it should be mentioned here that long-term morphine administration upregulates TRPV1 expression in the spinal cord in a mitogen-activated protein kinase–dependent manner (Chen et al., 2008c). This is intriguing because morphine tolerance is often associated with the development of thermal hyperalgesia. In fact, intrathecal pretreatment with the TRPV1 antagonist SB366791 [N-(3-methoxyphenyl)-4-chlorocinnamamide] has been shown to attenuate morphine tolerance and to prevent thermal hyperalgesia (Chen et al., 2008c). It is hoped that TRPV1 antagonists will reduce the need for opioids and, as an added benefit, also prevent tolerance to opioids. Interestingly, acute morphine administration has the opposite effect since it negatively modulates TRPV1 via inhibition of adenylate cyclase (Vetter et al., 2006). Withdrawal symptoms in patients addicted to opiates, by contrast, may include cold hyperalgesia, which appears to be mediated by TRPM8 (Shapovalov et al., 2013). Indeed, activation of μ-opioid receptors induces internalization of TRPM8 with a rebound after naloxone treatment. These “cold turkey” patients may be helped to overcome their cold sensitivity by TRPM8 antagonists. Of note, the painful adverse effect of apomorphine (a nonnarcotic morphine
derivative used in the pharmacotherapy of patients with Parkinson disease) involves TRPA1 activation (Schulze et al., 2013).

2. Transient Receptor Potential Channels in Dental Pain. The tooth is a unique tissue in that it is subject to extreme changes in temperature from ice cold to burning hot, depending on the food or drink consumed. In contrast with other tissues, noxious hot or cold temperatures do not evoke nociception in the teeth under normal circumstances due to the thermal insulating capacity of enamel, the outer protective shell of the tooth (reviewed in Chung and Oh, 2013; Chung et al., 2013). However, when the enamel is decayed and dentin is exposed (e.g., due to caries or trauma), small changes in temperature and/or light touch can evoke sudden and intense toothache.

Three complementary, rather than mutually exclusive, hypotheses have been put forward to explain the generation of dental pain, all potentially involving TRP channels (summarized in Fig. 23; reviewed in Chung and Oh, 2013): these are the neuronal, hydrodynamic (Fig. 24A) and odontoblast transducer (Fig. 24B) theories of dental nociception. The neuronal theory postulates the transduction of noxious temperatures by nerves innervating the dentin and pulp that express thermoTRPs. Indeed, the tooth pulp is densely innervated by primary sensory neurons with somata in trigeminal ganglia. The majority (70%–90%) of intrapulpal axons are unmyelinated C fibers which may conduct dull, drawing dental pain. The remaining myelinated intrapulpal fibers are mostly Aδ fibers that are responsible for the rapid, sharp, lancinating, well localized nociception (Abd-Elmeguid and Yu, 2009).

To elucidate the role of thermoTRPs in dental nociception, retrograde labeling with a fluorescent dye was used to identify dental primary afferent neurons. A combination of molecular [single-cell reverse-transcription polymerase chain reaction (RT-PCR)], immunohistochemical, and functional (patch clamping) studies revealed that most (up to 85%) trigeminal ganglion neurons innervating
the tooth pulp express TRPV1 (Park et al., 2006; Kim et al., 2011b). Importantly, these neurons respond to noxious heat (and also capsaicin) with elevated intracellular calcium levels (Chaudhary et al., 2001). Of note, TRPV1 expression was reported to be upregulated in trigeminal ganglion neurons during LPS-induced experimental pulpitis (Chung et al., 2011). This increased TRPV1 expression might contribute to the thermal hyperalgesia in patients with chronic pulpitis. In support of this hypothesis, desensitization of TRPV1-expressing sensory neurons to RTX reduced bone loss and suppressed the generation of proinflammatory cytokines in periodontitis-susceptible Fischer 344 rats, a rodent model of human periodontal disease (Breivik et al., 2011).

TRPV2 is predominantly expressed by medium to large-size dental afferent neurons in culture (Ichikawa and Sugimoto, 2000). Interestingly, the expression level of TRPV2 is higher in labeled dental afferent neurons (37%) than in unlabeled trigeminal ganglion neurons (14%). Indeed, half of the neurons that innervate the periodontal ligament express TRPV2 (Gibbs et al., 2011).

Molecular and immunocytochemical studies also revealed the presence of cold-responsive TRPA1 and TRPM8 channels in retrogradely labeled rat dental afferent neurons (Park et al., 2006). Decayed tooth is usually more sensitive to cold than heat; thus, the expression prevalence of both TRPM8 and TRPA1 in dental afferents was unexpectedly lower than that of TRPV1. However, TRPA1 expression is increased in trigeminal ganglion neurons after injury applied to rodent teeth (Haas et al., 2011). Some of the dental afferents appeared to coexpress TRPM8 and/or TRPA1 with TRPV1 (Park et al., 2006). If this controversial observation holds true, such neurons may respond to temperature changes in either direction (warm or cold) with nerve impulses. Indeed, TRPA1 was reported to respond to both heat and cold (Hoffmann et al., 2013).

A sensory role for odontoblasts has also been postulated (see Chung and Oh, 2013). Odontoblasts form the outermost cell layer between the dentin and the tooth pulp. The main function of odontoblasts is to deposit a mineralized Ca\(^{2+}\) matrix, which, in turn, is converted into dentin during tooth development and repair. Whereas it is generally accepted that odontoblasts may double as cellular sensors [in support of this hypothesis, they express both voltage-gated Na\(^+\) channels (Allard et al., 2006) and store-operated Ca\(^{2+}\) channels (Shibukawa and Suzuki, 2003)], the expression of thermoTRP channels in odontoblasts (and their role in toothache, the so-called odontoblast transducer theory of dental pain; Fig. 24B) remains controversial. For example, odontoblasts obtained by in vitro differentiation of neonatal rat pulpal cells were reported to express a variety of TRP channels including TRPV1, TRPV2, TRPV3, and TRPV4, along with TRPM3 (Son et al., 2009). Similarly, in human odontoblasts differentiated in vitro from the pulp of extracted molar teeth, expression of TRPV1, TRPA1, and TRPM8 was described (El Karim et al., 2011). By contrast, odontoblasts isolated short term from adult rat incisors showed no evidence of TRPV1, TRPV2, TRPV3, and/or TRPM8 expression (Yeon et al., 2009). To resolve these discrepant results, one might argue that either the actual odontoblasts are different from those obtained via in vitro differentiation or that the procedure of short-term isolation itself eliminates the odontoblasts that express TRPV1 or TRPV2. Finally,
the discrepancy may simply reflect developmental differences between adult and neonatal rats. For instance, TRPV1 appears to be widely expressed in neonatal (Ritter and Dinh, 1992), but not adult (Cavanaugh et al., 2011), mouse brain. Furthermore, TRPC1, TRPC3, and TRPC6 are highly expressed in adult, but not neonatal, rodent DRG neurons; by contrast, TRPC2 and TRPC5 show an age-dependent decline in expression levels (Elg et al., 2007).

Although thermoTRP channels expressed on dental afferents may explain the heat sensitivity of decayed teeth, temperature transduction alone cannot account for the sudden and intense dental pain induced by normally innocuous stimuli such as exposure to air puffs and water spray or consumption of sweets. Furthermore, patients with chronic pulpitis describe a pulsating pain sensation in response to hydrostatic pressure (Heyeraas and Berggren, 1999). To explain the mechanical allodynia in patients with toothache, the hydrodynamic theory of dental pain (Fig. 24A) was formulated (Brannstrom, 1986; Lin et al., 2011). According to this hypothesis, the movement of dentinal fluid (which is restricted when both ends of the dentinal tubule are closed by the pulp and enamel layer) gets exaggerated when dentin is exposed by dental caries or tooth crack (see Fig. 24A).

Of TRP channels that exhibit mechanosensitivity and thus may respond to dentinal fluid movement, the expression of TRPV1 (Chaudhary et al., 2001; Park et al., 2006), TRPV2 (Ichikawa and Sugimoto, 2000; Gibbs et al., 2011), and TRPA1 (Park et al., 2006) has been demonstrated in retrogradely labeled dental afferent nerves. Furthermore, the expression of TRPV4 (Wei et al., 2011b) and TRPM3 (Vriens et al., 2011) was reported in trigeminal ganglion neurons. With regard to toothache, TRPA1 is of particular interest because of the broad range of stimuli to which it responds (chemical, cold, and mechanical), including bacterial endotoxins (Meseguer et al., 2014). Of note, TRPA1 is found at the tip of the inner hear hair bundles, where it was suggested to play a crucial role in detecting fluid movements (Corey et al., 2004). Thus, it is a reasonable assumption that TRPA1 may play a similar role in detecting fluid movements within dentinal tubules. However, the role of TRPA1 in mechanotransduction remains controversial because TRPA1-null mice showed normal response to loud noise (Kwan et al., 2006); therefore, the involvement of TRPA1 in the generation of dental pain by mechanical stimuli needs to be verified.

Dentin formed before the tooth eruption is called primary dentin. However, dentin formation continues throughout the lifetime with (secondary dentin) or without (tertiary dentin) periodontal disease. The mechanism underlying ongoing dentin formation is unclear. It was suggested that human dental pulp stem cells may differentiate into odontoblastic cells in response to hydrodynamic pressure (Yu et al., 2009a). If so, mechanosensitive TRP channels expressed in odontoblasts might play an important role in this process.

In summary, the contribution of TRP channels to gum disease and dental pain is most likely complex and still poorly understood. According to the US Centers for Disease Control and Prevention, more than half of the adult population in the United States suffers from gum disease (gingivitis, periodontitis). Desensitization to RTX suppresses inflammation and resultant bone loss in a rodent model of periodontal disease (Breivik et al., 2011). Furthermore, the TRPV1 antagonist AZD1386 relieves acute dental pain after molar extraction in humans (Quiding et al., 2013). These observations suggest that TRPV1 (and maybe also related thermoTRPs) is a viable therapeutic target in patients with periodontal disease; for example, it can be exploited by topical RTX (applied to the gingiva) or TRPV1 antagonist treatment.

3. Transient Receptor Potential Channels in Visceral Pain. With up to 10% of the general population affected, chronic pain and discomfort arising from the viscera represent a large unmet medical need. A hallmark of chronic visceral pain is mechanical hyperalgesia and allodynia (perceived as colicky pain) in response to peristaltic movements and/or distension that may be secondary to (or at least exacerbated by) inflammatory disorders. Thus, compounds that simultaneously block mechanosensory transduction and inflammation may prove particularly useful in relieving visceral pain. There is good evidence that TRP channels are involved in both of these processes (Fig. 25; see Holzer, 2004; Blackshaw et al., 2010, 2013; Holzer, 2011a,b).

a. Esophagus and gastrointestinal tract. Sensory afferent nerve endings in the mucosa of the gastrointestinal tract respond to chemical or fine tactile stimulation of the epithelium, whereas those innervating the muscularis
and the serosa mostly function as mechanosensitive tension receptors (see Brierley et al., 2004; Holzer, 2011a). The majority of sensory fibers (e.g., > 80% of splanchnic afferents originating in thoracolumbar DRG) that project into the visceral mucosa possess TRPV1 (see Robinson and Gebhart, 2008) and convey sensation of bloating, discomfort, and pain to the CNS (Chen et al., 2013b). Indeed, capsaicin administered topically into the colon evokes intense pain and referred hyperalgesia (Laird et al., 2001).

A major subset (40%–60%) of vagal afferents arising from nodose ganglia is TRPV1 positive and is typically associated with satiety and nausea (Patterson et al., 2003). In accord, desensitization of these nerves to RTX exerts a marked antiemetic effect in different species, including dogs and ferrets (Yamakuni et al., 2002). Furthermore, mice fed capsaicin-containing chow gain less weight compared with littermates on a regular diet (Zhang et al., 2007b; Leung, 2008). In addition, there is anecdotal evidence that human capsaicin consumption leads to early satiety and weight loss (see Whiting et al., 2012). On the basis of these observations, the use of dietary capsaicin to boost weight loss as part of a comprehensive weight management program was advocated. The phenotype of the Trpv1 knockout mouse is, however, controversial. Early reports indicated no difference in body weight between Trpv1 knockout and wild-type mice (Caterina et al., 1997). However, when kept on a high-fat diet, the TRPV1-null animals stayed lean compared with control subjects and showed less fatty change in the liver (Motter and Ahern, 2008). By contrast, another study found increased obesity (presumably secondary to less physical activity) in aged Trpv1 knockout mice (Wanner et al., 2011). Apparently, both age and diet influence the phenotype of the TRPV1-null mouse.

The finding that TRPV1 is directly activated by low pH (Fig. 4) makes this channel a prime candidate for sensing heartburn, caused by the reflux of acidic stomach contents into the gastroesophageal junction (reflux esophagitis, also known as GERD). Indeed, TRPV1 is present on afferents innervating the mucosa of the human esophagus (Matthews et al., 2004; Bhat and Bielefeldt, 2006), where its expression is increased in mucosal biopsies taken from patients with GERD. Mice whose TRPV1 has been deleted by genetic manipulation develop less esophagitis after acid exposure compared with wild-type control subjects (Fujino et al., 2006). This is important because persistent reflux esophagitis is believed to play a pivotal role in the development of intestinal metaplasia (Barrett esophagus), a premalignant condition. Furthermore, desensitization to RTX of gastric mucosal sensory afferents was shown to protect against ulcer formation in response to acids (Szolcsányi, 1990). These observations formed the experimental foundation of the clinical trials with TRPV1 antagonists in patients with GERD. It should be kept in mind, however, that TRPV1 is a major, but by no means exclusive, acid sensor in the gastrointestinal tract. Indeed, proton-induced responses were reduced (by approximately 50%), but not abolished, in Trpv1 knockout animals (Kichko and Reeh, 2009). Thus, the minimal benefit of TRPV1 antagonism in patients with GERD (ClinicalTrials.gov identifier D9127C00002) was hardly unexpected (Kraru et al., 2013). Parenthetically, the first-generation TRPV1 antagonist capsazepine was reported to paradoxically aggravate acid-induced gastric ulcer formation in the rat (Horie et al., 2004). If this observation holds true for humans, it may represent another problematic adverse effect for per os TRPV1 antagonists.

TRPV1-positive nerves appear to mediate visceral pain in response to noxious rectal distension (Spencer et al., 2008). This is somewhat surprising since TRPV1 is not supposed to have mechanosensitive properties. Indeed, genetic deletion of TRPV1 has no significant effect on the mechanosensory function of somatic nociceptors (Caterina et al., 2000). For unclear reasons, visceral TRPV1-expressing afferents seem to function differently. For example, TRPV1-null animals show deficits in visceral mechanoreceptors, and their behavioral responses to colorectal distension are markedly reduced (Jones et al., 2005a). Of note, Trpv1(−/−) mice exhibit decreased mechanical hyper-reactivity of the bladder during cystitis (Wang et al., 2008c), implicating a role for TRPV1 in visceral pain associated with inflammatory disorders. Silencing by RNA interference of TRPV1 has been reported to ameliorate visceral pain in rats (Christoph et al., 2006). Moreover, the first-generation TRPV1 antagonist, capsazepine, diminished discomfort to colorectal distension in mice (Sugiyura et al., 2007), similar to the decrease seen in TRPV1-null animals (Jones et al., 2005a). Interestingly, TRPV1 and TRPA1 antagonists synergistically attenuate caerulein-induced pain behavior and pancreatic inflammation (Schwartz et al., 2011; Terada et al., 2013) and prevent the transition from acute to chronic pain during experimental pancreatitis (Schwartz et al., 2013); after this transition, TRP antagonism is ineffective. Alcohol abuse is a well known cause of acute pancreatitis. In this context, it is worth mentioning that ethanol is capable of activating TRPV1 (Trevisani et al., 2002) and ethanol-induced acute pancreatitis is attenuated in TRPV1-null mice (Vigna et al., 2014). These findings argue for early intervention by TRPV1 (and also TRPA1) antagonists.

Increased TRPV1-immunoreactivity was observed in colonic sensory afferents in patients with IBD, both Crohn disease and ulcerative colitis (Yangou et al., 2001), and in rectal sensory fibers with rectal hypersensitivity and fecal urgency (Chan et al., 2003). It is currently unclear whether these changes in TRPV1 expression are pathogenic or adaptive. In a rat model
of irritable bowel syndrome, TRPV1 antagonists prevent the development of visceral hypersensitivity initiated by acetic acid treatment during the neonatal period (Winston et al., 2007). These findings imply a pathogenic role for the dysfunction of TRPV1-positive colonic fibers in irritable bowel syndrome (Keszthelyi et al., 2013). In accord, one study reported a positive correlation between the number of TRPV1-immunoreactive fibers in the rectosigmoid colon and the abdominal pain score in patients with irritable bowel syndrome (Akbar et al., 2008). A second study also noted increased pain perception to rectal capsaicin application in patients with irritable bowel syndrome but no evidence of TRPV1 upregulation (van Wanrooij et al., 2014). Taken together, these observations suggest that TRPV1 is a relevant therapeutic target for treatment of visceral pain. TRPV1 may even mediate (at least in part) the well-documented beneficial effect of cannabinoids on visceral pain (De Petrocellis et al., 2012).

In a rat model of IBD, topical capsaicin treatment reduces bowel ulceration in response to trinitrobenzene sulfonic acid (TNBS) (Goso et al., 1993a). In this model, the small molecule TRPV1 antagonist JYL1421 [N-(4-tertbutylbenzyl)-N-[3-fluoro-4-(methylsulfonylamino) benzyl]-thiourea] suppressed microscopic colitis and significantly reduced (but did not completely abolish) visceromotor response to colorectal distension (Miranda et al., 2007). TRPV1 also appears to be involved in the postinflammatory hyperalgesia that occurs after resolution of dextran sodium sulfate–induced experimental colitis (Eijkelkamp et al., 2007). Nonetheless, it may be a premature conclusion that TRPV1 is exclusively responsible for the beneficial effect of capsaicin desensitization in preclinical models of colitis. Indeed, in a murine model of visceral pain TRPV1-null mice showed only partial (approximately 60%) reduction in pain response magnitude compared with wild-type control subjects (Jones et al., 2005a).

What is responsible for the remaining 40% of the pain behavior? The amiloride-sensitive acid-sensing ion channels may be a major contributor (Sugiura et al., 2007). Indeed, ASIC(−/−) mice display a reduction in pain behavior that is similar in magnitude to that observed in the TRPV1 knockouts (Jones et al., 2005a). Furthermore, it has been shown that TRPA1 (presumably present on TRPV1-positive fibers) is markedly upregulated during TNBS-evoked colitis (Yang et al., 2008). Consistent with a pathogenic role of the increased TRPA1 expression, both intrathecal administration of TRPA1 antisense oligodeoxynucleotide (Yang et al., 2008) and intraperitoneal blockade of TRPA1 by the small molecule antagonist TCS-5861528 (Vermeulen et al., 2013) was reported to reverse hyperalgesia to colonic distension. Furthermore, colitis induced by TNBS is markedly attenuated in TRPA1-null mice (Engel et al., 2011a,b). Importantly, the analgesic activity of TRPV1 and TRPA1 antagonism was synergistic (Vermeulen et al., 2013), implying a therapeutic potential for a dual TRPV1/TRPA1 antagonist in the pharmacotherapy of IBD. In further support of this concept, TRPV1 blockade ameliorated early, inflammatory pain, whereas TRPA1 blockade suppressed sensitization leading to chronic visceral pain in a rat model of ulcerative colitis (Chen et al., 2013b). Analysis of TRPA1 mutants revealed that TNBS covalently binds to cysteine residues in the TRPA1 protein in the cytoplasmic N terminus (Engel et al., 2011a). Of note, peripheral blood mononuclear cell supernatants obtained from patients with diarrhea-predominant IBD, but not constipation-predominant patients or healthy individuals, caused mechanical hypersensitivity of mouse colonic afferents that was partially inhibited by TRPA1 antagonists (Hughes et al., 2013).

In the rat, TRPA1 (via activation of p38 mitogen-activated protein kinase) seems to play an important role in gastric distension-induced visceral pain (Kondo et al., 2013), raising the possibility that TRPA1 may be involved in the development of epigastric pain in patients with functional dyspepsia (FD). This hypothesis was recently tested in a rat model of FD. Adult rats subjected to inflammatory insult by intracolonic TNBS administration as neonates were reported to develop FD-like gastric hypersensitivity with no apparent involvement of TRPA1 (Winston and Sarna, 2013). In this context, it is worth mentioning that a combination of caraway and peppermint oil attenuates postinflammatory visceral hyperalgesia in rats (Harrington et al., 2011). Caraway oil is used as a dietary supplement for digestive problems, including bloating and mild spasms of the stomach and intestines. In a multicenter, double-blind clinical study, the combination of peppermint and caraway oil was reported to provide symptomatic relief in patients with FD (Madisch et al., 1999). Peppermint oil (and its active ingredient, menthol) is a known TRPM8 agonist. Indeed, TRPM8 is expressed on a distinct subset of colonic afferents with a possible (and rather controversial) coexpression of TRPV1 (Harrington et al., 2011). This is interesting because dietary capsaicin was reported to cause visceral pain in patients with FD. It was postulated that TRPM8 activation may interfere with nociceptive transduction via TRPV1 and TRPA1 (Blackshaw et al., 2010). Taken together, these observations imply a role for TRPV1, TRPA1, and TRPM8 in the pathomechanism of FD; however, as yet, no unequivocal evidence has been presented to support this concept.

H2S is an irritant colorless gas with offensive odor, which is overproduced in the feces of patients with ulcerative colitis presumably as a metabolic aberration due to altered colonic bacterial flora. H2S was recently shown to function as a gasotransmitter acting on...
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various channels, including TRPA1 (Miyamoto et al., 2011). Intraluminal colonic H$_2$S evokes visceral nociceptive behavior in the mouse (Matsunami et al., 2009). TRPA1 is a well established target for the somatic pronociceptive actions of H$_2$S. Indeed, the H$_2$S-donor NaHS stimulates DRG neurons obtained from wild-type, but not Trpa1$^{-/-}$, mice (Andersson et al., 2012), and NaHS-induced mechanical hyperalgesia was significantly suppressed (although not completely eliminated) by both pharmacological blockade with AP18 [4-(4-chlorophenyl)-3-methyl-3-buten-2-one oxime; Fig. 10] and genetic silencing of TRPA1 (Okubo et al., 2012). The role of TRPA1 in H$_2$S-induced visceral pain, however, remains controversial, with one group finding similar pain behavior in wild-type and Trpa1 knockout mice (Andersson et al., 2012) and another group reporting the involvement of both TRPA1 and Ca$_2$-3.2 channels in H$_2$S-induced colonic pain and referred hyperalgesia (Tsubota-Matsunami et al., 2012).

Another gasotransmitter in the gastrointestinal tract is NO. In behavioral assays, peripheral NO-evoked nociception is absent when both TRPV1 and TRPA1 are ablated such as in TRPV1/Trpa1 double-knockout mice (Miyamoto et al., 2009). As was the case for H$_2$S, the participation of TRPA1 in NO-induced visceral pain is unclear. Of note, in the bladder, the TRPA1 antagonist HC-030031 [2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4-isopropylphenyl)acetamide; Fig. 10] failed to inhibit the pain reaction induced by ifosfamide (an NO donor) (Okubo et al., 2013).

An intriguing, newly identified target for NO in the gastrointestinal tract is TRPV2. In the mouse intestine, TRPV2 is expressed in both sensory afferents and inhibitory motor neurons (Mihara et al., 2010). In dissociated myenteric neurons, TRPV2-like currents were detected in association with increased intestinal motility. In the stomach of the mouse, approximately 84% of neuronal NOS-expressing myenteric neurons coexpressed TRPV2, and gastric emptying was accelerated by the TRPV2 activator probenecid (Mihara et al., 2013).

TRPV4 has emerged as a molecular osmotransducer and mechanotransducer candidate on visceral afferents (Brierley et al., 2008). Compared with TRPV1, the anatomic distribution of TRPV4 in the gastrointestinal tract is more discrete: TRPV4 appears to be preferentially expressed in colonic sensory neurons with a virtual absence on vagal afferents (Brierley et al., 2008). Behavioral responses to painful colonic distension are significantly reduced in TRPV4($^{-/-}$) mice (Brierley et al., 2008), as is the mechanical hyperalgesia that occurs in response to PAR-2 (Grant et al., 2007). Indeed, TRPV4 has been implicated as a primary target in PAR-2–mediated sustained inflammatory signaling (Poole et al., 2013). Of note, PAR-2 also sensitizes TRPV1 (Amadesi et al., 2004, 2006) and TRPA1 (Tarada et al., 2013). Thus, PAR-2 appears to function as a regulator of TRP channels (Surprenant, 2007). Since gut bacteria produce high amounts of PAR-2, TRPV4 (along with TRPV1 and TRPA1) may be an attractive pharmacological target to relieve visceral pain. This hypothesis has gained good experimental support by the demonstration of increased TRPV4 mRNA expression in patients with IBD and the efficacy of pharmacological TRPV4 antagonism in a mouse model (induced by TNBS) of colitis (Fichna et al., 2012). Of note, Src inhibitor-1 suppressed PAR-2–induced activation of TRPV4, indicating the importance of tyrosine phosphorylation (Poole et al., 2013).

The literature on the neuroimmune link between sensory neurons and the aberrant immune response in colitis (see Engel et al., 2011b) is growing (but still highly speculative). As discussed above, capsaicin-sensitive C fibers coexpress SP with CGRP (see Holzer, 1991). Unexpectedly, these neuropeptides seem to exert opposite effects in colitis. Indeed, SP-null mice are protected against experimental (oxazolone-induced) colitis, whereas, conversely, the CGRP knockout animals show increased susceptibility (Engel et al., 2012). Chemical ablation of SP-positive nerves by capsacin pretreatment, or pharmacological blockade of SP receptors (NK1R) by CAM 4092, abolished the T cell–mediated transfer colitis in severe combined immunodeficiency (SCID) mice (Gad et al., 2009). Unfortunately, the clinical data are equivocal and confusing since patients with IBD were reported to have decreased (Jarcho et al., 2013), increased (Goode et al., 2000), or similar (ter Beek et al., 2007) NK1R expression compared with healthy subjects. Furthermore, SP-like immunoreactivity was markedly reduced in gastrointestinal biopsies taken from patients with ulcerative colitis (Kimura et al., 1994).

Most recently, TRPC4 has been added to the list of potential therapeutic targets in visceral pain (Westlund et al., 2014). In rats, genetic inactivation of TRPC4 by transposon-mediated knockout mutation, or pharmacological blockade by the TRPC4 antagonist ML-204 [4-methyl-2-(1-piperidinyl)quinoline], induced tolerance to visceral pain evoked by intracolonic mustard oil exposure.

b. Genitourinary tract. Multiple TRP channels are expressed in the bladder (in urothelium, nerve endings, fibroblasts, and detrusor muscle; Fig. 26), where they are thought to function as sensors of stretch and chemical irritation (see Charrua et al., 2010; Skryma et al., 2011; Avelino et al., 2013). There is a dense network of nerve fibers that express TRPV1 in the suburothelium and muscular layer of both rat and human renal pelvis, ureter, bladder, and urethra (see Avelino et al., 2013) but the existence of functional TRPV1 in urothelial cells and detrusor smooth muscle remains controversial (recently reviewed in Boudes
and De Ridder, 2012). As discussed elsewhere in this article, the involvement of neuronal TRPV1 (and nonneuronal TRPV4) in the micturition reflex is well established; here, we focus on the role of TRPs in nociception.

The pain of interstitial cystitis is thought to be amenable to TRPV1 antagonist therapy. In a feline model of interstitial cystitis, abnormally enhanced capsaicin responses were detected (Sculptoreanu et al., 2005a) secondary to TRPV1 phosphorylation by PKC (Sculptoreanu et al., 2005b). In addition, increased TRPV1 expression was reported in bladder afferents of patients with interstitial cystitis (Mukerji et al., 2006b). An interesting, alternative molecular pathway to increase TRPV1 activity during cystitis is by down-regulation of TRPV1b, a TRPV1 splice variant that acts as a dominant negative modulator (Charrua et al., 2008). In mice, genetic manipulation of the TRPV1 gene prevents spinal c-fos overexpression (a surrogate biochemical marker of nociceptive pain) and ameliorates mechanical hyperalgesia in experimental cystitis models (Wang et al., 2008c). Furthermore, the TRPV1 antagonist GRC-6211 (structure shown in Fig. 9) ameliorates nociceptive behavior and micturition reflex activity in chronically inflamed rat bladder (Charrua et al., 2009a). Combined, these findings imply a therapeutic potential for TRPV1 and TRPV4 blockade and TRPM8 activation in the management of painful and/or overactive bladder disorders. Reprinted with permission from Moran et al. (2011).

**Fig. 26.** Roles of TRP channels in bladder function. The micturition reflex is mediated by TRPV1-positive afferents and probably, albeit to a lesser degree, also by TRPM8-expressing nerves. The same neurons convey nociceptive information (e.g., bladder pain secondary to cystitis) to the CNS. TRPV4 is thought to be a key player in bladder functions because it is present in both the urothelium and the detrusor muscle where it is activated by stretch (bladder distension) and hypo-osmolar urine. TRPV1 may be activated by protons and the inflammatory milieu during cystitis (although the existence of functional TRPV1 in the urothelium remains controversial). The micturition reflex is under the control of a descending CNS pathway. When this pathway is disrupted (e.g., as a result of spinal cord injury or MS), the micturition reflex becomes autonomous and partly driven by TRPV1-expressing nerves. Collectively, these findings imply a therapeutic potential for TRPV1 and TRPV4 blockade and TRPM8 activation in the management of painful and/or overactive bladder disorders. Reprinted with permission from Moran et al. (2011).
worrisome disconnect between preclinical models (robust effect for TRP targeting) and patients (no clinical benefit for the same approach).

A complex change in mRNA levels encoding TRP channels was recently reported in bladder biopsies taken from 22 patients with classic interstitial cystitis and 17 patients with nonclassic interstitial cystitis (Homma et al., 2013). In patients with nonclassic (i.e., nonulcerated) interstitial cystitis, TRPV2 was the only TRP channel to show increased expression. By contrast, classic interstitial cystitis tissues showed increased levels of TRPA1, TRPM2, TRPM8, TRPV1, and TRPV2 mRNAs and a concomitant decrease in TRPV4. This supports the notion that interstitial cystitis is a heterogenous disease (Peeker and Fall, 2000).

As discussed elsewhere in this review, in the rat, TRPA1 is highly coexpressed with TRPV1 on bladder afferents and is especially abundant in the outflow region, implying a role in micturition (Streng et al., 2008). Although it is tempting to speculate that TRPA1 (a shared target for a broad range of chemical irritants) may contribute to bladder pain under inflammatory conditions, the experimental data to support this hypothesis are weak. In fact, even visceral pain evoked by mustard oil (a well established activator of TRPA1) appears to be mediated via TRPV1 and not TRPA1 (Everaerts et al., 2011). Parenthetically, allyl-isothiocyanate, the active ingredient in mustard oil, both directly activates TRPV1 (Gees et al., 2013) and sensitizes it to heat stimulation (Alpizar et al., 2014). Although TRPA1 was implicated in the hyperalgesia and urinary bladder overactivity that accompany cyclophosphamide-induced cystitis (Meotti et al., 2013), these animals also show elevated TRPV1 expression (Dang et al., 2013) and diminished nociceptive behavior in the presence of TRPV1 blockade (Charrua et al., 2009a).

TRPM8 is expressed both in urothelium and on thin bladder afferents (Fig. 26; Stein et al., 2004). TRPM8 is an interesting target to explore for bladder pain in that 1) it is increased on the bladder afferents, but not the urothelial cells, of patients with idiopathic detrusor overactivity, and 2) the increase in TRPM8 correlates with pain severity (Mukerji et al., 2006b). Indeed, these patients show higher pain scores during cold water instillation (Mukerji et al., 2006c). The TRPM8 agonist menthol evokes the micturition reflex in humans. Conversely, the TRPM8 antagonist AMTB (structure shown in Fig. 11) decreases the frequency of volume-induced bladder contractions in a rat model of painful bladder syndrome (Lashinger et al., 2008). These findings imply a therapeutic potential for TRPM8 antagonists in the management of bladder disorders that are characterized by pain and/or overactivity of the detrusor muscle (see Andersson et al., 2010).

In the bladder, TRPV4 is the most abundant TRP channel, especially in the urothelium and detrusor muscle (see Avelino et al., 2013). Perplexingly, TRPV4 is present in lumbar DRG neurons, where its expression is increased in transgenic animals overexpressing NGF (Girard et al., 2013), but not in bladder nerve endings. TRPV4(-/-) mice show an altered micturition pattern that is characterized by increased intermicturition intervals and spotting behavior due to spontaneous (not reflex-driven) detrusor muscle contractions (Gevaert et al., 2007). In addition, TRPV4 knockout mice show reduced urinary frequency and increased void volume after bladder damage caused by intravesical administration of cyclophosphamide (Everaerts et al., 2010b). On the basis of these findings, it was postulated that TRPV4 plays a crucial role in the mechanosensory pathway in the bladder by detecting changes in intravesical pressure. Indeed, mechanical stretch activates urothelial TRPV4 in vitro (Mochizuki et al., 2009). Of note, TRPV4 is increased in the urothelium and detrusor muscle of rats with experimental bladder outlet obstruction (Cho et al., 2013). Furthermore, TRPV4 is osmosensitive and the osmolarity of urine changes during cystitis. However, there is no evidence that TRPV4 contributes to bladder pain in humans (as discussed above, some “TRPV4pathy” patients show abnormal micturition but none complain of painful bladder).

4. Contribution of Transient Receptor Potential Channels to Neuropathic and Cancer Pain. Chronic pain affects a large segment of the population, an estimated 50 million Americans, and costs the United States billions of dollars in health care expenditures and lost productivity. Although the Decade of Pain Control and Research has given new impetus to pain research, the translation of preclinical research into clinical practice is slow to occur. This is evident by the limited number of mechanistically novel therapeutic agents that have entered into the clinic for the treatment of pain in recent years. Consequently, agents that have been around for decades, such as opiates and nonsteroidal anti-inflammatory drugs (NSAIDs), still represent a mainstay in current pain therapy. However, NSAIDs have only modest effects on moderate to severe pain and their use is limited by a combination of gastrointestinal and cardiovascular side effects. Opiates are effective pain killers but their use is complicated by sedation, constipation, and itch, as well as concerns regarding tolerance and abuse. Clearly, new potent analgesic drugs with an acceptable safety profile are needed.

a. Targeting vanilloid transient receptor potential 1 for pain relief: A brief overview of clinical studies. Preclinical research has identified an array of new molecular mechanisms that are involved in the development and maintenance of chronic pain and may represent attractive targets for pharmacological intervention. Since the molecular cloning of the vanilloid receptor TRPV1 (Caterina et al., 1997), overwhelming experimental evidence has been accumulated that sensory neurons expressing TRP channels, in particular
TRPV1, are important mediators of pathologic pain (Patapoutian et al., 2009; Brederson et al., 2013). For instance, rats desensitized to systemic RTX (300 μg/kg s.c.) are devoid of both the ongoing spontaneous pain (manifested in the guarding behavior of the injured paw) and the evoked pain (quantified as thermal hyperalgesia in the hot plate test) that develop after mechanical damage of the sciatic nerve (Bennett model) (Fig. 18) (unpublished data). Strikingly, RTX also abolishes pain behavior when given to rats in a therapeutic fashion (i.e., in animals already in discomfort after the operation) (Fig. 18).

Despite 25 years of intensive research, the mechanism of action of RTX is still poorly understood. In the rat, systemic RTX (up to 300 μg/kg s.c.) induces a peculiar constellation of molecular changes. For example, the expression of endogenous compounds that suppress pain (e.g., galanin) is increased; by contrast, the expression of proinflammatory and algogenic agents (e.g., SP and CGRP) is downregulated (Farkas-Szallasi et al., 1995, 1996; Szallasi et al., 1999). These changes, collectively referred to as “vanilloid-induced messenger plasticity,” appear to be fully reversible and parallel the loss and restoration of the pain behavior (see Szallasi, 1996). Indeed, in patients with overactive bladder (similar to rats), intravesical RTX induces a long-lasting (several months) but fully reversible improvement in bladder functions (Cruz et al., 1997; Silva et al., 2000, 2001, 2005). This reversible desensitization (defunctionalization) contrasts with the irreversible loss (RTX as a “molecular scalpel”) of nociceptive behavior that follows intrathecal RTX administration (Jeffry et al., 2009; Iadarola and Mannes, 2011).

TRPV1 can be both upregulated and sensitized during inflammation and injury (Bishnoi and Premkumar, 2013). Indeed, TRPV1 was suggested to play a central role both in peripheral (Immeke and Gavva, 2006) and central sensitization (Palazzo et al., 2012). In keeping with this concept, the “wind-up” phenomenon that develops after repeated C fiber stimulation (believed to be a marker of central sensitization) is abolished in rats desensitized to RTX (Xu et al., 1997). In a trigeminal neuropathic pain model, extensive TRPV1 hyperactivity was detected in central terminals innervating the dorsal horn (Kim et al., 2014b). This hyperactivity was maintained by descending serotonergic input from the brainstem. This is in accord with the report that TRPV1 antagonists that enter the CNS [e.g., A-784168 (3,6-dihydro-3’-(trifuoromethyl)-N-[4-[(trifuoromethyl)sulfonyl]phenyl]-[1(2H),2’-bipyridine]-4-carboxamide)] are superior in their analgesic activity compared with those that are peripherally restricted [e.g., A-795614 (1-(1H-indazol-4-yl)-3-[(1R)-5-piperidin-1-yl-2,3-dihydro-1H-inden-1-yl]urea)] (Cui et al., 2006).

A key mechanism of peripheral TRPV1 sensitization is phosphorylation by PKC (Premkumar and Ahern, 2000), in particular PKCζ (Numazaki et al., 2002; Mandadi et al., 2006), that requires the interaction of TRPV1 with the scaffolding protein AKAP79/150 (Jeske et al., 2009). Indeed, small inhibitory peptides that interfere with this interaction exhibit analgesic potential in rodent models of neuropathic pain (Fischer et al., 2013). Small molecule antagonists that selectively block sensitized (i.e., phosphorylated by PKC) TRPV1 channels were also reported (Sugimoto et al., 2013).

As detailed above, in the periphery, TRPV1 functions as a polymodal receptor with complex regulation (Fig. 12; see Szallasi et al., 2007; Lázár et al., 2009; Szolcsányi and Sándor, 2012). Importantly, there is emerging evidence that TRPV1 may also play an important role in the modulation of synaptic transmission in the spinal cord (first sensory synapse in the dorsal horn where TRPV1 is coexpressed with μ-opioid receptors), as well as in supraspinal nuclei (Maione et al., 2009b; McGaraughty et al., 2009). Indeed, a study comparing the analgesic effects of TRPV1 antagonists with and without access to the CNS provided compelling evidence that a dual (both peripheral and central) action is required for full analgesic action (Cui et al., 2006). It was speculated that TRPV1 in the dorsal horn of the spinal cord contributes to central sensitization and is subject to regulation by endogenous agents, the so-called “endovanilloids” (Palazzo et al., 2013).

Two independent gene-targeting studies deleting TRPV1 alleles conclusively showed that TRPV1 is a critical channel for mediating thermal hyperalgesia under inflammatory pain conditions in mice (Caterina et al., 2000; Davis et al., 2000). In addition, one study showed that TRPV1-null mice are significantly less sensitive to acute noxious heat stimulation (Bölskei et al., 2010). Rats with nonfunctioning TRPV1 (desensitized to RTX) also show profound hypalgesia in the hot plate test (Broberger et al., 2000). Indeed, increased cutaneous heat pain perception is a biomarker to quantitate analgesic capsaicin activity in men (Bley, 2013). In light of these findings, it was not completely unexpected that TRPV1 antagonists increased the noxious heat pain threshold in humans (discussed later in the clinical trials section).

The endogenous fatty acid OEA, which is synthesized and released from the intestine upon feeding, evokes visceral pain-related behavior in wild-type mice. This effect was prevented by TRPV1 antagonism and was absent in TRPV1 knockout mice (Wang and Wang, 2005). Other loss-of-function studies, such as transgenic mice expressing TRPV1 shRNA, have conclusively shown that silencing the gene encoding for TRPV1 by RNA interference significantly attenuates capsaicin-induced pain behavior and sensitivity toward noxious heat (Kasama et al., 2007).

In a rat model of partial-thickness cutaneous thermal injury, pharmacological blockade of TRPV1
almost completely (by 98%) blocked thermal allodynia (Green et al., 2013), indicating that pain evoked by a second-degree burn is predominantly TRPV1 mediated. This observation implies a clinical benefit for TRPV1 antagonist-containing lotions in the pain control of burn patients. Authors speculated that oxidized LA metabolites generated during burn injury are capable of activating TRPV1 as endogenous algogenic agents. Of note, sunburn pain also involves epidermal TRPV4 (Moore et al., 2013). Activation of TRPV4 by UVB in keratinocytes upregulates endothelin-1 expression. TRPV1-expressing neurons express endothelin-1 receptors and endothelin-1 potentiates capsaicin-evoked currents in sensory neurons (Plant et al., 2007). Although not directly related to pain, it is worth mentioning here that sunburn activates TRPA1 in human melanocytes and thereby increases pigmentation (suntan) (Bellono and Oancea, 2013; Bellono et al., 2013). One might speculate that creams containing TRPA1 agonists may help prevent sunburn, an important cause of skin cancers (if so, one can also easily visualize an interest by tanning salons in this approach).

An entire book was recently devoted to the analgesic activity of TRPV1 antagonists in preclinical chronic (inflammatory and neuropathic) pain models (Gomtsyan and Faltynek, 2009). For an update on this topic, interested readers are referred to a number of comprehensive reviews (Kort and Kym, 2012; Szallasi and Sheta, 2012; Bredersee et al., 2013; Szolcsányi and Pintér, 2013). As a representative example of the problems that plague this field, here we briefly review the OA data from rodents to clinical trials.

In a rat model of OA (induced by intra-articular monooiodoacetate [MIA] injection), DRG neurons back-labeled from the affected joints showed increased TRPV1-like immunoreactivity (Fernhough et al., 2005). Compared with naïve rats, these animals displayed enhanced TRPV1-mediated CGRP release in response to capsaicin (Pütterfarcken et al., 2010). Three weeks after MIA injection, spinal neurons obtained from these rats showed increased spontaneous firing activity (supposedly corresponding to ongoing pain), which was inhibited by the TRPV1 antagonist A-889425 [1-(5-methylpyridin-2-yl)-N-(4-(trifluoromethylsulfonyl)phenyl)-1,2,3,6-tetrahydropyridine-4-carboxamide] (Chu et al., 2011). Intra-articular administration of the TRPV1 antagonist JNJ17203212 [4-[3-(trifluoromethyl)-2-pyridinyl]-N-[5-(trifluoromethyl)-2-pyridinyl]-1-piperazinecarboxamide; Fig. 9] almost completely abolished the weight-bearing asymmetry in mice with MIA-induced OA 2 hours after treatment (Kelly et al., 2013). Furthermore, desensitization to capsaicin reduced both pain and bone damage induced by MIA (Kalff et al., 2010). In mice, genetic deletion of TRPV1 reduced (but did not eliminate) tissue damage and mechanical hyperalgesia in the complete Freund's adjuvant (CFA) model of chronic arthritis (Szabó et al., 2005). TRPV1 is increased in synovial samples taken from patients with OA (Engler et al., 2007; Kelly et al., 2013), and the loss-of-function TRPV1 variant I585V has been linked to a decreased risk for painful OA (Valdes et al., 2011). Taken together, these findings identify TRPV1 as a promising target to relieve OA pain. However, the TRPV1 antagonist ABT-116 [urea, N-(2-(3,3-dimethylbutyl)-4-(trifluoromethyl)phenyl)methyl]-N'-(1-methyl-1H-indazol-4-yl) did not provide any significant pain relief in dogs with experimental synovitis (Cathcart et al., 2012) or naturally occurring, age-related hip OA (Malek et al., 2012), and AZD1386 (Fig. 9) was withdrawn from clinical trials in patients with OA after a periodic review of the data failed to show any clinical benefit (Svensson et al., 2010; Miller et al., 2014).

It is not easy to reconcile these discrepant findings. Clearly, they question the relevance of the rodent (MIA) model of human OA pain (Zhang et al., 2013c). It is worth recalling here that in other preclinical pain models, the analgesic efficacy of TRPV1 blockade was dependent on the duration (stage) of the disease. For instance, TRPV1 is increased in the early stage of experimental diabetic neuropathy but is reduced (and eventually lost) with disease progression (Pabbidi et al., 2008). Moreover, insulin (which is initially increased in the circulation of patients with T2DM) increases TRPV1 expression in the plasma membrane (Van Buren et al., 2005). In skin biopsies taken from patients with long-standing diabetic neuropathy, TRPV1-like immunoreactivity is, however, markedly reduced (Lauria et al., 2006). Of note, TRPV1 antagonists block acute pancreatitis pain (as well as the transition from acute to chronic pain) in rats, but become ineffective when chronic pancreatitis is established (Schwartz et al., 2013). On the basis of these findings, one might argue that TRPV1 is involved in the development, but not maintenance, of chronic neuropathic pain. One may also make an argument that TRPV1 antagonists, if given early, might prevent (or at least) delay the development of chronic pain. This is consistent with the observations that capsaicin reduces hyperalgesia and bone damage when given prophylactically (i.e., before MIA injection) in the rat (Kalff et al., 2010); however, in multiple clinical trials it showed no proven clinical benefit in patients with OA (reviewed in Remadevi and Szallasi, 2008). Clearly, OA is a complex disease with redundancy in pain pathways, and TRPV1 is only one of many pain targets in patients with OA (Killock, 2013; Zhang et al., 2013c).

TRPV1-expressing sensory afferents remain a promising therapeutic target in neuropathic pain. Indeed, targeted silencing by QX-314 and capsaicin of TRPV1-positive axons abolishes thermal, mechanical, and (somewhat surprisingly) cold hyperalgesia in the rat (Brenneis et al., 2013), and perineural (Kissin and Szallasi, 2011) or epidural RTX (a molecular scalpel...
that selectively abolishes TRPV1-expressing nerves) produces lasting analgesia in rats with neuropathic pain secondary to spinal nerve ligation (Lee et al., 2012). Importantly, intra-articular RTX injections ameliorate pain behavior and restore ambulation in dogs with severe OA pain (Fig. 19A; D. Cimino-Brown and M. Iadarola, personal communication). As discussed below, intrathecal RTX is undergoing clinical trials in patients with chronic, intractable cancer pain. Although epidural RTX is well tolerated at doses at which it induces analgesia (ED\textsubscript{50} = 0.265 mg in the rat), it can cause sedation and hyperventilation at approximately 10-fold higher doses (>2 μg) and the treatment can be even fatal if the dose exceeds 10 μg (Lee et al., 2012). Localized injection (into the affected painful joints) should minimize the risk for these adverse effects. Finally, it is worth mentioning that RTX does not influence the level of TRPV1 mRNA and protein in non-neural cells (Kun et al., 2012).

Cancer pain is another promising indication for TRPV1 blockade. Here it suffices to mention that TRPV1 expression is enhanced in DRG neurons ipsilateral to bone cancer (osteosarcoma) in the mouse (Niiyama et al., 2007). In mice and dogs, treatment with RTX to desensitize TRPV1-containing neurons ameliorates bone cancer pain (Fig. 19; Brown et al., 2005; Menendez et al., 2006). In the mouse, this effect was mimicked by both genetic disruption of the TRPV1 gene and pharmacological TRPV1 blockade by the selective antagonist JNJ17203212 (Fig. 9; Ghilardi et al., 2005). It is noteworthy that intrathecal RTX injection was reported to provide long-lasting (several weeks) pain relief in dogs with advanced osteosarcoma, although it had no effect on the natural progression of the disease (Fig. 19B; reviewed in Iadarola and Mannes, 2011; Iadarola and Gonnella, 2013). The treatment was well tolerated. These findings paved the way for the clinical trials at the National Cancer Institute with intrathecal RTX in patients with intractable cancer pain (ClinicalTrials.gov identifier NCT00804154).

Of note, based on coimmunoprecipitation and fluorescence resonance energy transfer interaction experiments, the existence of functional TRPA1/TRPV1 heteromers was postulated in sensory neurons (Staruschenko et al., 2010). The cannabinoid agonists WIN 55,212-2 [(R)-(+)-2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthenylmethanone and AM1241 [(2-iodo-5-nitrophenyl)-[1-[(1-methylpiperidin-2-yl)methyl]indol-3-yl]methanone] were shown to directly activate TRPA1 (Akopian et al., 2008). Unexpectedly, TRPA1 activation by these compounds also blocked the capsaicin-induced nocifensive behavior, implying heterologous desensitization between TRPA1 and TRPV1. Indeed, WIN 55,212-2 caused profound changes in the phosphorylation status of the TRPV1 protein (Jeske et al., 2006). This effect was absent if TRPA1 was silenced via siRNA knockdown. Taken together, these findings suggest that the peripheral antinociceptive actions of cannabinoids are mediated by TRP channels, in particular TRPA1/TRPV1 heteromers (see Akopian et al., 2008). In other words, TRPA1/TRPV1 heteromers may represent novel "ionotropic cannabinoid receptors" (Akopian et al., 2009).

The clinical value of capsaicin (and its less pungent synthetic congeners, olvanil; Fig. 5) was extensively discussed elsewhere by us (Knotkova et al., 2008; Szallasi and Sheta, 2012) and others (Wong and Gavva, 2009; McCormack, 2010; O’Neill et al., 2012; Bley, 2013), including in a recent systematic review of the Cochrane Database by Derry et al. (2013). Although ALGRX-4975 (patent owner, AlgoRx; Adlea), an injectable capsaicin preparation, showed promise in early clinical studies to relieve OA and postoperative pain (e.g., after bunectomy or knee replacement surgery; see Remadevi and Szallasi, 2008), Anesiva (the company developing Adlea) ran out of funds to complete the clinical trials and filed for bankruptcy in 2010 (www.fiercebiotech.com/story/anesiva-ends-2009-bankruptcy-petition/2010-01-04).

Another major setback for capsaicin therapy was the recent unanimous decision by a US Food and Drug Administration (FDA) panel not to recommend Qutenza, a high-dose (8%) capsaicin patch, for HIV-related pain for lack of proof for clinical efficacy (www.bloomberg.com/news/2012-03-08/neurogesx-fails-to-win-u-s-approval-to-sell-patch-for-hiv-related-pain.html). Qutenza is still available for postherpetic neuralgia (PNH) in the United States (Acorda, Ardsley, NY), and for nondiabetic peripheral neuropathic pain in Europe (Astellas Europe, Chertsey, UK). Each 280 cm\textsuperscript{2} patch contains a total of 179 mg capsaicin (i.e., 640 μg/cm\textsuperscript{2}; www.qutenza.com). In the United States, a kit of two patches retails for $1430.87. Clinical trials with Qutenza in patients with PNH and other forms of neuropathic pain were detailed elsewhere (Noto et al., 2009). In brief, Qutenza induced a reversible loss of TRPV1-positive dermal afferents (Fig. 27). It was well tolerated (with a few exceptions, all subjects were able to complete 30- to 60-minute treatment duration without asking for early removal of the patches), and it gave a modest analgesic effect over placebo (Peppin et al., 2011; Bley, 2013). Although herpes zoster is not uncommon (it affects about 800,000 adults in the United States annually) (Schmader, 2002), the probability of long-standing pain of clinical importance is low (Hergason et al., 2000); therefore, the market for Qutenza is limited. Indeed, Qutenza is projected to garner peak sales of just $50 million across the seven major markets. NGX-1998 (Neuroges-X) is a liquid formulation of high-concentration capsaicin (20% w/w). It has a shorter application time (5 minutes compared with Qutenza, which needs to be left on the painful area for at least 30 minutes); therefore, it is more...
convenient for the patients. NGX-1998 was reported to provide modest pain relief up to 12 weeks in 165 patients with PHN (www.empr.com/liquid-capsaicin-ngx-1998-provides-pain-relief-after-five-minutes/article/241907/#).

Civamide (synthetic zu-capsaicin) is being developed by Winston Pharmaceuticals (Vernon Hills, IL; www.winstonlabs.com) for indications such as cluster headache, migraine (Diamond et al., 2000), and OA (Schnitzer et al., 2012). CivaneX (a cream containing 0.075% civamide) is intended to be used in combination with COX-2 inhibitors in patients with moderate to severe OA pain. In 2010, Health Canada issued a Notice of Compliance for CivaneX (as of today, the FDA approval is still pending).

There is a recent revival of interest in RTX as a molecular scalpel to achieve permanent analgesia in patients with intractable cancer pain, fueled by the ability of the compound to relieve pain and restore ambulation in dogs with osteosarcoma (Fig. 19). Canine osteosarcoma is a naturally occurring bone tumor that usually affects the long bones in one limb. In the dog, RTX injections (1–3 μg/kg) can be made into either the lumbar cistern (for hind limb tumors) or the cisterna magna (for forelimb tumors). At this dose, intracisternal RTX administration caused nearly complete loss of sensitivity to noxious thermal stimulation when tested 2 days after treatment (Brown et al., 2005). Dogs with intractable osteosarcoma pain (that had become unresponsive or intolerant to conventional pain management) were enrolled into the intrathecal RTX administration studies. Most of the animals were candidates for euthanasia because of poor pain control. Because intrathecal RTX causes an acute pain reaction, the injection is performed under general anesthesia. The RTX treatment was well tolerated and the animals were discharged to home the day after treatment. Intrathecal RTX administration resulted in a profound analgesic action (Fig. 19B): The animals became ambulatory, walking on four legs. RTX administration, however, had no influence on disease progression. By 14 weeks, only four dogs stayed alive (Iadarola and Gonnella, 2013). The first human patient with intractable cancer pain was enrolled into the phase I RTX trials at the National Cancer Institute in October 2009 (ClinicalTrials.gov identifier NCT00804154).

With more than 50 pharmaceutical companies filing over 1000 patents (for survey of the patent literature, see Voight and Kort, 2010), the preclinical literature on small molecule TRPV1 antagonists is vast and extensively reviewed both by us (Appendino and Szallasi, 2006; Gharat and Szallasi, 2007; Khairatkar-Joshi and Szallasi, 2009; Szallasi and Sheta, 2012; Brederson et al., 2013) and others (Gunthorpe and Chizh, 2009; Kort and Kym, 2012; Palazzo et al., 2012). Although a number of small molecule TRPV1 antagonists have been advanced to clinical trials, thus far none have progressed beyond phase II. GlaxoSmithKline was among the first to disclose its phase I results obtained with SB705498 [1-[(2-bromophenyl)-3-[(3-(trifluoromethyl)pyridin-2-yl)pyrrolidin-3-yl]urea] (Chizh et al., 2007). In a proof-of-concept study, a single oral dose of 400 mg of SB705498 (structure shown in Fig. 9) substantially reduced pain and flare evoked by cutaneous capsaicin challenge (0.075% capsaicin cream applied to the forearm) compared with placebo. SB705498 did not show any serious adverse effects in the study. Subsequently, the effect of per os SB705498 on dermal heat sensitivity after capsaicin or UVB challenge was evaluated. Somewhat unexpectedly, SB705498 caused a modest, but significant (up to 1.3°C), increase in the noxious heat perception threshold both in rodents and humans. In December 2005, an active-controlled, placebo-controlled, randomized, single-blind, phase II trial was initiated in subjects with dental pain after third molar tooth extraction. The subjects were to receive a single oral dose of SB705498, placebo, or co-codamol. The study was completed in February 2008, and results have not yet been revealed (ClinicalTrials.gov identifier NCT00281684).

In 2008, Amgen announced the early termination of a phase Ib dental pain (molar extraction) study with their clinical candidate molecule, AMG517 [N-[4-[[6-[4-[(trifluoromethyl)phenyl]pyrimidin-4-yloxy]-1,3-benzothiazol-2-yl]acetamide; Fig. 9], because it caused a lasting (1–4 days) marked hyperthermia response (up to 40.2°C) in human volunteers (Gavva et al., 2008).

AstraZeneca was developing AZD1386 (Fig. 9) for the potential oral treatment of chronic nociceptive pain and GERD. In April 2008, an active-controlled, placebo-controlled, randomized, double-blind phase II trial was initiated in subjects with pain due to third molar
AZD1386 (55 mg p.o.) caused significant pain relief (Krarup et al., 2011). The drug was well tolerated although a modest increase in body temperature (approximately 0.4°C in average) was noticed in most patients, exceeding 38°C in one individual (Krarup et al., 2011). Disappointingly, AZD1386 did not provide any clinically meaningful pain relief in patients with OA (Svensson et al., 2010; Miller et al., 2014) or GERD (Krarup et al., 2013). Of note, in 2012 AstraZeneca announced the closing of its neuroscience division, inclusive of the TRPV1 antagonist program (www.fiercebiotech.com/story/astrazeneca-cutting-2200-rd-jobs-slaashing-neuroscience-restructuring).

A phase II trial with GRC-6211 (Glenmark-Eli Lilly) for OA pain was suspended due to undisclosed reasons (see Fig. 9 for structure) (Kitagawa et al., 2012). No body temperature or thermosensation data were reported for this compound (www.glenmarkpharma.com/GLN_NWS/news.aspx?res=P_GLN_NWS_RLS).

Merck/Neurogen was developing MK-2295 (Fig. 9) for the potential treatment of pain and cough. MK-2295 markedly increased the noxious heat pain threshold in humans (unlike the hyperthermia response, this did not show any attenuation with repeated dosing), placing the study participants at the risk of scalding injury (reviewed in Eid, 2011).

In 2011, Abbott reported that its clinical candidate ABT-102 (structure shown in Fig. 9) caused the elevation of mean core body temperature by 0.6°C in healthy volunteers after short-term administration of a 4-mg dose (Rowbotham et al., 2011). ABT-102 was well tolerated with repeated dosing in humans; by day 7, temperature increases were no longer significant for any dose tested. Abbott also reported that ABT-102 caused an increase in cutaneous and oral heat pain thresholds measured by quantitative sensory testing, and that the deficit in noxious heat perception did not attenuate with the 7-day twice-daily dosing regimen (Rowbotham et al., 2011).

Compound PHE377 has completed a phase I clinical trial (www.pharmeste.com; PharmEste). PHE377 (structure not revealed) is interesting in that it was reported not to cause any detectable hyperthermia in rats or dogs (see Trevisani and Szallasi, 2010). JTS-653 by Japan Tobacco (Fig. 9) was reported to ameliorate PNH pain in rodent models (Kitagawa et al., 2013a). In the rat bladder, JTS-653 suppressed overactivity without affecting normal micturition (Kitagawa et al., 2013b). According to the company website, this compound is now in phase II clinical trials in Japanese patients with painful overactive bladder (www.jt.com/investors/results/S_information/pharmaceuticals/pdf/P.L.20220207_E.pdf).

Although it is not intended to be a pain medication, for the sake of completeness, it should be mentioned here that Provesica (a subsidiary of Xentia) successfully completed its phase I clinical trials with XEN-D0501. According to the company website, XEN-D0501 is about to enter phase II development for the indications of overactive bladder and chronic cough (www.provesica.com). In healthy subjects, XEN-D0501 was rapidly absorbed (t_{max} between 0.5 and 4 hours postdose), well tolerated, and caused only mild hyperthermia (0.74°C) at the highest dose (5 mg) tested (Round et al., 2011). Finally, Amorepacific, a skincare company based in South Korea, is developing PAC-14028 ([E]-N-((R)-1-(3,5-difluoro-methanesulfonyl-phenyl)-ethyl)-3-(2-propyl-6-trifluoromethyl-pyridine-3-yl)-acrylamide; Fig. 9) for atopic dermatitis (Lim and Park, 2012).

There are two schools of thought with regard to the major adverse effects (febrile reaction and impaired heat pain sensation) of TRPV1 antagonists. The first considers these on-target side effects. Many proponents of this theory believe that side effects of TRPV1 blockade can be adequately managed by sensible precautions. Indeed, the hyperthermia response shows attenuation after repeated dosing, and the initial increase in body temperature can be adequately managed by common antipyretic drugs such as acetaminophen (Gavva et al., 2007). Moreover, burns (that are usually mild) can be prevented by simply warning the patients to be careful.

A more attractive approach is to eliminate the undesirable side effect of TRPV1 antagonists by chemical modification of the pharmacophore. In the rat, it was feasible to eliminate hyperthermia while preserving antihyperalgesia by differential modulation of distinct modes of TRPV1 activation (Lehto et al., 2008). Subsequently, several companies (Astellas, Muchida, Grünenthal, etc) described so-called “modality-specific” TRPV1 antagonists that do not raise body temperature or thermosensation data were reported for this compound (www.glenmarkpharma.com/GLN_NWS/news.aspx?res=P_GLN_NWS_RLS).

Merck/Neurogen was developing MK-2295 (Fig. 9) for the potential treatment of pain and cough. MK-2295 markedly increased the noxious heat pain threshold in humans (unlike the hyperthermia response, this did not show any attenuation with repeated dosing), placing the study participants at the risk of scalding injury (reviewed in Eid, 2011).

In 2011, Abbott reported that its clinical candidate ABT-102 (structure shown in Fig. 9) caused the elevation of mean core body temperature by 0.6°C in healthy volunteers after short-term administration of a 4-mg dose (Rowbotham et al., 2011). ABT-102 was well tolerated with repeated dosing in humans; by day 7, temperature increases were no longer significant for any dose tested. Abbott also reported that ABT-102 caused an increase in cutaneous and oral heat pain thresholds measured by quantitative sensory testing, and that the deficit in noxious heat perception did not attenuate with the 7-day twice-daily dosing regimen (Rowbotham et al., 2011).

Compound PHE377 has completed a phase I clinical trial (www.pharmeste.com; PharmEste). PHE377 (structure not revealed) is interesting in that it was reported not to cause any detectable hyperthermia in rats or dogs (see Trevisani and Szallasi, 2010). JTS-653 by Japan Tobacco (Fig. 9) was reported to ameliorate PNH pain in rodent models (Kitagawa et al., 2013a). In the rat bladder, JTS-653 suppressed overactivity without affecting normal micturition (Kitagawa et al., 2013b). According to the company website, this compound is now in phase II clinical trials in Japanese patients with painful overactive bladder (www.jt.com/investors/results/S_information/pharmaceuticals/pdf/P.L.20220207_E.pdf).

Although it is not intended to be a pain medication, for the sake of completeness, it should be mentioned here that Provesica (a subsidiary of Xentia) successfully completed its phase I clinical trials with XEN-D0501. According to the company website, XEN-D0501 is about to enter phase II development for the indications of overactive bladder and chronic cough (www.provesica.com). In healthy subjects, XEN-D0501 was rapidly absorbed (t_{max} between 0.5 and 4 hours postdose), well tolerated, and caused only mild hyperthermia (0.74°C) at the highest dose (5 mg) tested (Round et al., 2011). Finally, Amorepacific, a skincare company based in South Korea, is developing PAC-14028 ([E]-N-((R)-1-(3,5-difluoro-methanesulfonyl-phenyl)-ethyl)-3-(2-propyl-6-trifluoromethyl-pyridine-3-yl)-acrylamide; Fig. 9) for atopic dermatitis (Lim and Park, 2012).

There are two schools of thought with regard to the major adverse effects (febrile reaction and impaired heat pain sensation) of TRPV1 antagonists. The first considers these on-target side effects. Many proponents of this theory believe that side effects of TRPV1 blockade can be adequately managed by sensible precautions. Indeed, the hyperthermia response shows attenuation after repeated dosing, and the initial increase in body temperature can be adequately managed by common antipyretic drugs such as acetaminophen (Gavva et al., 2007). Moreover, burns (that are usually mild) can be prevented by simply warning the patients to be careful.

A more attractive approach is to eliminate the undesirable side effect of TRPV1 antagonists by chemical modification of the pharmacophore. In the rat, it was feasible to eliminate hyperthermia while preserving antihyperalgesia by differential modulation of distinct modes of TRPV1 activation (Lehto et al., 2008). Subsequently, several companies (Astellas, Muchida, Grünenthal, etc) described so-called “modality-specific” TRPV1 antagonists that do not raise body temperature or thermosensation data were reported for this compound (www.glenmarkpharma.com/GLN_NWS/news.aspx?res=P_GLN_NWS_RLS).

Modality specificity is an attractive concept. TRPV1 is unique in that it functions as a “multisteric nocisensor” (Szolcsányi and Sándor, 2012). Side-directed mutagenesis experiments furnished strong evidence that point mutations can selectively eliminate TRPV1 activation mechanisms (Fig. 4). For example, the Y511A mutant (affecting the TM3 region) responds to heat and protons but not capsaicin (Jordt et al., 2004). Conversely, the T633A mutant (that changes the pore) shows normal capsaicin activation but does not respond to heat or protons (Ryu et al., 2007). These findings support that such modality-selective antagonists may be synthesized that leave heat sensing intact. For example, the R114E mutant (in the N terminus) shows normal heat responses but is insensitive to both protons and acetaminophen (Gavva et al., 2007). Moreover, burns (that are usually mild) can be prevented by simply warning the patients to be careful.

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and capsaicin (Jung et al., 2002a). Indeed, a new methodology was developed to screen for TRPV1 antagonists that do not inhibit capsaicin activation (Zicha et al., 2013). Added to this complexity are reports of allostERIC TRPV1 modulators (Kaszas et al., 2012; Lebovitz et al., 2012) and partial agonists/antagonists (Blumberg et al., 2011). The Amgen group postulated that hyperthermia is a function of proton activation (Lehto et al., 2008): Antagonists that do not interfere with the proton activation of TRPV1 are devoid of hyperthermia. This concept appears to be true for some compounds (AS1928370 and A-1165442) but not others (PHE377 and JTS-653) (compare structures in Fig. 9).

In summary, we still do not understand why some TRPV1 antagonists induce a marked febrile reaction, whereas others do not. It may be of relevance that TRPV1 is expressed in arteriolar smooth muscle (Cavanaugh et al., 2011). The TRPV1 agonist RTX evokes “reddening” (hyperemia) when applied to the rodent ear (Szallas and Blumberg, 1989). Although this effect is traditionally attributed to neurogenic inflammation, a direct action on TRPV1 expressed on vascular smooth muscle cells cannot be ruled out (vasodilatation/vasoconstriction in the skin is an important mechanism of body temperature regulation). The literature is, however, confusing. Functional TRPV1 expression was reported in murine skeletal muscle (Lotteau et al., 2013) where capsaicin was noted to induce hypertrophy (Ito et al., 2013). In the perfused rat hind limb, RTX causes vasoconstriction and increased O₂ consumption (Eldershaw et al., 1994). Taken together, these observations imply that TRPV1 agonism (and possibly antagonism) may influence body temperature regulation via non-neuronal TRPV1 receptors. Neuronal and non-neuronal TRPV1 may have distinct structure-activity relations. If this hypothesis holds true, such antagonists can be synthesized that block TRPV1 in sensory neurons (important for pain relief) but do not affect non-neuronal TRPV1 involved in body temperature regulation.

b. Vanilloid transient receptor potential 3. Although TRPV3 is most abundantly expressed in the skin keratinocytes, expression has been also reported in mouse peripheral and central nervous systems (Smith et al., 2002; Xu et al., 2002; Facer et al., 2007). However, the functional significance of central TRPV3 receptors is largely unknown. In humans, no clear picture has yet emerged with regard to non-neuronal (keratinocyte) TRPV3 expression in painful conditions. For example, TRPV3 is increased in keratinocytes of patients with mastalgia (painful breast tissue) (Gopinath et al., 2005), but is decreased in the skin of patients with diabetic neuropathy (Facer et al., 2007). More consistent observations were reported with regard to neuronal TRPV3 expression after nerve injury: TRPV3 is increased in small and medium diameter peripheral nerve axons in the brachial nerve plexus of patients with traumatic nerve injury (Facer et al., 2007). TRPV3 is also increased in the spinal nerve ligation model of neuropathic pain in the rat (see Bevan and Andersson, 2009).

No robust phenotype has been reported for Trpv3 knockout mice in inflammatory pain; however, several lines of evidence support a role for TRPV3 in pain processing. Trpv3 knockout mice showed deficits in response to acute noxious thermal stimuli in water immersion (>48°C) and hot plate (55°C) assays and exhibited deficits in responses to innocuous and noxious heat (within the first hour) in a thermal place preference assay (Moqrich et al., 2005). Knockdown of TRPV3 with shRNA reduced nocifensive behavior induced by intraplantar injection of farnesyl pyrophosphate (structure shown in Fig. 8), a TRPV3 agonist, in inflamed rats and also blocked farnesyl pyrophosphate–induced thermal hyperalgesia (Bang et al., 2010). Hydra Biosciences has reported efficacy of TRPV3 receptor antagonists in models of inflammatory pain (CFA), formalin-induced flinching, thermal injury, and spinal sensitization (see Reilly and Kym, 2011). Glenmark Pharmaceuticals has reported efficacy of TRPV3 antagonists in inflammatory and nerve injury models (see Khairatkar-Joshi et al., 2010).

Pharmacological interest in the role for TRPV3 in pain has lead to the discovery of small molecule TRPV3 antagonists and their characterization in preclinical pain models (see Huang and Chung, 2013). Hydra Biosciences reported a selective (>40-fold versus other TRP channels) and moderately potent tetrahydroquinolone amide TRPV3 antagonist (IC₅₀ of 0.2–1 μM) with in vivo efficacy (200 mg/kg i.p.) in decreasing thermal hyperalgesia in inflammatory or burn models of pain (see Reilly and Kym, 2011). No effect was observed in the contralateral paw, suggesting that the compound did not have a systemic effect on noxious thermosensation. Hydra Biosciences disclosed a quinazolinone as a second TRPV3 chemotype with moderate potency (IC₅₀ of 0.2–1 μM) and good selectivity against other thermoTRP channels (10-fold versus TRPA1 and TRPM8; 20-fold versus TRPV1). This chemotype was also efficacious in reducing thermal hyperalgesia in the carrageenan model of inflammatory pain.

Glenmark Pharmaceuticals has disclosed TRPV3 antagonists with antinociceptive efficacy in preclinical inflammatory and neuropathic pain models (see Khairatkar-Joshi et al., 2010; Preti et al., 2012). GRC15300 (structure not disclosed) was described as a potent (IC₅₀ = 79.5 nM in 2-APB–induced ⁴⁵Ca²⁺ uptake in hTRPV3/Chinese hamster ovary (CHO) cells, and 137 nM in human keratinocytes) and selective (>100× versus TRPV1, TRPV4, TRPA1, and TRPM8) TRPV3 antagonist (Khairatkar-Joshi et al., 2010). GRC15300 demonstrated a dose-dependent (ED₅₀ = 1.66 mg/kg) reversal of mechanical hyperalgesia in the
induced OA (ED50 = 11.9 mg/kg) models of pain. The repeated dosing (ED50 = 8.25 mg/kg p.o. after 8 days of decreasing mechanical hyperalgesia with short-term and In the in the CCI model of neuropathic pain, GRC15300 also demonstrated antinociceptive efficacy in postoperative paw incision pain (ED50 = 0.92 mg/kg), as well as in the MIA-induced OA (ED50 = 11.9 mg/kg) models of pain. The safety profile of GRC15300 was described with no significant effects at hERG (human ether-a-go-go-related gene) K+ channel current, no alteration in response to noxious heat in naive rat tail flick assays, and no locomotor deficits in the rotarod assay (up to 30 mg/kg). In 2010, Glenmark Pharmaceuticals announced a partnership with Sanofi-Aventis to advance GRC15300/ SAR 292833 into clinical trials for neuropathic pain (www.glenmarkpharma.com/GLN_NWS/pdf/Sanofi-Aventis&Glenmark_sign_license_agreement.pdf). One year later, the phase I trial was successfully completed (it was well tolerated with good pharmacokinetic profile) and the molecule was advanced into proof-of-concept studies.

c. Melastatin transient receptor potential 8. Although TRPM8 is a well established molecular pain target (see McKemy, 2010), its medicinal chemistry is rudimentary compared with TRPV1 or TRPA1 (see DeFalco et al., 2011). It is often debated in the literature whether TRPM8 activation is proalgesic and analgesic. In other words, should we focus our efforts on developing specific TRPM8 agonists or antagonists? In our opinion, this is not an either/or question. It is not unlikely that (as is the case for TRPV1) both TRPM8 agonists and antagonists have therapeutic potential.

TRPM8 is expressed in a subpopulation of primary afferent sensory neurons originating from both DRG and trigeminal ganglia (Dhaka et al., 2008). Most studies agree that TRPM8-positive neurons are distinct from those expressing TRPV1 and/or TRPA1 (Fig. 17; Takashima et al., 2007). A number of studies have investigated the expression of TRPM8 in experimental models of chronic pain with conflicting findings. For example, TRPM8 levels (measured by in situ hybridization) decreased in the injured L4 DRG but remained unchanged in the neighboring (uninjured) L4 DRG in the spinal nerve ligation model of neuropathic pain (Ohata et al., 2006). By contrast, in the CCI model, TRPM8 RNA levels showed a delayed increase (reaching statistical significance only at 14 days after CCI) in affected DRG neurons (Frederick et al., 2007). In the same model, immunostaining revealed a rapid (already noticeable 4 days after surgery) increase in the percentage of TRPM8-immunoreactive neurons compared with the sham-operated group (Xing et al., 2007; Su et al., 2011). Importantly, the development of cold allodynia paralleled the increase in TRPM8 staining after CCI (Rossi et al., 2012).

TRPM8 is a cold-sensitive nociceptor (McKemy et al., 2002). Indeed, conditional ablation of TRPM8 in adult mice renders the animals unable to distinguish warm from cool or to avoid noxious cold (Knowlton et al., 2013). It is noteworthy that cold allodynia is markedly diminished in these animals. By contrast, both Pirt (Tang et al., 2013) and artemin (Lippoldt et al., 2013) enhance TRPM8-dependent cold pain. Indeed, Pirt-null mice exhibit decreased behavioral responses to cold temperatures (Tang et al., 2013). [Somewhat confusingly, Pirt also potentiates the heat-responsive TRPV1 channel (Kim et al., 2008a).] Cooling (e.g., ice packs) is a time-honored analgesic approach to soothe acute pain secondary to skin burn or muscle strains. Although cooling can clearly activate TRPM8, the beneficial effects of ice packs on pain are probably not TRPM8 mediated. Menthol (Fig. 6), a naturally occurring TRPM8 agonist isolated from peppermint leaves, has been used for centuries in folk medicine as an analgesic and antipruritic agent. Today, menthol (similar to capsaicin) is used in over-the-counter (OTC) pain medications without a clinically proven benefit. Of note, menthol is also broadly used in lozenges, nasal sprays, inhalers, and cough syrups for the relief of nasal congestion associated with rhinitis and upper respiratory tract irritation, and it is added to certain cigarette brands to reduce the respiratory irritation that the smoke causes (see Preti et al., 2012). Menthol has, therefore, been claimed as an adjuvant agent in formulations addressing rhinitis and upper tract airway inflammation (Preti et al., 2012). However, there is good evidence that menthol has no measurable effect on nasal airflow (Lindemann et al., 2008).

In animal experiments, the behavioral responses to topical menthol are complex and often conflicting. For example, a recent study found that low (0.01%–1%) and high (10%–40%) concentrations of menthol had opposite effects on thermal preference in rats, with decreased cold avoidance at high concentrations (Klein et al., 2010). Proudfoot et al. (2006) showed that TRPM8 agonists, including menthol, relieved pain in certain neuropathic models; however, a second study was unable to replicate this finding (Caspiani and Heppenstall, 2009; Caspani et al., 2009). The interpretation of these studies is further complicated by the findings that menthol is not selective for TRPM8. In fact, menthol was shown to inhibit voltage-gated Na+ (Haeseler et al., 2002) and Ca2+ channels (Swandulla et al., 1987), which could reduce nociceptive transmission. This may explain the observation that responses to menthol were markedly reduced, but not abolished, in TRPM8-null mice (Colburn et al., 2007). To complicate the picture even further, cold hypersensitivity to menthol was abolished in TRPA1-deficient
mice but developed normally in TRPM8-null animals (Gentry et al., 2010).

The pharmacology and medicinal chemistry of TRPM8 modulators were recently reviewed in detail (DeFalco et al., 2011; Fernández-Peña and Viana, 2013). Here we highlight only a few interesting findings related to analgesic potential of these agents. In 2006, Bayer HealthCare reported a series of 2-benzoxo-benzoic acid amide derivatives such as AMTB (Alonso-Alija et al., 2006) for the treatment of airway disorders (reviewed in Preti et al., 2012). Relevant to our review, AMTB (structure shown in Fig. 11) was reported to attenuate the bladder nociceptive reflex responses in the rat (Lashinger et al., 2008), potentially opening a new therapeutic window in the treatment of painful bladder syndrome.

In 2007, Janssen Pharmaceuticals described a series of benzothiophene phosphate TRPM8 antagonists. In vitro, phosphate 25 inhibited TRPM8 receptors in various species (rats, dogs, and humans) with similar IC_{50} values of 29, 53, and 54 nM, respectively (Matthews et al., 2012). In vivo, this compound inhibited icilin-induced “wet-dog shakes” in rats with an ED_{50} value of 24 mg/kg p.o., and also suppressed neuropathic pain (ED_{50} = 14.8 mg/kg) in the CCI model. The substitution of the benzothiophene core with a benzimidazole ring increased potency: a representative compound in this series blocked canine TRPM8 with an IC_{50} value of 6 nM (Parks et al., 2011). Vinylicycloalkyl-substituted benzimidazole TRPM8 antagonists were reported to suppress both icilin-induced wet-dog shakes and CCI-induced neuropathic pain behavior by approximately 80% at a dose of 10 mg/kg p.o. (Calvo et al., 2012). The selective TRPM8 blocker PBMC [1-phenethyl-4-(benzoxo)-3-methoxybenzyl(2-aminoethyl)carbamate; structure shown in Fig. 11] inhibited cold hypersensitivity in the CCI model of chronic pain but, disappointingly, was ineffective against chemotherapeutic agent (oxaliplatin)-induced neuropathic cold hypersensitivity (Knowlton et al., 2011).

Additional notable TRPM8 antagonists in the preclinical stage of drug development include JNJ41876666 [3-[7-trifluoromethyl-5-(2-trifluoromethyl-phenyl)-1H-benzoimidazol-2-yl]-1-oxa-2-aza-spiro[4,5]dec-2-yl] | Hydrochloride| and AM09678 [(R)-1-(4-(trifluoromethyl)phenyl)-N-((S)-1,1,1-trifluoropropan-2-yl)-3,4-dihydroisoquinoline-2(1H)-carboxamide] (structures shown in Fig. 11).

Similar to TRPV1, TRPM8 is involved in thermoregulation. Menthol increases body temperature, whereas pharmacological TRPM8 blockade decreases body temperature (Knowlton et al., 2011; Gavva et al., 2012). The hypothermic response is, however, mild (<1°C) and disappears upon repeated dosing; therefore, it may not pose a problem for developing TRPM8 antagonists as therapeutics (unlike the febrile response to TRPV1 antagonists) (Gavva et al., 2012).

d. Ankyrin transient receptor potential 1. TRPA1 is expressed both in neuronal and non-neuronal tissues. In neurons, TRPA1 is expressed by TRPV1-positive, peptidergic, small diameter neurons in DRG (Figs. 17, 20, and 21; Story et al., 2003; Kobayashi et al., 2005). Ablation of TRPV1* neurons by RTX in mice results in an almost complete loss of sensitivity to allyl-isothiocyanate, a TRPA1 agonist (Pecze et al., 2009). These data support a high degree of colocalization between TRPV1 and TRPA1 in sensory neurons. In human DRG, TRPA1 is localized primarily to small and medium DRG neurons (Fig. 17), although some expression in large neurons was also reported (Anand et al., 2008). Neuropathic injury increases neuronal expression of TRPA1 (Anand et al., 2008; Ji et al., 2008). TRPA1 localizes to central terminals of primary afferent neurons in the dorsal spinal cord of rats and humans (Anand et al., 2008; Kim et al., 2010), where it is believed to play an important role in central sensitization and the development of mechanical allodynia (Fig. 20; see McGaraughty et al., 2010; Koivisto et al., 2014). Non-neuronal localization of TRPA1 mRNA and protein was also reported in human skin keratinocytes (Anand et al., 2008; Atayan et al., 2009) and melanocytes (Bellono et al., 2013). TRPA1 as a target for analgesic drugs has been extensively reviewed elsewhere (Garrison and Stucky, 2011; Strassmaier and Bakhavatchalam, 2011; Andrade et al., 2012; Bautista et al., 2013; Brederson et al., 2010; Radresa et al., 2013; Koivisto et al., 2014). The potential role of TRPA1 in chemotherapy-induced neuropathic pain is described below. An emerging indication for TRPA1 blockade is pain secondary to diabetic neuropathy. Methylglyoxal, a toxic offshoot of glycolysis, is held responsible by some for the development of long-term diabetic complications, including diabetic neuropathy (Vander Jagt, 2008). Indeed, in mice, methylglyoxal activates Na_{+}1.8 channels and evokes pain that is absent in the Na_{+}1.8-null Scn(-/-) animals; in patients, a plasma level of 600 nM methylglyoxal is required for the development of pain (Bierhaus et al., 2012). Methylglyoxal is a potent activator of murine TRPA1 (Andersson et al., 2013). Importantly, methylglyoxal also activates human TRPA1 (Ohkawara et al., 2012) and this interaction was implicated in the pathomechanism of metabolic neuropathies (Eberhardt et al., 2012). Another interesting aspect of the TRPA1-diabetes link is the ability of the antidiabetic drug glibenclamide to activate TRPA1 on sensory neurons (Babes et al., 2013). Glibenclamide is known to cause abdominal pain as a side effect in some patients, and it was suggested that its direct effect on TRPA1 is responsible.

TRPA1 antagonists ameliorate mechanical hyperalgesia and (even more important) prevent cutaneous nerve fiber loss in diabetic rodents (Wei et al., 2009b, 2010, 2011a, 2013; Koivisto et al., 2012). Paclitaxel, a common cause of chemotherapy-induced peripheral neuropathy (CIPN), worsens cold hyperalgesia in
diabetic rats via mitochondrial ROS generation and resultant TRPA1 activation (Barrière et al., 2012). Parenthetically, a similar mechanism (i.e., sensory nerve terminal mitochondrial dysfunction causing TRPA1 activation) was recently linked to asthma (Nesuashvili et al., 2013).

Another promising indication for pharmacological TRPA1 blockade is OA pain. The selective TRPA1 antagonist A-967079 ([1E,3E]-1-(4-fluorophenyl)-2-methyl-1-pentene-3-one oxime; see Fig. 10 for structure) blocks OA pain in rats with an ED50 of 23.2 mg/kg p.o. without altering physiologic noxious cold sensation (Chen et al., 2011).

Several companies (e.g., Hydra Biosciences, Abbott, Glenmark Pharmaceuticals, Janssen Pharmaceuticals, and Merck) have reported TRPA1-selective antagonists (representative structures for HC-030031, CHEM58611528, AP18, A-967079, and JNJ41477670 are shown in Fig. 10). The medicinal chemistry of TRPA1 antagonists was reviewed elsewhere (Rech et al., 2010; Strassmeier and Bakthavatchalam, 2011). In brief, the Hydra Biosciences antagonists contain a xanthine alkaloid core that is also present in caffeine. TRPA1 potency was enhanced through modifications of the purine core and manipulation of the ary1 group from phenyl to thiazole. Further modification of a second ary1 functionality attached to aliphatic pyrrolidine capping group led to exceptionally potent TRPA1 antagonists such as HC-03001 (structure shown in Fig. 10; reviewed in Strassmeier and Bakthavatchalam, 2011; De Petrocellis and Schiano Moriello, 2013).

Glenmark Pharmaceuticals also described TRPA1 antagonists that were based on the modifications of the xanthine alkaloid core. Chemists at Glenmark Pharmaceuticals modified this ring by replacing the five-membered imidazole fragment of the purine core with different heterocycles, including isothiazole, pyrrole, thiophene, pyridine, and imidazole (reviewed in Preti et al., 2012). Abbott reported TRPA1 antagonists based on an oxime lead series as exemplified by A-967079. In vitro, A-967079 is more potent for human TRPA1 (IC50 = 67 nM) than rat TRPA1 (IC50 = 290 nM) and demonstrates good oral bioavailability (Chen et al., 2011). In preclinical studies, A-967079 inhibited spontaneous and mechanically evoked firing of spinal wide dynamic range and nociceptive-specific neurons in OA rats (McGaraughty et al., 2010). Janssen Pharmaceuticals described two lead compound series: 1) highly potent biaryl pyrimidines that contain a pyrrolidine carboxamide side chain, and 2) compounds based on the tricyclic thioxodihydroindenopyrimidinone core (see Strassmeier and Bakthavatchalam, 2011). Finally, Merck described TRPA1 antagonists based on a hydroxyl-substituted aminodecalin core.

To date, two TRPA1 antagonists have been advanced into clinical trials. Cubist Pharmaceuticals and Hydra Biosciences were jointly testing CB-625 in the Netherlands in dose-escalating studies in healthy volunteers (phase Ia). Reportedly, the compound was well tolerated and Hydra/Cubist now plan to test the compounds in patients with acute, postsurgical pain (www.painresearchforum.org/news/22419-filling-pain-drug-pipeline). Of note, in recent animal studies, TRPV1, but not TRPA1, blockade inhibited cutaneous incision–mediated hypersensitivity (Barabas and Stucky, 2013). The second TRPA1 antagonist to enter clinical trials was GRC15736 by Glenmark Pharmaceuticals. In phase I studies, the compound was well tolerated at plasma levels comparable to those that were analgesic in the preclinical models (www.rttnews.com/1819388/glenmark-s-novel-molecule-grc-17536-completes-phase-i-clinical-trials-in-europe). Glenmark Pharmaceuticals plans to initiate phase II proof-of-concept studies with this compound for pain and respiratory indications.

An intriguing alternative approach to silence TRPA1-expressing neurons is by a combination of cinnamaldehyde and QX-314. Cinnamaldehyde (a TRPA1 agonist) activates TRPA1 and renders it permeable to QX-314 (Lennertz et al., 2012). This approach is very similar to (although probably less effective than) that previously described to silence TRPV1* afferents by a combination of capsaicin and QX-314 (Binshtok et al., 2007; Ries et al., 2009) since TRPA1 is less permeable to QX-314 than TRPV1 (Nakagawa and Hiura, 2013).

Of note, TRPA1 was suggested to be a target for the analgesic action of acetaminophen (Andersson et al., 2011) and etodolac (Wang et al., 2013c), two time-honored NSAIDs.

5. The Contribution of Transient Receptor Potentials to Chemotherapy-Induced Neuropathic Pain. CIPN represents a major dose-limiting side effect for many commonly used antineoplastic agents, both platinum-based (e.g., cisplatin and oxaliplatin) and other (e.g., vincristine, paclitaxel and thalidomide) agents. CIPN can be especially severe in patients with coexisting independent risk factors for neuropathic pain such as diabetes and/or alcohol abuse (see Nassini et al., 2013). Cisplatin combination chemotherapy is a cornerstone of the treatment of many cancers. Cisplatin is known to cause a chronic peripheral sensory neuropathy that is manifested as neuropathic pain and sensory ataxia and may develop off-therapy with a delayed onset, even several months after the completion of treatment (Park et al., 2008). This form of cisplatin-induced neuropathy is often irreversible, is progressive, and shows signs of spinal demyelinating disease. The initial response rate to cisplatin is high, but many patients will eventually relapse with cisplatin-resistant cancer. The third-generation platinum drug oxaliplatin is typically administered in a combination known as FOLFOX (with fluorouracil and leucovorin) for the treatment of advanced colorectal cancer. Oxaliplatin has less ototoxicity and nephrotoxicity than cisplatin but it can cause an acute painful neuropathy soon after administration.
Paclitaxel (originally derived from the bark of the Pacific yew, *Taxus brevifolia*) is a mitotic inhibitor that is also effective against cisplatin-resistant tumors. Paclitaxel acts by promoting the assembly of abnormal bundles of microtubules in the mitotic machinery. Microtubules are, however, also important for the development and maintenance of neurons. In the rat, paclitaxel was shown to accumulate both in Schwann cells and DRG neurons, causing the formation of unusual microtubule aggregates with resultant demyelination and loss of axoplasmic transport. These findings suggest that paclitaxel causes a combination of sensory axonopathy and ganglioneuropathy with an end result of severe sensory peripheral neuropathy (Dougherty et al., 2004). Signs and symptoms of peripheral neuropathy may begin as early as 24 hours after administration of a single, high dose of paclitaxel. Affected patients commonly complain of numbness, tingling, and burning pain, which typically occurs in a “glove and stocking” distribution (Dougherty et al., 2004). Sensory symptoms usually start symmetrically in the feet but can also appear simultaneously in both hands and feet. Although mild cases have been reported to resolve after discontinuation of therapy, the sensory abnormalities can persist in patients who develop severe neuropathy. Despite attempts to minimize paclitaxel-induced neuropathy by changes in dosing and formulation, this adverse effect remains a major barrier to achieving the desired clinical response in many patients.

Although antineoplastic agents linked to CIPN vary in both their chemical structure and mechanism of action, they seem to share a common target, mitochondria (Jaggi and Singh, 2013). For example, paclitaxel-induced painful peripheral neuropathy is associated with the presence of swollen and vacuolated axonal mitochondria (Flatters et al., 2006). Likewise, bortezomib was shown to cause intracytoplasmic vacuolization in DRG satellite cells, probably due to mitochondrial and endoplasmic reticulum enlargement (Cavaletti et al., 2007). The molecular targets that mediate paclitaxel- and bortezomib-induced mitochondrial toxicity are, however, different. Whereas paclitaxel appears to gate the mitochondrial permeability transition pore (Flatters et al., 2006), bortezomib activates mitochondrial-based apoptotic pathways, including activation of caspases (Cavaletti et al., 2007; Broyl et al., 2010). A major player in mitochondrial toxicity is Ca$^{2+}$ overload. Indeed, Ca$^{2+}$-chelating agents were shown to reverse paclitaxel-evoked pain (Siau and Bennett, 2006). Of note, mitochondrial dysfunction has been linked to neuropathic pain (Botzem and Herrmann, 2010; Reichling and Levine, 2011; Vincent et al., 2011) and migraine (Stuart and Griffiths, 2012), pointing to TRP channels as downstream targets for the oxidative stress.

Finally, significant changes in the expression of various genes, including those controlling mitochondrial dysfunction due to vincristine- and bortezomib-associated peripheral neuropathy, have been demonstrated in humans (Broyl et al., 2010). Given their role in both generating neuropathic pain and regulating mitochondrial calcium homeostasis, TRP channels are attractive targets to explore in CIPN (see Nassini et al., 2013).

Patients on platinum-based chemotherapy regimens often complain about heat hypersensitivity (Carozzi et al., 2010). There is a growing body of evidence that this adverse effect is predominantly mediated by TRPV1. In the mouse, cisplatin was reported to upregulate TRPV1 mRNA both in vivo and in cultured DRG neurons (Ta et al., 2010). In cisplatin-treated mice, the increase in TRPV1 mRNA was associated with enhanced nociceptor responsiveness, and these animals (similar to the patients) developed thermal, but not mechanical, hyperalgesia. Likewise, enhanced capsaicin responses were seen in rats treated with oxaliplatin (Anand et al., 2010).

Patients with CIPN also exhibit mechanical allodynia. This has been attributed to TRPV4 activation, mostly based on the observation that TRPV4(-/-) mice exhibit reduced mechanical hyperalgesia compared with wild-type animals in response to treatment with paclitaxel (Alessandri-Haber et al., 2004) and vincristine (Alessandri-Haber et al., 2008). This is in keeping with the reports that in various models of painful peripheral neuropathy, including those that develop in diabetic animals, the mechanical hyperalgesia is markedly reduced by intrathecal administration of oligodeoxynucleotides that are antisense to TRPV4 (Alessandri-Haber et al., 2008). Paclitaxel and vincristine do not activate TRPV4 directly, nor do they enhance TRPV4 mRNA levels (Alessandri-Haber et al., 2004). So how do these agents sensitize TRPV4? According to a recent theory, a unique signaling cascade is activated by integrins during CIPN that, in turn, leads to membrane insertion and/or activation of the TRPV4 channel in sensory neurons via Src tyrosine kinase (Odell et al., 2005). An alternative model focuses on PAR-2 (Chen et al., 2011). This model holds that paclitaxel releases mast cell tryptase and thereby activates PAR-2 in DRG neurons. There is good evidence that PAR-2 activation may indirectly sensitize (via PKA, PKCε, and PLC) a number of TRP channels, including TRPV1, TRPA1, and TRPV4, thereby contributing to the molecular
pathomechanism of mechanical allodynia and thermal hyperalgesia. The extrapolation of these observations to humans is, however, hindered by the apparent lack of painful phenotype in patients carrying gain-of-function TRPV4 mutations.

In summary, the participation of TRPV4 in the development and maintenance of mechanical allodynia is controversial. Furthermore, neither TRPV4 nor TRPV1 can account for the cold hyperalgesia that patients with CIPN often display (see Nassini et al., 2013).

Geppetti and co-workers recently explored the role of TRPA1 in rodent CIPN models (Nassini et al., 2011; Materazzi et al., 2012; Trevisan et al., 2013). They showed that TRPA1 entirely mediates both the oxidative stress and cold hypersensitivity that develops in mice and rats in response to oxaliplatin and cisplatin administration. Moreover, in paclitaxel-treated animals, the TRPV4-resistant component of the mechanical hyperalgesia was also mediated by TRPA1 (Nassini et al., 2011). Oxaliplatin and paclitaxel do not directly gate TRPA1 because they do not cause any Ca^{2+} response in mouse or rat DRG neurons. However, in CHO cells transfected with the mouse TRPA1 channel, oxaliplatin evokes intracellular Ca^{2+} mobilization in a glutathione-sensitive manner that is absent in untransfected CHO cells. To explain these seemingly contradictory results, the authors have hypothesized that the Ca^{2+} response to oxaliplatin challenge requires two conditions: 1) the presence of TRPA1, and 2) cells that generate sufficient levels of oxidative stress in response to oxaliplatin to activate TRPA1 (see Nassini et al., 2013). It is possible that DRG neurons do not produce oxidative stress products in amounts sufficient to activate TRPA1, whereas CHO cells possess the metabolic and enzymatic repertoire to produce high enough ROS levels (Nassini et al., 2011). Cells that are in the proximity of TRPA1-expressing nerve terminals may release oxidative stress by-products generated by paclitaxel and thus activate TRPA1. In support of this hypothesis, paclitaxel was reported to worsen cold hyperalgesia in diabetic rats compared with normoglycemic animals and this effect was prevented both by the ROS scavenger N-acetylcysteine and the selective TRPA1 antagonist, HC-030031 (Nassini et al., 2011). Paclitaxel treatment was associated with an accumulation of atypical mitochondria and an increase in mitochondrial ROS production. Interestingly, platinum-based drugs also increase TRPA1 expression in DRG neurons. In rats, the novel TRPA1 antagonist ADM-09 ameliorates oxaliplatin-induced neuropathic pain (Nativi et al., 2013).

In sensory neurons, TRPM8 receptor expression is increased after nerve injury (Frederick et al., 2007). A similar increase in TRPM8 mRNA was noted in DRG neurons of mice treated with oxaliplatin (Gauchan et al., 2009). Importantly, this increase in TRPM8 mRNA correlated to the development of cold hypersensitivity. These findings were interpreted to imply that oxaliplatin-induced cold hypersensitivity is, at least in part, mediated by TRPM8. If so, TRPM8 activators such as menthol and icilin should exacerbate CIPN symptoms. Indeed, wet-dog shake and jumping behaviors elicited by icilin were enhanced in mice treated with oxaliplatin. Icilin is, however, not selective for TRPM8. Icilin also activates TRPA1 and this response seems to be potentiated by oxaliplatin (Anand et al., 2010). Furthermore, topical menthol application was reported to show a paradoxical analgesic effect in CIPN induced by bortezomib (Colvin et al., 2008). In addition, menthol was able to significantly reverse CIPN induced by carboplatin, and its prolonged application during chemotherapy appeared to prevent the worsening of CIPN. Clearly, more basic and clinical investigation is needed to clarify the role of TRPM8 in CIPN.

6. Transient Receptor Potential Channels in Migraine.

a. The role of vanilloid transient receptor potential 1 in migraine and cluster headache. Migraine is a common, debilitating episodic disorder characterized by unilateral throbbing headache, phonophobia, photophobia, and nausea, which is sometimes preceded by premonitory symptoms (so-called “aura”) (reviewed in Nassini et al., 2010a; Benemei et al., 2013). For unclear reasons (that may include the hormonal milieu), migraine is more common in women (18%) than in men (6%). The initiation of migraine attacks has been attributed to a number of trigger mechanisms (reviewed in Kelman, 2007), including both environmental (e.g., air pollution, odors, alcohol consumption, as well as changes in temperature or weather) and physiologic factors (e.g., hormonal milieu or stress). Migraine headache is believed (at least in part) to reflect the activation of the trigeminovascular system, resulting in neurogenic inflammation of the meninges and the release of CGRP, a potent vasodilator (Geppetti et al., 2005; Raddant and Russo, 2011; Messlinger et al., 2012). This concept has gained strong experimental support by the efficacy of CGRP agonists in clinical trials (Olesen and Ashina, 2011; Salvatore and Kane, 2011). TRP channels are promising targets in migraine (see Nassini et al., 2010a, 2013; Dux et al., 2012; Oxford and Hurley, 2013), both in preventing attacks (e.g., TRPA1 is a well established target for air pollutants and odors) and in relieving headache (TRPV1 is thought to play a central role in neurogenic inflammation including CGRP release).

Retrograde labeling studies demonstrated that a distinct subset (approximately 25%) of the trigeminal ganglion cells projecting to the dura express TRPV1, and 80% of these neurons are positive for CGRP (Shimizu et al., 2007; Chatchaisak et al., 2013). This high level of colocalization is consistent with the

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concept that TRPV1 activation in the dura stimulates CGRP release and subsequent vasodilatation (Dux et al., 2003). In addition to its presence in presynaptic terminals of trigeminal afferents, TRPV1 has also been detected, albeit at a much lower abundance, in arteriolar endothelium and smooth muscle (Kark et al., 2008; Cavanaugh et al., 2011; Czikora et al., 2012). Indeed, TRPV1 expressed in cochlear arterioles has been proposed to mediate the inner ear disturbances in migraine (Vass et al., 2004).

It is noteworthy that migraine is comorbid with a number of CNS disease states such as epilepsy, which implies that central sites might also be involved in the triggering of migraine attacks. In a murine model of temporal lobe epilepsy, increased TRPV1 expression was noted in the dentate gyrus compared with control brain (Bhaskaran and Smith, 2010). This is in keeping with the report of increased TRPV1 expression in the cortex and hippocampus of patients with mesial temporal lobe epilepsy (Sun et al., 2013). A similarly increased TRPV1 expression was described in cortical lesions removed from tuberous sclerosis patients to ameliorate epileptic seizures (Shu et al., 2013). Taken together, these findings imply that TRPV1 may represent a novel antiepileptogenic target, also relevant for preventing migraine attacks. However, conflicting reports have made it difficult, if not impossible, to assign any pathophysiologic role for TRPV1 in the CNS. Recent experimental data (obtained by TRPV1 reporter mice, a sensitive genetic approach for lineage tracing) have even questioned the very existence of brain TRPV1, except for a few small regions in the CNS, most notably the hypothalamus (Cavanaugh et al., 2011). Other studies reported a dramatic (almost complete) loss of TRPV1 in the rat brain by postnatal day 26 (Koles et al., 2013). These discrepant reports must be reconciled before brain TRPV1 can be validated as a potential target to prevent migraine attacks.

In preclinical studies, a large body of evidence implicates TRPV1 in migraine pain (reviewed in Szallasi et al., 2006; Goadsby, 2007; Meents et al., 2010). In the mouse, TRPV1 is highly expressed in trigeminal dural afferents, where it mediates neurotransmitter release (Huang et al., 2012). This provides the anatomic basis for understanding the functional significance of TRPV1 activation in headache pathophysiology. Capsaicin evokes CGRP release from dural tissue via TRPV1 activation, which in turn leads to CGRP-dependent vasodilatation (Eltorp et al., 2000). Furthermore, in trigeminal nucleus caudalis slices, capsaicin produces repetitive action potential formation and subsequent CGRP release that is inhibited by the antimigraine drug sumatriptan (Evans et al., 2012). Importantly, in the cat, application of the inflammatory soup to the dura increased blood flow and led to neuronal sensitization to mechanical stimuli; these responses were reversed by the TRPV1 antagonist SB705498 (Lambert et al., 2009). Finally, in humans, desensitization to intranasal civamide application was reported to ameliorate cluster headache (Saper et al., 2002), a pain condition that shares some pathophysiological characteristics of migraine, including involvement of the trigeminovascular system.

Combined, these findings imply an important role for TRPV1 in the initiation and propagation of migraine, as well as in the development and maintenance of hyperalgesia and allodynia that often accompany migraine attacks. It was speculated that TRPV1 is an important mediator of CGRP release, responsible for vasodilatation and plasma extravasation in the meninges (see Szallasi et al., 2006). In turn, mediators generated during neurogenic inflammation sensitize trigeminal nociceptors presumably by potentiating TRPV1 channel function via phosphorylation and increased membrane trafficking (Meents et al., 2010). In support of this theory, NGF (a component of the inflammatory soup and a proven TRPV1 modulator) was found to be elevated in the cerebral spinal fluid (Sarchielli et al., 2007), plasma and saliva (Jang et al., 2011) of migraineurs. However, the TRPV1 antagonist A-993610 [\((S)-1-(3-chloropyridin-2-yl)-3-methyl-N-(4-(trifluoromethyl)phenyl)-1,2,3,6-tetrahydropyridine-4-carboxamide] failed to prevent cortical spreading depression in a rat model of migraine, arguing against a therapeutic potential for TRPV1 antagonism in the management of acute migraine (Summ et al., 2011). Of note, a clinical trial with the TRPV1 antagonist SB705498 in patients with migraine was completed but the outcome has not yet been made public.

Chronic migraine (defined as 15 or more headache days a month with headache episodes lasting 4 hours or longer for at least 3 consecutive months) is a debilitating condition that affects approximately 3% of patients with migraine with. As of today, it is unclear why migraine becomes chronic but functional magnetic resonance imaging studies suggest a role for the dysfunction of the descending antinociceptive systems. It was speculated that TRPV1 activation in the trigeminal nucleus caudalis might contribute to the chronification of migraine by altering microglia (Shibata, 2012). If this hypothesis holds true, TRPV1 antagonists may prove useful in preventing the development of chronic migraine in at-risk patients even if these antagonists provide no benefit during the acute attacks.

Finally, capsaicin-sensitive trigeminal afferents innervating the nasal mucosa were implicated in the pathomechanism of sinus headache and hemiangina. There is anecdotal evidence that capsaicin-containing nasal sprays may relieve sinus headache (indeed, a number of OTC capsaicin preparations are commercially available for this indication). Clinical trials with intranasal civamide (a synthetic capsaicin congener)
are now complete, but the results have not yet been disclosed (ClinicalTrials.gov identifiers NCT00069082 and NCT00033839). Preliminary studies suggest a link between migraine and TRPV1 variants (Carrero et al., 2012; Rainero et al., 2013).

b. Ankyrin transient receptor potential 1 as a candidate sensor for environmental irritants that provoke migraine attacks. TRPA1 is highly coexpressed with TRPV1 in a subpopulation of dural afferents. TRPA1 detects environmental irritants such as acrolein (found in cigarette smoke, a known inducer of headache) (reviewed in Bessac and Jordt, 2008; Facchinetti and Patacchini, 2010; Nilius et al., 2011, 2012) and umbellulone (structure shown in Fig. 7), the active agent in the Californian “headache tree” Umbellularia californica (Zhong et al., 2011; Edelmayer et al., 2012; Nassini et al., 2012a). Moreover, TRPA1 is activated by endogenous products of inflammation and oxidative stress (see Bautista et al., 2013). Importantly, these TRPA1 agonists induce CGRP release from dural tissue and stimulate meningeal vasodilatation (Kunkler et al., 2011). There is good evidence that activation by irritants of TRPA1 receptors at trigeminal nerve terminals in the nasal mucosa and subsequent activation of the trigeminovascular system play an important role in air pollution–induced headache (see Nassini et al., 2013; Oxford and Hurley, 2013). Studies on the potential role of TRPA1 in migraine are, however, still in their infancy (reviewed in Benemei et al., 2013).

c. Possible Involvement of other transient receptor potential channels in migraine. In addition to TRPV1, three other members of the vanilloid subfamily of TRP channels (TRPV2, TRPV3, and TRPV4) are expressed on trigeminal neurons innervating the dura, but no specific evidence has yet appeared that either TRPV2 or TRPV3 is important in migraine (Rainero et al., 2013). By contrast, TRPV4 is strongly implicated in some migraine symptoms. TRPV4 has been identified as a probable mechanosensor and osmosensor on dural afferents (Wei et al., 2011b), and it was hypothesized that activation of TRPV4 may be the source of the throbbing head pain seen in migraine after movement or coughing. Indeed, activation of TRPV4 on dural afferents was shown to produce headache-related behavior in a rat model of migraine (Wei et al., 2011b). In this study, approximately 50% of dural afferents identified by retrograde labeling showed sensitivity to hypotonic solutions and/or 4α-PDD (Fig. 8), both known chemical activators of TRPV4. Topical application of these substances to the dura produced allodynia that could be blocked by a TRPV4 antagonist in vivo. Unfortunately, adverse effects will probably preclude systemic TRPV4 antagonist therapy, but topical administration (e.g., nasal spray) may be of therapeutic value in patients with migraine.

TRPM8 might be interesting with regard to migraine in that it appears to be more highly expressed in trigeminal ganglion than in DRG neurons (Kobayashi et al., 2005). TRPM8 knockout mice exhibit significant deficiencies in behavioral responses to a range of cold temperatures, but the importance of TRPM8 in the development of cold hyperalgesia and allodynia is still hotly debated. Although OTC menthol patches are available for migraine, the evidence for their clinical efficacy is at best anecdotal.

d. Migraine as a transient receptor potential channelopathy? The observation that more than half of patients have at least one first-degree relative with migraine implies a strong genetic predisposition (Shyti et al., 2011). Indeed, some rare familial forms of migraine are due to SNPs in genes encoding ion channels, including the Ca_{2.1} voltage-gated calcium channel, the Na_{1.1} voltage-gated sodium channel, and the TWIK-related spinal cord K^{+} channel (TRESK) (reviewed in Van den Maagdenberg et al., 2010; Silberstein and Dodick, 2013). These mutations are all predicted to be gain of function, leading to an increase in neuronal excitability. For example, mice bearing familial hemiplegic migraine mutations in the Ca_{2.1} protein exhibit enhanced susceptibility to cortical spreading depression, presumably due to increased release of neurotransmitters (Van den Maagdenberg et al., 2010). On the basis of these observations, it is a reasonable assumption that gain-of-function TRP channel gene mutations may also contribute to migraine.

Thus far, a genetic polymorphism in two TRP genes, TRPV1 (Carrero et al., 2012) and TRPM8 (Chasman et al., 2011), has been implicated in the pathomechanism of migraine, although the available evidence is rather weak and indirect. Cortical hyperexcitability is associated with both migraine and epilepsy. Indeed, antiepilepsy drugs that were useful in migraine prophylaxis reduced susceptibility to cortical spreading depression in animal studies. A recent study describes a SNP allele in the TRPV1 channel that is associated with enhanced synaptic transmission in the cerebral cortex and produces larger currents in heterologous expression systems (Mori et al., 2012). When subjected to transcranial magnetic stimulation using a paired pulse stimulus in the motor cortex, individuals homozygous for this allele exhibited larger short-interval intracortical facilitation. Again, this report is difficult to reconcile with the apparent absence of TRPV1 in the mouse brain cortex (Cavanaugh et al., 2011).

7. Transient Receptor Potential Channels and Pruritus. Itch (pruritus) is an unpleasant sensation that elicits the desire (or reflex) to scratch. Itch has many similarities to pain; indeed, it was referred to as the “skin equivalent of chronic pain” (Kini et al., 2011). Chronic itch is a large, unmet medical need. According to a questionnaire-based German study, the lifetime prevalence of chronic pruritus exceeds 20% (Matterne et al., 2009), although the real prevalence is unknown because many patients never seek medical attention.
The similarities and differences between itch and pain sensation (Fig. 28) are only beginning to be understood (reviewed in Goutos, 2013). We scratch ourselves until it hurts to relieve itch, suggesting a common neuronal pathway for pain and itch (Akiyama et al., 2012). Indeed, scratching blocks responses of spinal second-order neurons to pruritic, but not algesic, stimuli (Nishida et al., 2013). The intensity theory states that the very same primary afferents are capable of triggering both pruritus and pain (reviewed in Ross, 2011; Tóth and Bíró, 2013; Tóth et al., 2014). This model is supported by the observations that 1) intradermal capsaicin injection can cause both itch and pain (Shimada and LaMotte, 2008; Klein et al., 2011; Sikand et al., 2011) in a dose-dependent manner, and 2) desensitization to topical capsaicin creams alleviates both pain and pruritus (see Szallasi and Blumberg, 1999). However, modern research points to the existence of an autonomous pruriceptive system (Fig. 28), organized more or less independently from nociception (labeled-line theory) (reviewed in Ma, 2012; Tóth et al., 2014). This pruriceptive system is thought to have two functionally and anatomically distinct subdivisions, distinguished by a characteristic response (or the lack of it) to histamine (see Tóth and Bíró, 2013).

Histamine released from activated mast cells and basophils plays a central role in allergic responses. Histamine was shown to directly activate sensory neurons by interacting at H1 and H4 receptors to evoke itch in humans (see Shim and Oh, 2008). Histamine-sensitive neurons are unmyelinated sensory C-afferent neurons that respond to both chemical and thermal, but not mechanical, stimuli and exhibit a combination of low conduction velocity and high transcutaneous electrical threshold (Schmelz et al., 2003; Ständer et al., 2003, 2011). These neurons express TRPV1 (Fig. 29). Indeed, in the itch pathway, the H1 histamine receptor is indirectly coupled downstream to TRPV1 via PLA2 (Kim et al., 2004; Shim and Oh, 2008; Imamachi et al., 2009). Histamine plays a pivotal role in pruritic, urticarial skin diseases in which antihistamines provide almost instantaneous symptomatic relief (Kiss and Keserü, 2012). However, antihistamines are without any clinical benefit in many disorders characterized by chronic pruritus (including atopic dermatitis/eczema, and allergic as well as dry skin itch), implying the existence of a second, histamine-insensitive pruriceptive pathway (Handwerker, 2010).

Intradermal insertion of spicules from the pods of the cowhage plant Mucuna pruriens (broadly used in Ayurvedic medicine) was shown to activate mechanosensitive, but not mechanoinsensitive, C fibers, as well as a subset of nociceptive, myelinated A fibers (Ringkamp et al., 2011). Although both evoke a similar itchy sensation, the skin reaction to histamine and cowhage is different in that histamine produces a wheal reaction, whereas cowhage does not (Johanek et al., 2007). The active ingredient in cowhage was identified as mucunain (Shelley and Arthur, 1955), a novel cysteine protease that activates PAR-2 and PAR-4 (Reddy et al., 2008).

PAR-2 is a major itch mediator: PAR-2 agonists enhance, whereas an anti-PAR-2 antibody attenuates, scratching behavior (Akiyama et al., 2010). When
applied to the human skin, mucunain clearly evokes a histamine-independent itch reaction. However, the possibility could not be excluded that both mucunain and histamine target TRPV1 via different second messengers, PAR-2 and PLA2, respectively. Indeed, it was postulated that endogenous pruritogenic substances act on TRPV1 indirectly via PAR-2 activation, based on a rat model of hepatogenic pruritus induced by bile duct ligation (Belghiti et al., 2013).

A recent breakthrough in pruritus research was the observation that the antimalarial drug chloroquine evokes scratching behavior in wild-type, but not TRPA1-null, mice via the Mas-related G protein–coupled receptor A3 (MrgprA3) (Fig. 29; Wilson et al., 2011). Similarly, SLIGRL (an itch-producing peptide that, such as cowhage, activates PAR-2) causes itch by activating TRPA1 receptors via MrgprC11 (Fig. 29; Wilson et al., 2011; Liu et al., 2013a). Thus, in a much simplified manner, it can be postulated that the pruriceptive system has two major subdivisions: a histamine-sensitive/TRPV1-positive subdivision, in which the histamine receptor H1 is coupled to TRPV1 via PLA2; and a histamine-insensitive/TRPA1-expressing subdivision, in which TRPA1 is the downstream target of MrgprA3 and MrgprC11 (see Fig. 29). In support of this model, activity-dependent silencing of TRPV1- and TRPA1-positive afferents selectively blocks histamine- and chloroquine-induced scratching, respectively (Roberson et al., 2013). The real situation is, however, probably more complex because genetic inactivation of Pirt abolishes both histaminergic and nonhistaminergic itch (Patel et al., 2011). Indeed, TRPV1 appears to be coexpressed with TRPA1 in both nociceptive and pruriceptive afferents, both of which are positive for CGRPα (McCoy et al., 2013b). Genetic ablation of these CGRPα-positive afferents reduce sensitivity to thermal and chemical (capsaicin) pain, as well as itch induced by histamine and chloroquine. By contrast, ablation of MrgprA3-positive neurons by genetic manipulation leads to a substantial decrease in scratching behavior, whereas pain sensitivity remains intact (Han et al., 2013). Furthermore, only a minority (43%) of chloroquine-sensitive DRG neurons was found to express TRPA1; the majority (57%) seemed to respond to chloroquine in a TRPC3-mediated fashion (Than et al., 2013). If confirmed, this observation will furnish TRPC3 with a previously unsuspected function in itch pathogenesis.

There is increasing experimental support for TRPA1 as a novel target for antipruritic drugs. Urushiol, the contact allergen in poison ivy, evokes pruritogenic responses in wild-type, but not in TRPA1-null, mice (Liu et al., 2013a). Chronic itch evoked by impaired skin barrier is also attenuated in the TRPA1-deficient animals (Wilson et al., 2013a). TRPV1 as an itch target is more problematic. Topical capsaicin relieves pruritus (see Papoiu and Yosipovitch, 2010); however, somewhat paradoxically, chemical ablation of sensory afferents by systemic capsaicin administration results in chronic pruritic dermatitis in mice with skin ulceration due to extensive scratching behavior (www.abstractstosubmit.com/IBRO2011/abstracts/main.php?do).

The TRPV1 antagonist PAC-14028 (Fig. 9) reduces, but does not eliminate, scratching behavior in a murine model of atopic dermatitis (Yun et al., 2011a,b). By contrast, SB705498 (compare structures in Fig. 9) did not provide any symptomatic relief in patients with seasonal allergic rhinitis (Bareille et al., 2013). Finally, comparable attenuation of the intradermal LTβ4-induced scratching behavior by TRPV1 [SB366791, 97% inhibition] and TRPA1 [TCS-5861528, 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-[4-(1-methylpropyl)phenyl]acetamide; 82% inhibition] antagonism in mice was recently reported (Fernandes et al., 2013).

IL-31 produced by Th2 cells evokes itch when injected into the skin of mice (Dillon et al., 2004) or dogs (Gonzales et al., 2013). Several lines of experimental evidence point to IL-31 as a critical neuro-immune link between pathogenic T cells and pruriceptive afferents. When overexpressed in transgenic mice, IL-31 induces a pruritic skin condition that resembles
human atopic dermatitis (Dillon et al., 2004). Importantly, TH2 cells overproduce IL-31 in both canine (Gonzales et al., 2013) and human (Sonkoly et al., 2006) atopic dermatitis. A large subset of sensory neurons that coexpress TRPV1 with TRPA1 carry a receptor for IL-31 (Cevikbas et al., 2013); indeed, the IL-31-induced pruritus is ameliorated in both TRPV1- and TRPA1-deficient mice.

It was suggested that pain- and itch-transmitting afferents are distinguished by the central mediators that they use. Indeed, TRPV1-expressing pruriceptive afferents contain gastrin-releasing peptide (GRP) in humans (Timmes et al., 2013). Mice whose GRP receptors have been deleted by genetic manipulation were reported to show normal pain response but no scratching (itch) behavior (Sun and Chen, 2007). By contrast, TRPV1-positive nociceptive afferents lack GRP but carry the vesicular glutamate transporter-2. Importantly, loss of vesicular glutamate transporter-2 renders mice less sensitive to pain but, paradoxically, more sensitive to itch (Lagerström et al., 2010; Liu et al., 2010b). Mice that express a constitutively active form of the serine/threonine kinase BRAF (member B of the Raf-kinase family of growth signal transduction proteins kinases) in their sensory neurons gated by Na+,1.8 (so-called BRAFNa1.8 mice) show spontaneous scratching behavior indicative of chronic pruritus (Zhao et al., 2013b). A subset of TRPV1+ neurons contains the neuropeptide natriuretic polypeptide b (Nppb). Nppb evokes potent scratching when injected intrathecally to mice (Misra and Hoon, 2013); conversely, Nppb(−/−) mice show markedly reduced responses to various itch-producing agents.

To resolve these somewhat conflicting findings, it was postulated that specific subsets of interneurons may help distinguish itch from pain in the spinal cord. For example, mice that lack Bhlhb5-positive (a transcription factor whose absence leads to abnormal neuronal differentiation) interneurons show dramatically enhanced scratching behavior in response to pruritogenic agents but no change in nociception (Ross et al., 2010). This model is in keeping with the observation that morphine, which activates μ-opioid receptors in the spinal cord, may relieve pain and cause/enhance pruritus at the same time.

The processing of itch in the CNS is complex and poorly understood. In primates, two distinct populations of spinothalamic tract neurons were found to transmit histaminergic and nonhistaminergic (e.g., cowhage-evoked) itch. In accord, positron emission tomography studies revealed different cerebral activity patterns during itch evoked by histamine and cowhage. Generally speaking, in the human brain, the activity in the posterior cingulate cortex correlates to itch, whereas the thalamus is active during pain only (Mochizuki et al., 2007).

An intriguing and possibly underappreciated pruritogenic target is TRPV3 (recently reviewed in Nilius and Bíró, 2013; Nilius et al., 2014). Indeed, Olmsted syndrome, a rare genetic disorder that is due to a gain-of-function (G573S) mutation in TRPV3 (Lin et al., 2012), is characterized by debilitating itch. This is in keeping with the phenotype of transgenic mice over-expressing human TRPV3 G573S that includes loss of hair, dermatitis, and severe scratching behavior (Huang et al., 2008). DS-Nh mice that carry a gain-of-function TRPV3 isoform also display dermatitis and a hairless phenotype (Asakawa et al., 2006). Conversely, TRPV3 knockout mice exhibit amelioriated scratching response in a model of dry skin–induced pruritus (Cheng et al., 2010b). Importantly, TRPV3 appears to be under the negative control of Mg2+ and it was postulated that dietary Mg2+ deficiency may liberate TRPV3 from this tonic inhibitory control, leading to a constitutively active TRPV3 channel (Luo et al., 2012a). Indeed, a Mg2+-deficient diet causes dermatitis and scratching behavior in rats (Choi et al., 2008). Taken together, these findings imply a therapeutic potential for topically applied TRPV3 antagonists in the management of pruritus secondary to (atopic) dermatitis.

The role of TRPV3 in the development of atopic dermatitis is, however, controversial. The pathogenesis of atopic dermatitis is thought to involve compromised skin barrier formation and the skin barrier is clearly defective in the Trpv3(−/−) animals (Cheng et al., 2010b). To explain the phenotype of the Trpv3(−/−) mice, it was postulated that TRPV3 forms a complex with ADAM17 (A Disintegrin And Metalloproteinase 17), EGFR, and TGase1 that is required for normal terminal keratinocyte differentiation (Cheng et al., 2010a). Dysregulation of this complex is believed to impair barrier function and lead to dry skin. Thus, a certain level of basal TRPV3 activity seems to be essential for normal skin function, with both increased and decreased activity leading to skin disease. In accord, keratinocyte migration and wound healing are impaired in the Trpv3(−/−) mice. If so, one would not want to apply a TRPV3 antagonist cream on pruritic but damaged (scratched/ulcerated) skin. On the other hand, topical TRPV3 agonist administration might be useful in the management of nonhealing skin ulcers (although it could cause pruritus as an on-target side effect). Recent data implicate TRPA1 as the primary itch mediator in atopic dermatitis (Oh et al., 2013; Wilson et al., 2013a,b). Diseased (atopic dermatitis) keratinocytes release thymic stromal lymphopoietin (TSLP) via the ORAI1/NFAT pathway; in turn, TSLP activates TRPA1-expressing cutaneous afferents to trigger itch (Wilson et al., 2013b). Importantly, TSLP also regulates immune cells that are responsible for
the development of asthma in patients with atopic dermatitis, the so-called “atopic march.” It is noteworthy that keratinocytes isolated from DS-Nh mice carrying a gain-of-function TRPV3 mutation also show increased responses to TSLP (Yamamoto-Kasai et al., 2013). As a note of caution, the pathobiology of chronic itch is complex and still poorly understood (see Raap et al., 2011); therefore, it is probably prudent to temper our expectations with regard to the therapeutic potential of blocking individual TRP channels.

B. Transient Receptor Potential Channels in Airway Disorders

1. What Should We Target in Patients with Chronic Cough: Ankyrin Transient Receptor Potential 1, Vaniloid Transient Receptor Potential 1, or Both? In a somewhat simplified manner, the airways transport gases to and from the pulmonary alveoli where the gas exchange with the circulating blood takes place. Functionally, the lungs can be divided into the airways and the pulmonary vasculature. The airways are a gateway not only to gas exchange but also to airborne pollutants, allergens, and infectious agents. The main airways are lined by a ciliated columnar epithelium covered by mucus, the main function of which is to filter out and remove harmful particulate matters. Motile cilia directly sense noxious substances entering the airways and initiate defensive mechanisms (Shah et al., 2009). Indeed, ciliary dysfunction (“ciliopathy”) is now recognized as an early sign of chronic inflammatory lung diseases (Horváth and Sorscher, 2008). The possible connection between smoking, ciliopathy, and COPD is now subject to intensive research (weill.cornell.edu/news/releases/wcmc/wcmc_2012/07_12_12b.shtml).

The airway mucus is produced by goblet cells, and goblet cell hyperplasia is a hallmark of allergic asthma. The columnar respiratory epithelium may undergo squamous metaplasia in response to long-term irritation such as cigarette smoke. This can not only compromise mucociliary airway clearance but can also create an environment for the emergence of squamous cell carcinoma (SQCC).

Large airways (bronchi) are held open by cartilage rings, whereas smaller airways (bronchioles) are supported by the surrounding lung tissue. Both contain circular smooth muscle that can regulate the airway diameter by constricting or dilating. Bronchiole terminal in alveolar sacs in which a single layer of pneumocytes line delicate fibrous septae containing a dense capillary network, the site of gas exchange between the pulmonary circulation and the external milieu. Destruction of these septae creates large air spaces called emphysema, whereas the proliferation of fibroblasts in the septae leads to pulmonary fibrosis. Both of these pathologic processes will eventually compromise gas exchange, leading to hypoxemia and hypercapnia.

The pulmonary vessels are lined by endothelial cells. When these cells become “leaky,” it will manifest as pulmonary edema. The proliferation of vascular smooth muscle (in particular, the occurrence of smooth muscle in small arterioles that do not normally have a muscular layer) is a hallmark of pulmonary hypertension.

Last but not least, the mammalian respiratory tract is lined with a dense plexus of nerve fibers by irritant and/or inflammatory stimuli triggers multiple reflexes such as sneezing, coughing, mucus secretion, bronchospasm, and apnea that limit ventilation and dilute/expel foreign materials. Analogous to pain, in which inflammatory mediators produce a hypersensitivity to various stimuli, reflexes including cough are proposed to be sensitized by mediators of inflammation and oxidant stress such that they become triggered by innocuous stimuli (see Undem and Carr, 2010; O’Neill et al., 2013; Spina and Page, 2013). This is believed to be the underlying mechanism of sensory hyper-reactivity syndrome (see Millqvist, 2011).

In summary, the airways are built of epithelium, smooth muscle, and connective tissue (fibroblasts), with an important contribution of sensory nerves and resident immune cells. The pulmonary vasculature consists of endothelial cells, smooth muscle, and connective tissue. TRP channels are expressed in all of these tissues and cells (see Fig. 30) and there is good evidence that they play a pivotal role in the development and maintenance of chronic lung diseases, ranging from chronic cough through COPD and asthma to idiopathic pulmonary fibrosis (IPF) and IPAH (see Nassini et al., 2010c; Abbott-Banner et al., 2013).

Combined, these diseases already affect hundreds of millions of patients worldwide and their prevalence is on the rise. By the end of this decade, COPD alone is predicted to be the third leading cause of death and fourth commonest cause of disability. Unfortunately, available COPD treatment options (including inhaled corticosteroids, long-acting bronchodilators, and roflumilast, a phosphodiesterase-4 inhibitor) provide only symptomatic relief and do not halt (or reverse) disease progression. With regard to IPF and IPAH, the therapeutic options are even less satisfactory. Clearly, there is an urgent need for novel therapeutics and, as discussed below, there is a cautious optimism in the field that targeting certain TRP channels/functions may have important therapeutic benefits in selected patients with chronic respiratory disease (see Takemura et al., 2008; Moran et al., 2011; Preti et al., 2012; Abbott-Banner et al., 2013). A number of tools (e.g., selective TRPV1 and TRPA1 antagonists and TRPV4 agonists) are already available for clinical studies and now the major challenge (similar to the pain field) is to identify the patients who are most likely to benefit from these molecules. For example, asthma is a postulated therapeutic target for TRPA1 (and maybe also...
TRPV1, TRPV4, TRPM4, TRPC1, and TRPC4) antagonists (see Facchinetti and Patacchini, 2010; Abbott-Banner et al., 2013). Asthma is, however, a heterogeneous disease with at least six different forms and patients belonging to these different forms are likely to respond differently to TRPA1 blockade.

a. Vanilloid transient receptor potential 1. The most common type of sensory nerves in the mammalian respiratory tract are C fibers (see Canning, 2006) that express TRPV1 (see Geppetti et al., 2006). These fibers are relatively unresponsive to mechanical changes that occur during respiration but can be selectively stimulated by BK (and other inflammatory mediators), acidic solutions, and capsaicin. Parenthetically, a recent study reported the presence of TRPV1 in bronchial epithelium with a marked upregulation in individuals with asthma (McGarvey et al., 2014). Moreover, TRPV1 expression was detected in airway smooth muscle cells with a similarly increased expression in asthmatic rats (Zhao et al., 2013a). These are unexpected and controversial findings that need to be confirmed. Below, we focus on neuronal TRPV1 receptors in the airways, the existence of which is firmly established.

There are at least two distinct types of vagal C fibers, bronchial and pulmonary, that innervate different regions of the respiratory tract and also differ in their activation profiles and neurochemistry. A third (and probably the least understood) population of C fibers in the respiratory tract originates from the DRG. Adding to this anatomic complexity is the fact that capsaicin-sensitive C fibers show striking species-related differences in the airways. For example, when exposed to inhaled capsaicin, rats do not cough or show bronchoconstriction; however, they develop a marked neurogenic inflammatory (edema) response that is absent in animals desensitized to capsaicin (Lundberg and Saria, 1983).

Guinea pigs are very sensitive to inhaled capsaicin: they severely cough and display both airway edema and constriction (see Canning, 2006). Capsaicin-evoked cough occurs only in conscious (but not in anesthetized) animals (see Undem and Carr, 2010). Humans also respond with cough to inhaled capsaicin (Fig. 31A). Study subjects describe an itchy, urge-to-cough sensation that slowly builds up in intensity and is relieved by voluntary coughing. This is very different from the explosive, involuntary cough response that occurs in response to aspiration even in anesthetized animals. Humans also develop a neurogenic inflammatory response in the airways (see Joos et al., 1995). There is good evidence (as reviewed in Baraniuk, 2001; Lacroix and Landis, 2008) that in upper airway mucosa, neurogenic inflammation is involved in the
pathogenesis of rhinosinusitis. Indeed, TRPV1 expression is increased in the nasal mucosa of patients with vasomotor rhinitis (Van Gerven et al., 2013) and therapy employing intranasal capsaicin desensitization provides symptomatic relief in these individuals (Marabini et al., 1991). This procedure is, however, poorly tolerated (painful) and thus has not been widely adopted. It is still hotly debated whether neurogenic inflammation in humans can play any significant role in lower airway diseases such as asthma and/or COPD (for different views, please see Barnes, 2001; Widdicombe, 2003; Groneberg et al., 2004b; Butler and Heaney, 2007; Ramalho et al., 2011). According to the majority opinion, it does not.

There are limited data on capsaicin effects in human airways. In vitro, capsaicin was shown to induce contractions in isolated human bronchi; however, this effect required high (10 \( \mu \text{M} \)) capsaicin concentrations and was very weak compared with the contractile response detected in the guinea pig (Lundberg et al., 1983). In human volunteers, inhaled capsaicin evokes cough and causes mild retrosternal discomfort, as well as a transient and mild increase in airway resistance (Fuller et al., 1985). This bronchoconstrictor response did not differ between patients with asthma and smokers or nonsmokers who were otherwise healthy (Fuller et al., 1985).

Vagal \( \text{AD} \) cough afferents and bronchopulmonary C fibers converge in the nucleus of the solitary tract (see Canning, 2006). This was suggested to form the anatomic basis by which increased C fiber drive may enhance cough reflex sensitivity. The control of cough reflex by descending neural pathways was reviewed elsewhere (Mazzzone et al., 2011). Of note, the increased C fiber drive may also originate outside of the respiratory tract. GERD is a well recognized cause of chronic cough (see Pauwels et al., 2009), and antireflux surgery eliminates chronic cough in a subset of patients with GERD (Hoppo et al., 2013). Yet, intraluminal esophageal challenge with capsaicin or acids causes only pain but no cough response in humans (Wu et al., 2002). The pH challenge in the esophagus, however, sensitizes the cough response to inhaled capsaicin (Ing and Ngu, 1999). In keeping with this model, capsaicin (or SP) microinjected into the commissural subnucleus of the nucleus of the solitary tract was shown to facilitate the cough reflex (Canning and Mori, 2011). Of note, at least in rodents, \( \text{AD} \) fibers that do not respond to capsaicin under physiologic conditions may acquire capsaicin sensitivity after ovalbumin sensitization (Zhang et al., 2008b). Furthermore, the “silent majority” of TRPV1+ C fibers (that are inactive under physiologic conditions) start discharging during disturbances (Andresen et al., 2012).

Capsaicin is a prototypical respiratory irritant that evokes protective reflexes such as cough, sneeze, and fluid secretion when applied to the human respiratory mucosa. Increased sensitivity to capsaicin aerosols occurs in a number of respiratory disorders of varying severity and etiology. For instance, inhaled capsaicin

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**Fig. 31.** Increased sensitivity to inhaled capsaicin identifies a specific subset of patients with chronic cough (B) compared with healthy control subjects (A). Bronchial biopsies reveal enhanced TRPV1 expression in these patients (C), implying the therapeutic potential for TRPV1 antagonists. (A) and (B) are original experimental notes kindly contributed by K.F. Chung. (C) is reprinted with permission from Groneberg et al. (2004a).
provokes exaggerated cough response in patients with cough variant, but not classic asthma (Fig. 31B; Nakajima et al., 2006), as well as in sensory airway hyper-reactivity syndrome (SHR) (Ternesten-Hassèus et al., 2013). Indeed, the capsaicin inhalation test is broadly used as a diagnostic tool to identify patients with SHR (Johansson et al., 2002). Of note, a reduced urge-to-cough threshold to inhaled capsaicin was observed in otherwise healthy smokers (Dicpinigaitis, 2003).

Theoretically, the increase in capsaicin-induced cough may be due to the following: 1) enhanced TRPV1 expression and/or activity in airway sensory neurons, 2) airway epithelial barrier permeability alterations that allow capsaicin to more readily access airway nociceptor terminals, and 3) heightened CNS responsiveness to afferent input. Bronchial biopsies obtained from a subset of patients with chronic cough revealed an increase in TRPV1-like immunoreactivity (Fig. 31C) that correlated to the sensitivity of the patients to capsaicin-evoked cough (Groneberg et al., 2004a; Mitchell et al., 2005). Furthermore, in cultured sensory neurons, increased TRPV1 expression was observed 2–4 hours after rhinovirus infection (Abdullah et al., 2014). Intriguingly, treatment with the nonselective COX inhibitor indomethacin increased capsaicin-induced cough thresholds in patients with asthma or chronic bronchitis but not in healthy subjects (Fujimura et al., 1995). By contrast, at least in rodents, acetaminophen causes airway hyper-reactivity through TRPA1 (Nassini et al., 2010b). These results offer compelling evidence that inflammatory mediators produced in diseased airways enhance airway reflex sensitivity in a manner that can be at least partially reversed by pharmacological intervention. In a large European study, six TRPV1 gene SNPs appeared to confer higher risk for chronic cough (Smit et al., 2012). In preclinical models, various TRPV1 antagonists were reported to potently inhibit the evoked (e.g., by inhaling aerosolized citric acid) tussive response (reviewed in Geppetti et al., 2006; McLeod et al., 2008; Preti et al., 2012). Moreover, TRPV1 antagonists attenuated antigen-provoked cough in guinea pigs sensitized to ovalbumin (McLeod et al., 2006), and reduced epithelial injury in a mouse model of asthma (Rehman et al., 2013). In a clinical study, the TRPV1 antagonist SB705498 (Fig. 9), however, did not block “spontaneous” cough in patients afflicted with chronic cough, indicating that the clinically important cough may be mediated by a target on capsaicin-sensitiveafferents, which is unrelated to TRPV1 (Smith et al., 2012; Khalid et al., 2014). This has refocused attention on TRPA1 as a promising therapeutic target in airway disorders.

b. Ankyrin transient receptor potential 1. Many of the irritants that activate TRPA1 are air pollutants produced by the combustion of materials (including tobacco products) that cause pronounced respiratory irritation in humans (see Bessac and Jordt, 2008). Since tobacco smoking is thought to be the single most important factor responsible for the development of COPD, it was a breakthrough observation that cigarette smoke extract (CSE) activates DRG neurons obtained from wild-type, but not TRPA1 knockout, mice (of note, deleting TRPV1 has no effect on CSE activation) (Andrè et al., 2008). Parenthetically, airway TRPA1 is also a target for wood smoke (Shapiro et al., 2013). Indeed, cigarette smoke activates pulmonary C fibers by interacting at TRPA1 (Lin et al., 2010). Interestingly, nicotine itself activates both TRPA1 (Talavera et al., 2009) and TRPV1, albeit at much higher concentrations at which it activates nicotinic acetylcholine receptors (Kichko et al., 2013). Parenthetically, nicotine also appears to activate TRPC1 and TRPC6 channels (Wang et al., 2014a). The implications of this finding to smoking-associated lung diseases will be discussed later. Activation of sensory neurons by high-concentration nicotine was markedly reduced in the TRPA1/TRPV1 double-null animals. One may argue that TRPA1 activation in the airways might mediate the irritant sensation (perceived as pleasurable by smokers) that is (at least in part) responsible for the tobacco addiction. If so, inhaled TRPA1 antagonists (by masking this pleasant feeling) might aid smokers in kicking the habit. In keeping with this hypothesis, TRPA1 polymorphism has been linked to menthol preference in heavy smokers (Uhl et al., 2011).

Of note, off-target TRPA1 activation seems to mediate the undesirable side effects of many broadly used drugs. For example, the antidiabetic drug glibenclamide was proposed to evoke abdominal pain via TRPA1 activation (Babes et al., 2013). Moreover, multiple classes of anesthetic molecules (including lidocaine and propofol) have been shown to cause pain in a TRPA1-mediated fashion (Fischer et al., 2010). Although these data raise the possibility that surgical anesthesia may paradoxically increase postoperative pain, their more immediate impact is to identify TRPA1 as a possible mediator of the respiratory complications of gas anesthetics, which can include coughing and laryngospasm (Eilers et al., 2010). In support of this hypothesis, the TRPA1 blocker HC-030031 prevents desflurane-induced increases in airway resistance in guinea pigs (Mutoh et al., 2013).

Additional hazardous irritants, including isocyanates, ozone, and chlorine, activate TRPA1 in vitro and cause respiratory irritation in vivo (reviewed in Bessac and Jordt, 2008). These molecules are broadly toxic and exposure to them also causes marked symptoms in human airways. Importantly, interruption of TRPA1 function in rodents via gene disruption or pharmacological blockade nearly abolishes sensory neuron activation and/or respiratory reflexes, including cough produced by short-term exposure to these.
irritants, pointing to TRPA1 as the sole (or at least most important) molecular target for a wide range of respiratory irritants. One noteworthy exception is nicotine, which (as we saw above) activates sensory neurons via both TRP (TRPA1 and TRPV1) and nicotinic acetylcholine receptors. The complex interplay between these two mechanisms in vivo, however, awaits further clarification because TRPA1 is necessary for increases in the Pernh (enhanced pause) respiratory measurement caused by intranasal nicotine; however, similar provocations produce robust respiratory sensory irritation responses in TRPA1-deficient mice (reviewed in Moran et al., 2011).

In preclinical models, increasing evidence supports a pivotal role for TRPA1 in airway allergic inflammation (reviewed in Nassini et al., 2010c; Abbott-Banner et al., 2013). Mice either lacking TRPA1 or pretreated with the TRPA1 blocker HC-030031 were protected from airway inflammation (both in bronchoalveolar lavage [BAL] and lung tissue), bronchial hyperreactivity to acetylcholine, and transcription of the gene product for the gel-forming mucin, MUC5AC (Caceres et al., 2009). Of note, knocking out TRPV1 did not alter any of these parameters, providing evidence that TRPA1 has a distinct role in this model of allergic disease. The TRPA1 blocker HC-030031 (but not the TRPV1 antagonist JNJ17203212) was also shown to attenuate the late asthmatic response in rodent models of allergic asthma (Raemdonck et al., 2012). TRPA1 has also been implicated in airway neurogenic inflammation caused by the acetylamphophen metabolite N-acetyl-p-benzo-quinoneimine in multiple small animal species (Nassini et al., 2010b). Neurogenic inflammation is clearly a key mechanism in experimental asthma, and the pivotal role of TRPA1 in the initiation and maintenance of the neurogenic inflammation in rodent and guinea pig airways is firmly established. Neurogenic inflammation, however, is unlikely to play any significant role in the pathomechanism of allergic disease or COPD in human airways (see Widdicombe, 2003). Therefore, it was an important recent discovery that non-neuronal cells in human airways (both epithelial cells and fibroblasts) express functional TRPA1 and respond to CSE exposure with IL-8 production in a TRPA1-mediated fashion (Nassini et al., 2012b). This finding is not completely unexpected since TRPA1 was originally cloned from human lung fibroblasts, and the presence of functional TRPA1 in lung fibroblasts was also demonstrated (Mukhopadhyay et al., 2011). Taken together, these compelling data demonstrate that TRPA1 can contribute to neurogenic inflammation in laboratory animal models and non-neurogenic inflammation in human airways.

CSE increases both matrix metalloprotease-1 (MMP1) and IL-8 production by human small airway epithelial cells; these effects are prevented by antioxidant treatment (Geraghty et al., 2011). TRPA1 is a major target for ROS in the respiratory tract. Moreover, as discussed above, TRPA1 is a main mediator of CSE-induced IL-8 production in respiratory epithelium. MMP1 is increased in the lungs of patients with emphysema. Indeed, transgenic mice expressing human MMP1 develop changes resembling human emphysema (D’Armiento et al., 1992; Foronjy et al., 2003). The promoter of the MMP1 gene has a CSE-responsive element (Mercer et al., 2009) and a polymorphism in the MMP1 gene (G1607GG) has been linked to rapidly declining lung function in smokers (Wallace et al., 2012). Interestingly, MMP1 also exhibits a neurotropic function in that it sensitizes vagal C fibers to chronic cough. On the basis of these observations, one might visualize a complex feedback loop in which smoking upregulates MMP1 expression via TRPA1 (leading to emphysema) and then MMP1 elevates C fiber drive (directly or indirectly involving TRPA1) to cause chronic cough.

Interestingly, increased IL-8 levels were also found in the BAL of patients with IPF (Willems et al., 2013), and polymorphism in the IL-8 gene has been linked to IPF (Ahn et al., 2011). It was recently speculated that COPD and IPF may be “distinct horns of the same devil” (Chilosi et al., 2012). Given the proposed role of non-neuronal TRPA1 (e.g., in lung fibroblasts) in IL-8 production, it might be argued that the relentless progression of IPF could be slowed down by TRPA1 antagonists.

Although it is technically not a chronic airway disorder (and is better suited to the section on cancer), it is worth mentioning here that human small cell (poorly differentiated neuroendocrine) carcinoma cells express TRPA1 (Schaefer et al., 2013). Small cell carcinoma is a dreaded smoking-related lung tumor. The TRPA1 agonist allyl-isothiocyanate evokes Ca2+ currents in small cell carcinoma cells and promotes the survival of tumor cells via ERK phosphorylation. TRPA1 is expressed in airway epithelial cells, where it serves as a receptor for toxic lung inhalants, many of which are carcinogenic (Büch et al., 2013). Thus, one might speculate that TRPA1 is instrumental for both the initiation and progression of lung cancer.

Chronic cough is a large, unmet medical need. The exact prevalence of chronic cough has proven difficult to determine, with reports ranging from 9% to 33% of the general population, depending on environmental factors such as smoke exposure and air pollution (reviewed in Chung and Pavord, 2008). In heavy smokers living in Delhi, a large metropolis with a problem of air pollution that is fairly common in the developing world, the prevalence of chronic cough approaches 70% (Chhabra et al., 2001, 2008). Bende and Millqvist (2012) recently reported an age-dependent increase in the prevalence of chronic cough in nonsmoking Swedish adults, with a peak (13%) occurring in middle-aged women (age 40–49 years).
The symptomatic management of chronic cough has changed surprisingly little during the past 50 years. It still heavily relies on codeine and its derivatives such as dextromethorphan. This is a problem because there is little evidence that codeine derivatives can provide any clinical benefit in cough relief over placebo in patients with chronic cough (Ramsay et al., 2008), yet they have significant adverse effects (ranging from itch to hallucinations) as well as a well documented abuse potential (www.nhtsa.gov/people/injury/research/job185drugs/dextromethorphan.htm).

The current American College of Chest Physicians guidelines recommend that cough treatment follow a thorough diagnostic work-up (Irwin, 2006). However, no underlying disease can be diagnosed in up to 46% of patients with chronic cough (effectivehealthcare.ahrq.gov/ehc/products/356/1370/CER_100_ChronicCough_ExecutiveSummary_20130102.pdf). This recognition has raised the provocative question of whether idiopathic chronic cough is a real disease or a failure of diagnosis (Morice et al., 2011). As of today, most experts seem to agree that idiopathic chronic cough is a real disease, although a consensus on terminology (variably referred to as idiopathic chronic cough, cough hypersensitivity syndrome, and SHR) is still lacking. This is most likely a heterogenous group of patients whose unifying feature is their unique sensitivity to inhaled capsaicin (compare Fig. 31, A and B).

Guinea pigs cough in response to inhaled capsaicin (or citric acid) and this response is exaggerated in ovalbumin-sensitized animals (reviewed in Canning and Mori, 2011). TRPV1 antagonists such as BCTC (McLeod et al., 2006) and V112220 (Leung et al., 2007) block cough evoked by capsaicin or acid challenge with an efficacy similar to that of codeine. Guinea pigs also cough when exposed to the inhaled TRPA1 agonist allyl-isothiocyanate (Andrè et al., 2009); this cough response is ameliorated by the TRPA1 antagonists HC-030031 (Andrè et al., 2009) and AP18 (Brozmanova et al., 2012). Inhaled allyl-isothiocyanate also provokes cough in human volunteers (Birrell et al., 2009).

Perhaps surprisingly, TRPV1 activation is far more effective than TRPA1 activation to evoke coughing in guinea pigs (Brozmanova et al., 2012). This is in keeping with the finding that capsaicin (1 μM) is more active than allyl-isothiocyanate (100 μM) in causing sustained action potential generation in isolated tracheal C fibers.

Thus, TRPV1 antagonists block cough response evoked by TRPV1 agonists, and TRPA1 antagonists inhibit cough evoked by TRPA agonists. Of course, it was expected; however, what about "spontaneous" (pathologic) cough? Spontaneous cough is difficult to study in experimental animals. A reasonably good model is cough evoked by inflammatory mediators such as BK and PGE₂. In the airways, BK was previously shown to be coupled to TRPV1 downstream of the B2 receptor (Carr et al., 2003), whereas PGE₂ was demonstrated to activate TRPA1 to evoke coughs (Grace et al., 2012). However, somewhat unexpectedly, it turned out that the complete elimination of BK- and PGE₂-evoked cough responses requires a simultaneous blockade of TRPV1 and TRPA1 (Brozmanova et al., 2012; Grace et al., 2012). Subsequently, it was reported that the blockade of cigarette smoke–induced activation of the laryngeal nerve similarly required the simultaneous antagonism of TRPV1 and TRPA1 (Liu et al., 2013b). These observations may be interpreted to imply that a dual TRPV1/TRPA1 inhibitor might be superior as an antitussive agent to selective inhibitors.

Theoretically, there are three strategies to relieve cough by targeting airway C fibers: 1) to prevent C fiber activation by inflammatory stimuli (e.g., by NSAIDs or antihistamines); 2) to block generator potential formation (in which TRPV1 and TRPA1 are believed to be the major players); and 3) to inhibit action potential formation (reviewed in Undem and Carr, 2010; Muroi and Undem, 2011). Regarding option 1, COX metabolites activate TRPA1 (Materazzi et al., 2008), and the nonselective COX inhibitor indomethacin was reported to ameliorate the cough response in experimental animals, as well as in patients with chronic bronchitis (Fujimura et al., 1995). Yet, indomethacin is also known to cause paradoxical cough as a side effect in a small subset (2.3%) of patients (www.ehealthme.com/ds/indomethacin/cough). In the guinea pig, Na₁,1.7 knockdown by shRNA abolishes citric acid–induced cough (Muroi et al., 2013); this observation supports the feasibility of the third approach. This is in keeping with the antitussive action of nebulized lidocaine in patients with intractable cough (reviewed in Slaton et al., 2013). Arguing against this approach is the lack of cough phenotype in patients with congenital lack of pain due to loss-of-function Na₈,1.7 mutations (Cox et al., 2006).

A popular (and controversial) theory holds that chronic cough is due to the airway remodeling (plasticity) that may occur in response to ongoing irritation (critically reviewed in Niimi, 2011). Certain agents generated during inflammation (e.g., NGF) were shown to upregulate TRPV1 (and also SP) expression in nociceptive DRG neurons (Ji et al., 2002). Furthermore, it was reported that Aδ fibers that do not express TRPV1 under normal conditions become capsaicin sensitive in preclinical pain models (reviewed in Ueda, 2006). It is not unlikely that similar changes in TRPV1 expression may also occur during airway inflammation. Indeed, vagal Aδ fibers (both slowly and rapidly adapting) were reported to acquire capsaicin sensitivity after ovalbumin sensitization; this stems from newly expressed TRPV1 in NF+ (i.e., myelinated) nodose ganglion neurons (Zhang et al., 2008b). Importantly, these findings in experimental animals correlate to the clinical observations. SP-like...
immunoreactivity is increased in bronchial biopsies taken from patients with cough variant, but not classic, asthma (Lee et al., 2003b). For TRPV1-like immunoreactivity (Fig. 31C), a similar phenomenon was described in patients with chronic cough (Groneberg et al., 2004a; Mitchell et al., 2005). These increases in SP- and TRPV1-like immunoreactivity are associated with greatly exaggerated cough in response to inhaled capsaicin (Cho et al., 2003; Ternesten-Hassèus et al., 2013).

Interestingly, elevated SP levels were also found in the plasma (Otsuka et al., 2011) and nasal secretions (Cho et al., 2003) of patients with chronic cough. Indeed, it was postulated that elevated SP in nasal secretions defines a distinct population of patients with chronic cough, those with “upper airway cough syndrome” (Lim et al., 2011). The interpretation of these observations is, however, not straightforward since inhaled SP does not provoke cough in the guinea pig (El-Hashim and Amine, 2005), nor do tachykinin NK1R (the receptor SP) antagonists relieve spontaneous coughing in men (Joos et al., 1996). Thus far, elevated SP appears to be marker of overactive sensory nerves in the respiratory tract, rather than a disease-causing substance.

Bronchial biopsies reveal increased inducible nitric-oxide synthase (iNOS) activity in the airway epithelium of individuals with asthma (Lane et al., 2004). Increased iNOS was also found in the nasal mucosa of patients with allergic rhinitis (Kawamoto et al., 1999; Yuksel et al., 2008). Exhaled NO is a good biomarker of chronic cough (Chatkin et al., 1999). Indeed, patients with chronic cough hypersensitivity syndrome show significant nitrosative stress in their nasal mucosa (Bae et al., 2012). It was recently recognized that OA-N02 is a highly electrophilic TRPA1 activator; thus, elevated NO may cause persistent airway inflammation by activating TRPA1 in the respiratory tract (Taylor-Clark et al., 2009). In addition, the long-term nitrative stress may exacerbate airway remodeling. The importance of iNOS in the pathogenesis of allergic airway disorders was highlighted by the finding that allergen (ovalbumin) exposure causes PARP-1 activation and oxidative damage in the airways of wild-type, but not iNOS knockout, mice (Naura et al., 2009). The nonselective NOS inhibitor N^+ -nitro-L-arginine methyl ester inhibits cough both in naïve and ovalbumin-sensitized guinea pigs whereas the selective iNOS antagonist ONO1714 [(1S,5S,6R,7R)-7-chloro-3-imino-5-methyl-2-azabicyclo[4.1.0]heptane hydrochloride] exerts antitussive activity only in the sensitized animals (Hori et al., 2011). On the basis of these observations, the selective iNOS inhibitor GW274150 was advanced into clinical trials, where, disappointingly, it blocked neither the early nor the late asthmatic response (Singh et al., 2007). To explain these seemingly contradictory findings, one might hypothesize that iNOS is required for an initiating event in asthma pathobiology but no longer plays a significant role in the maintenance of the established disease. If so, knockout animals are not good models to study chronic cough and/or asthma because they do not reflect the established disease. Indeed, a loss-of-function TRPV1 variant (I585V) was associated with a lower risk for childhood asthma (Cantero-Recasens et al., 2010). Yet there is no experimental evidence to imply a therapeutic potential for TRPV1 antagonists in the pharmacotherapy of allergic airway diseases. For example, the TRPV1 antagonist SB705498 when applied to the nasal mucosa reduced the capsaicin effect, proving target involvement (Holland et al., 2013); however, it did not provide any symptomatic relief in patients with seasonal allergic rhinitis (Alenmyr et al., 2012; Bareille et al., 2013). A number of TRPV1 antagonists have entered clinical trials as potential antitussive drugs (the latest addition is XEN-D0105; www.ariopharma.com); unfortunately, the results are not available.

2. Transient Receptor Potential Channels Other than Vanilloid Transient Receptor Potential 1 and Ankyrin Transient Receptor Potential 1 in Airway Structural and Inflammatory Cells: Implications for Therapy.

In addition to TRPV1 and TRPA1, a number of TRP channels belonging to the TRPV (TRPV2 and TRPV4), TRPM (TRPM4 and TRPM8), and TRPC (TRPC1, TRPC3, and TRPC6) subfamilies are expressed in the airways (see Abbott-Banner et al., 2013). Of these TRPs, TRPV4 appears to be the most intriguing as a therapeutic target but it is also probably the most problematic. TRPV4 is highly expressed in bronchial smooth muscle, where it is thought to contribute to Ca^{2+} mobilization (Jia et al., 2004). TRPV4 was reported to be activated (Chen et al., 2008a), or at least potentiated (Güler et al., 2002), by hypoosmolar solutions. Because individuals with asthma produce copious amounts of airway secretions with low osmolarity, one attractive hypothesis for further exploration is that activation of TRPV4 by hypo-osmolar secretions in bronchial smooth muscle is a major cause of bronchoconstriction in these patients (see Liedtke and Simon, 2004). If this model holds true, inhaled TRPV4 blockers may represent a new class of bronchodilator drugs. The possible link between TRPV4 and COPD is also gaining strength: TRPV4 is expressed in murine and human airway epithelial cells, where it is believed to play a key role in mucociliary clearance by regulating ciliary beats (Lorenzo et al., 2008; Alenmyr et al., 2014). Impairment of this airway protective function by toxic inhalants (Li et al., 2011b) and/or carrying a gain-of-function TRPV4 variant (Zhu et al., 2009) is thought to increase the risk for developing COPD.

In addition to bronchial epithelium and smooth muscle, TRPV4 is also expressed in vascular endothelium (Watanabe et al., 2002). It is worth mentioning here that in endothelial cells, TRPV4 is coexpressed...
with a number of TRPs such as TRPC1–TRPC6 and TRPM4 (see Nilius et al., 2003). Shear stress-induced vasodilation was absent in the TRPV4-null mice (Hartmannsgruber et al., 2007). However, the acute biology of TRPV4 most readily manifests itself in the alveolar septae of the lung (Alvarez et al., 2006). In isolated and perfused mouse lungs, activation of TRPV4 by elevated vascular pressure or injurious high-pressure mechanical ventilation causes extravascular leakage of fluid (i.e., pulmonary edema) reflected by increases in the lung’s filtration coefficient (Hamanaka et al., 2007; Huh et al., 2012). Consistent with these findings, intravenous administration of the TRPV4 agonist GSK1016790A (Fig. 8) causes circulatory collapse characterized by the failure of the alveolar septal barrier (Willette et al., 2008; Thorneloe et al., 2012).

The exact role of TRPV4 in lung injury is complex and only partially understood. The TRPV4 agonist GSK1016790A, which seems to act by recruiting inactive TRPV4 channels from intracellular depots (Sullivan et al., 2012), can produce dramatic deformation of cultured human endothelial cells (Willette et al., 2008), but it is only part of the story. It was recently shown that TRPV4 channels expressed on alveolar macrophages can play a critical role in ventilator-induced injury of mouse isolated lungs (Hamanaka et al., 2010). Although it might take some time to dissect out the relative contribution of different TRPV4-expressing cells to lung injury, TRPV4 antagonism promises to be a novel and effective approach to mitigate pulmonary edema. Indeed, TRPV4 appears to be upregulated in the lungs of patients with heart failure (Thorneloe et al., 2012).

TRPC channels appear to have functional roles in the pulmonary vasculature, particularly with regard to responses to hypoxia (reviewed in Moran et al., 2011). Regional alveolar hypoxia redirects blood flow to the well oxygenated areas. This reflex mechanism is impaired in TRPC6 knockout mice because their ex vivo lungs develop marked deficits in arterial oxygen saturation after airway instillation of saline to produce regional ventilatory failure (Weissmann et al., 2006). Moreover, a SNP (-254C→G) in the TRPC6 promoter has been linked to IPAH (Yu et al., 2009b). Consistent with this hypothesis, pulmonary arterial smooth muscle cells obtained from patients with IPAH showed significantly higher protein levels of TRPC6, higher resting levels of cytosolic Ca\(^{2+}\), and larger OAG (1-oleoyl-2-acetyl-sn-glycerol)-dependent cationic currents than samples from control subjects (Kunichika et al., 2004; Yu et al., 2004). Of note, hypoxia-induced proliferation of pulmonary arterial smooth muscle cells was also attenuated in TRPC1 knockout animals, and TRPC1 knockout mice did not develop pulmonary hypertension when kept in a chronically hypoxic environment (Malczyk et al., 2013). Taken together, these results imply that both TRPC1 and TRPC6 are important players in the pathogenesis of pulmonary hypertension. Indeed, chronic cigarette smoke exposure upregulates TRPC1 and TRPC6 in mice; in these animals, the normal vascular tone is restored only when both TRPC1 and TRPC6 are knocked down by siRNA (Wang et al., 2014a).

Smooth muscle cells in diseased airways typically display abnormalities in contraction and/or proliferation. Although bronchodilators are efficacious therapies capable of reversing airflow obstruction, treatments that reduce contractility and pathologic remodeling of airway smooth muscle remain targets of active investigation. In human airway smooth muscle cells, TRPC1, TRPC3, TRPC4, and TRPC6 mRNA were detected (see Abbott-Banner et al., 2013). After incubation with the inflammatory cytokine TNF\(\alpha\), human airway smooth muscle cells exhibit marked changes in Ca\(^{2+}\) homeostasis that are accompanied by increased expression of the TRPC3 protein (White et al., 2006). Moreover, enhanced acetylcholine-induced cytosolic Ca\(^{2+}\) elevations in human airway smooth muscle cells are blocked by RNA silencing of TRPC3. These data are consistent with findings in allergically inflamed mice in which TRPC3-directed antibodies inhibited nonselective cation conductances and restored resting membrane potentials of airway smooth muscle cells to more hyperpolarized values, similar to those seen in control subjects (reviewed in Wang and Zheng, 2011). Compensatory elevation of TRPC3 expression in Trpc6\(^{-/}\) mice may also explain why Trpc6-deficient mice have enhanced airway reactivity to a muscarinic agonist despite having reduced BAL inflammation (Sel et al., 2008). In a rat model of pulmonary arterial hypertension, genetic inactivation of TRPC4 also conferred a clear survival benefit (Alzoubi et al., 2013).

Menthol is added to certain cigarette brands to reduce airway irritation. There is an increasing recognition that menthol may make it easier for young people to start smoking and more difficult for smokers to quit (www.cancer.org/cancer/news/fda-investigates-menthol-in-cigarettes). Even worse, people who smoke menthol-flavored cigarettes inhale deeper and hold the smoke longer, which is believed to increase the risk for cancer by prolonging the exposure of the lung to carcinogens in cigarette smoke. Interestingly, menthol preference among smokers has been linked to TRPA1 (and not TRPM8) variants (Uhl et al., 2011).

In healthy volunteers, inhaled menthol relieves citric acid–evoked cough (Morice et al., 1994). Furthermore, inhaled menthol elevates the capsaicin cough threshold in patients with chronic cough (SHR) (Millqvist et al., 2013). Indeed, the menthol receptor TRPM8 is expressed in trigeminal ganglion neurons (McKemy et al., 2002), including cold-sensitive afferents serving the upper (Abe et al., 2005; Keh et al., 2011; Zhou et al., 2012).
2011) and lower airways (Xing et al., 2008). Using a combination of functional and neurochemical (immuno- staining and single-cell RT-PCR) studies, it was shown that menthol exerts its antitussive effect in the guinea pig by interacting at TRPM8-expressing (and TRPV1 negative) trigeminal afferents (Plevkova et al., 2013). Cough is believed to be “plastic”: Up- and down-regulation of the cough reflex may be mediated by distinct sensory afferents. Sensitization of the cough reflex appears to be driven by nerves that coexpress TRPV1 and TRPA1. Conversely, the activation of TRPM8+ afferents may inhibit (down-regulate) the cough response. If this holds true, an ideal antitussive agent should block TRPV1 and TRPA1 and, at the same time, activate TRPM8. Menthol, however, was reported to evoke mucus secretion by airway epithelial cells (Li et al., 2011c). In patients with COPD, increased TRPM8-like immunoreactivity was found in bronchial epithelium (Li et al., 2011c). In Chinese children with asthma, elevated TRPV2 expression has given new impetus to investigate the proliferation of human lung fibroblasts in response to TGFβ1 (Yu et al., 2013).

Both TRPV2 (Nagasawa et al., 2007; Link et al., 2010) and TRPM2 (Knowles et al., 2011) are expressed in alveolar macrophages, where they are believed to play a role in phagocytosis and ROS production (reviewed in Knowles et al., 2013). The implications of these findings for respiratory disorders are unclear. In Chinese children with asthma, elevated TRPV2 expression was found in alveolar macrophages (Cai et al., 2013). In a murine model of COPD, no difference was observed between wild-type and TRPM2-null mice (Hardaker et al., 2012). Genetic deletion of TRPM2 did not change the shape and chemotaxis of neutrophils either. By contrast, impaired antigen-induced degranulation was observed in mucosal mast cells isolated from TRPM2 knockout animals (Oda et al., 2013). This observation might be interpreted to imply a therapeutic potential for TRPM2 blockers in patients with asthma (also food allergy) and other allergic airway disorders. For the sake of completeness, it is worth mentioning here that primary mast cells and mast cell–derived cell lines also express TRPC1, TRPC5, TRPM4, and TRPM7 (reviewed in Freichel et al., 2012). Studies with TRPM4-deficient mice suggest that this channel plays a critical role in mast cell degranulation inasmuch bone marrow–derived mast cells isolated from the knockout animals exhibited excessive release of histamine and other mediators in response to IgE-mediated stimulation (Vennekens et al., 2007). If lack of TRPM4 exacerbates mast cell degranulation, one might argue that TRPM4 agonism may exert the opposite effect, that is, it can block mast cells degranulation. Unfortunately (as discussed below), TRPM4 is highly expressed in the heart and gain-of-function TRPM4 mutations have been linked to cardiac arrhythmias and hypertension (reviewed in Mills and Milan, 2010; Abriel et al., 2012). These findings predict that TRPM4 agonists may exhibit unacceptable cardiovascular side effects.

C. Transient Receptor Potential Channels as Therapeutic Targets in Bladder Disorders

Multiple TRP channels are expressed in the bladder (Fig. 26), urothelium, nerve endings, and detrusor muscle, where they presumably function as sensors of stretch and chemical irritation (reviewed in Avelino and Cruz, 2006; Everaerts et al., 2008; Skryma et al., 2011; Avelino et al., 2013; Birder and Andersson, 2013). Intravesical administration of TRPV1 agonists (capsaicin and RTX) has been in use in the management of the overactive bladder for many years on a largely empirical basis (reviewed in Ozawa et al., 1999; Szallasi and Fowler, 2002). The recent recognition of disease state-related changes in TRP channel expression has given new impetus to investigate the roles of these channels in normal bladder function and dysfunction.

1. Vanilloid Transient Receptor Potential 1 and Vanilloid Transient Receptor Potential 4. There is a dense, TRPV1-expressing C fiber network in the suburothelium and muscularis of the human renal pelvis, ureter, bladder, and urethra (reviewed in Avelino et al., 2013) but the existence of functional TRPV1 in urothelial cells remains controversial. Urothelial TRPV1 expression is based on the following findings: 1) TRPV1-like immunoreactivity was described in all urothelial layers in the human bladder, and 2) capsaicin (as well as heat and low pH) evokes Ca2+ currents and ATP release in rat and human urothelial cells in culture (see Avelino et al., 2013). However, TRPV1-detecting antibodies are probably much less specific than previously thought; indeed, they stain cells in TRPV1 knockout animals (Everaerts et al., 2009). Capsaicin itself is not selective for TRPV1 either (see Szallasi and Blumberg, 1999). There is also a discrepancy between functional data: in particular, two independent groups were unable to replicate the capsaicin effects detected in human urothelial cells (Charrua et al., 2009b) using mouse (Yamada et al., 2009) and guinea pig (Xu et al., 2009b) urothelial cells, respectively (which may represent another striking species-related difference in TRP expression). Parenthetically, adding to the confusion, increased TRPV1 expression and capsaicin-evoked ATP release was recently reported in cultured human urothelial cells obtained from bladder biopsies of overactive bladder patients compared with control subjects (Birder et al., 2013). Of note, the electrophysiological properties of
capsaicin-evoked currents in the rat urothelium (linear current-voltage relation) were dissimilar to those detected in rat DRG neurons (outwardly rectifying). This controversy aside, an almost complete loss of specific [3H]RTX binding sites (a neurochemical measure of TRPV1 receptors) was noted after surgical (Szallasi et al., 1993) or chemical (Goso et al., 1993b) denervation of the rat bladder. This is in keeping with the loss of TRPV1 in the human bladder after botulinum toxin injections (Apostolidis et al., 2005). Thus, if nonneuronal TRPV1 exists in the bladder, its expression level relative to neuronal TRPV1 expression should be very low. Of note, immunohistochemical analysis also suggests the existence of TRPV1 in other nonneuronal cells of the human bladder, including detrusor muscle, fibroblasts, and endothelial cells (Lazzeri et al., 2004).

The involvement of neuronal TRPV1 in the physiologic micturition reflex is not clear. Rats whose TRPV1-expressing nerves have been ablated by neonatal capsaicin treatment develop hugely distended bladder (see Szallasi and Blumberg, 1999). The bladder phenotype of the TRPV1 knockout mouse is, however, less pronounced: these animals showed only mild spotty incontinence (Birder et al., 2002). Furthermore, TRPV1 antagonists did not alter micturition in naïve animals (Charrua et al., 2009a; Kitagawa et al., 2013b). When explaining these seemingly conflicting results, it should be kept in mind that neonatal capsaicin administration ablates not only TRPV1 but also other receptors coexpressed with TRPV1 on bladder afferents that may be involved in micturition. Clearly, further studies are need to elucidate the role (if any) of TRPV1 in the normal bladder reflex activity.

By contrast, the role of TRPV1 in micturition reflex dysfunction is well established (reviewed in Avelino and Cruz, 2006; Szallasi et al., 2006; Juszcak and Thor, 2012). In humans, intravesical TRPV1 agonist (capsaicin and RTX) desensitization therapy is based on the concept that the C fiber–driven micturition reflex, which is inactive in the adult life, resumes control of micturition in both neurogenic and nonneurogenic cases of overactive bladder (reviewed in Szallasi and Fowler, 2002; Avelino and Cruz, 2006). In patients with neurogenic detrusor overactivity disorders, intravesical capsaicin (Lazzeri et al., 1996; De Ridder et al., 1997) or RTX (Cruz et al., 1997; Lazzeri et al., 2000; Kim et al., 2003; Silva et al., 2005, 2007) provides symptomatic relief by increasing bladder capacity and decreasing the number of daily incontinent episodes. In some patients, intravesical RTX even fully restores continence (Cruz et al., 1997). Intravesical RTX appears to be superior to capsaicin in its initial excitation (burning pain) to lasting desensitization ratio. At doses at which it evokes only mild discomfort, RTX treatment results in an increase in bladder capacity that lasts for several months (Cruz et al., 1997; Silva et al., 2000, 2007). Importantly, this effect is fully reversible and then it can be achieved again by repeated RTX administration (Silva et al., 2000).

In keeping with these findings, biopsies taken from the bladder of patients undergoing intravesical RTX therapy showed no significant alterations at either light microscopic or electron microscopic levels (Silva et al., 2001).

Intravesical RTX reduces the number of incontinent episodes in patients with spinal cord injury (Kim et al., 2003; Silva et al., 2005). Likewise, intrathecal RTX blocks detrusor overactivity in rats with complete chronic spinal cord trans-section (Cruz et al., 2008). Taken together, these findings raise the possibility that intrathecal RTX can be used in neurogenic bladder patients in whom catheterization is contraindicated.

The therapeutic value of TRPV1 antagonists in managing detrusor overactivity is unclear since no endogenous agonist (“endovanilloid”) was identified in the bladder of patients with incontinence. By contrast, the pain and bladder hyperactivity that accompany interstitial cystitis are thought to be amenable to TRPV1 antagonist therapy. Most (>90%) bladder (lumbosacral and thoracolumbar) DRG neurons are capsaicin responsive (Dang et al., 2013). In rats with cyclophosphamide-induced cystitis, increased current density was observed in these neurons. In a feline model of interstitial cystitis, abnormally enhanced capsaicin responses were detected secondary to TRPV1 phosphorylation by PKC (Sculporeanu et al., 2005a).

In mice, genetic manipulation of the TRPV1 gene prevents bladder reflex hyperactivity and spinal c-fos overexpression in response to cystitis (Wang et al., 2008b). The TRPV1 antagonist GRC-6211 (structure shown in Fig. 9) ameliorates reflex activity in chronically inflamed bladder (Charrua et al., 2009a). Combined, these findings imply a therapeutic value for TRPV1 antagonists in the symptomatic treatment of interstitial cystitis.

In clinical trials, intravesical RTX yielded no clear benefits over placebo in patients with interstitial cystitis (reviewed in Mourtzoukou et al., 2008). This was unexpected and puzzling. RTX is a highly lipophilic compound, difficult to keep in aqueous solutions. The apparent affinity of RTX is hugely dependent on assay conditions. For example, low pH was reported to inhibit specific [3H]RTX binding by 50% at a pH value of 5.3 and by 90% at a pH value of 4 (Szallasi et al., 1995). Although the pH of urine is close to neutral in a pH-balanced body, dietary changes or certain medications can make the urine acidic (pH 4 to 5). It is not clear to what degree the bioavailability of RTX was controlled in the interstitial cystitis clinical trials [RTX sticks to plastics and is difficult to keep in aqueous solution (Szallasi and Blumberg, 1992)]. Another possible confounding factor is TRPV1b,
a dominant negative splice variant (Charrua et al., 2008). It was speculated that TRPV1 may be more (or less) active in the bladder of certain patients depending on the relative ratio of TRPV1b to TRPV1. In the rat, cystitis was shown to decrease the production of TRPV1b, leading to a more active TRPV1 phenotype (Charrua et al., 2008). It is possible (although not very likely) that patients participating in the interstitial cystitis trials had reduced RTX sensitivity due to an overproduction of TRPV1b. Finally, it is worth mentioning here that in bladder biopsies taken from patients with interstitial cystitis only TRPM2 and TRPV2 correlated with disease severity (Homma et al., 2013). Thus, it is entirely possible that (unlike in experimental animals) TRPV1 does not play any major role in the pathogenesis of interstitial cystitis.

In the bladder, TRPV4 is predominantly expressed in urothelium (Fig. 26), but it is also present in detrusor muscle (Birder et al., 2007; Gevaert et al., 2007; Xu et al., 2009b). In transgenic mice that overexpress NGF in their urothelium, increased TRPV4 levels were described in both urothelial cells and DRG neurons (Girard et al., 2013). The neuronal expression pattern of TRPV4 is puzzling. Whereas TRPV4 is present in the majority (>80%) of lumbar DRG neurons, no TRPV4-like immunoreactivity was detected in bladder afferents. In human urothelium, TRPV4 was localized adjacent to the adherence junctions (Janssen et al., 2011). Interestingly, TRPV4 seems to be highly coexpressed with TRPV2 and TRPM7 in both mouse (Everaerts et al., 2010a) and human (Shabir et al., 2013) urothelial cells.

TRPV4 knockout mice show an altered micturition pattern that is characterized by increased intermicturition intervals and spotting due to nonmicturition contractions (Gevaert et al., 2007). In other words, the TRPV4 knockout mice have an incontinent phenotype. In addition, TRPV4-null mice show reduced urinary frequency and increased void volume after bladder damage due to cyclophosphamide (Everaerts et al., 2010a). By contrast, a subset of patients with TRPV4 axonal neuropathy spectrum disorders due to gain-of-function TRPV4 mutations show bladder urgency/incontinence (reviewed in McEntagart, 2012). On the basis of these findings, it was postulated that TRPV4 plays a crucial role in the mechanosensory pathway in the bladder by detecting changes in intravesical pressure, and provides an important contribution to the pathogenesis of neurogenic overactive or underactive bladder (Everaerts and De Ridder, 2013).

Activation of TRPV4 located in detrusor muscle can lead to contractions. In accord, the TRPV4 agonist GSK1016790A is able to contract the bladder directly even in the absence of urothelium (Thorneroe et al., 2008). Of note, another study found GSK1016790A-induced bladder contractions to be dependent on urothelium (Aizawa et al., 2012). Importantly, GSK1016790A increased the amplitude of bladder contractions in a rat model of neurogenic underactive bladder (Young et al., 2013). As predicted by data from the TRPV4(−/−) mice, the TRPV4 antagonist HC-067047 (Fig. 11) improved bladder function in mice with cystitis (Everaerts et al., 2010a). Short-term dosing did not alter water intake, core body temperature, thermal selection behavior, heart rate, locomotion, or motor coordination in vivo (Everaerts et al., 2010a). Taken together, these observations imply a value for TRPV4 agonists and antagonists in the management of underactive and overactive bladder, respectively. Furthermore, TRPV4 antagonists may improve bladder function in patients with prostatic enlargement.

2. Melastatin Transient Receptor Potential 8 and Ankyrin Transient Receptor Potential 1. In the human bladder, TRPM8 is present both in urothelium and suburothelial myelinated nerve fibers (Fig. 26) (Du et al., 2008) with increased expression in patients with bladder pain or idiopathic idiopathic detrusor overactivity (Mukerji et al., 2006a,c). Of note, some recent studies confirmed (Kullmann et al., 2009; Shabir et al., 2013), whereas others questioned, the presence of TRPM8 in urothelial cells (Everaerts et al., 2010a). The TRPM8 agonist menthol activates the micturition reflex in the rat (Nomoto et al., 2008) and pig (Vahabi et al., 2013). Conversely, the TRPM8 antagonist AMTB (Fig. 11) decreases the frequency of volume-induced bladder contractions (Lashinger et al., 2008). These findings imply a therapeutic potential for TRPM8 antagonists in the management of bladder disorders characterized by pain and/or detrusor overactivity.

TRPA1 is highly coexpressed with TRPV1 on rat bladder afferents, especially in the bladder outflow region (Gratzke et al., 2009). Indeed, the TRPA1 agonist allyl-isothiocyanate contracts the rat bladder (Andrade et al., 2006). Moreover, TRPA1 appears to be expressed in the urothelium (Gratzke et al., 2009). The functional role of this dual (neuronal and urothelial) TRPA1 expression in the normal micturition reflex remains to be delineated.

In naive rats, the TRPA1 antagonist HC-030031 has no apparent effect on bladder functions (Minagawa et al., 2013). However, after spinal cord injury, TRPA1 mRNA and protein levels are elevated in rat bladder afferents and their perikarya in L5–S1 DRG (Andrade et al., 2011). This increase in TRPA1 expression parallels the development of bladder overactivity. Importantly, both genetic knockdown and pharmacological blockade of TRPA1 reduce the number of nonvoiding bladder contractions in these animals. Conversely, intravesical TRPA1 agonist administration induces detrusor muscle overactivity (Andrade et al., 2006). Of particular interest is the finding that H2S, a gasotransmitter produced during inflammation...
(e.g., bacterial cystitis), can act as a potent endogenous TRPA1 agonist (Streng et al., 2008). Taken together, these observations imply a therapeutic value for TRPA1 antagonism in the management of cystitis (Meotti et al., 2013) and patients with overactive bladder.

3. Canonical Transient Receptor Potential 1 and Canonical Transient Receptor Potential 4. Increased sprouting of sensory afferents and subsequent bladder overactivity was recently observed in wild-type, but not TRPC1/TRPC4 double-null, mice with cyclophosphamide-induced cystitis (Boudes et al., 2013). These findings imply a crucial role for TRPC1/TRPC4 in neuronal sprouting during cystitis that causes overactive bladder.

D. Transient Receptor Potential Channels in Cardiovascular Disorders

Cardiovascular disease includes a broad range of disorders (ranging from heart disease through vascular diseases of the brain, kidney, and lung to peripheral arterial disease) that affect the cardiovascular system. The causes of cardiovascular disease are diverse but atherosclerosis and hypertension are the most common. Although smoking remains the most important risk factor for cardiovascular disease, nonsmokers who are diabetic and/or obese or lead a sedentary lifestyle are also at increased risk. Cardiovascular disease kills an estimated 17 million people worldwide each year. Indeed, cardiovascular disease is the leading cause of death in most developed countries, including the United States. The majority of death is due to heart attack, or acute myocardial infarction (AMI), and cerebral stroke (both ischemic and hemorrhagic). The possible role of TRP channels in the pathogenesis of cardiac arrhythmias, pulmonary vascular disorders, cerebral stroke, and ocular neovascularization disorders is discussed elsewhere in this review. Here we focus on the contribution of TRP channels to cardiomyopathies (Fig. 32), cardiac fibrosis (Fig. 33), hypertension (Fig. 39), and atherosclerosis (also reviewed in Watanabe et al., 2009, 2013; Rowell et al., 2010; Vennekens, 2011; Zholos and Curtis, 2013). We will also touch on the emerging concept that TRP channels might play an important role in thrombotic disorders by regulating platelet aggregation (see Authi, 2007; Dionisio et al., 2012).

1. Transient Receptor Potential Channels and Heart Disease. The heart beats about 100,000 times daily and two and a half billion times over a 70-year lifetime. In a much simplified manner, the heart can be thought of as a pump with an energy supply (coronary vessels) and an electrical conduction system that connects the SA node (that orchestrates the pacemaker activity) to the cardiomyocytes (that do the pumping) via specialized muscle fibers known as the bundle of His, the fascicles of Tawara, and the fibers of Purkinje. Disorders that affect this conduction system are called heart block and can be subdivided into separate categories based on the anatomic location of the damage. Heart rhythm abnormalities (arrhythmias)

![Fig. 32. Schematic illustration of the roles that TRP channels are believed to play in the development of cardiac hypertrophy. See the text for details. Reprinted with permission from Watanabe et al. (2013).](image-url)
occur when the electrical impulses that coordinate the heart beats become disorganized. Diseases of the cardiomyocytes that result in deterioration of the pumping function are grouped together as cardiomyopathies. Alternatively, the heart may be weakened by increased deposition of cellular matrix proteins by fibroblasts, a process known as cardiac fibrosis. Abrupt blockage of the coronary vessels that deprive cardiomyocytes from their O₂ supply can cause sudden cardiac death (AMI), whereas sustained deprivation may lead to chronic ischemic cardiomyopathy. Various TRP channels have been implicated in all of these disorders (reviewed in Watanabe et al., 2009, 2013).

Under physiologic conditions, the heart rate is set by the rate of systolic depolarization that occurs in the pacemaker cells of the SA node. Although the existence of a number of TRP channels (TRPM4 and all TRPCs with the exception of TRPC5) have been reported in SA node cells (see Watanabe et al., 2009; Dietrich and Gudermann, 2011), it is unclear whether any of these channels is involved in the cation conductance that regulates the pacemaker activity. Transgenic mice that overexpress Gαq (a mouse model of heart failure) display premature ventricular contractions that are exacerbated by OAG (1-oleoyl-2-acetyl-sn-glyceryl) (Hirose et al., 2011), a DAG analog known to activate a functional subset of TRPC channels, namely TRPC3, TRPC6, and TRPC7 (Venkatachalam et al., 2003). Interestingly, these animals showed increased expression of TRPC3 and TRPC6 in their heart. Furthermore, the premature ventricular contractions were prevented by SK&F-96356 [1H-pyrrolo[3,2-c]quinolin-4-amine,2,3-dihydro-N,6-dimethyl-1-(2-methylphenyl)], a nonspecific inhibitor of receptor-mediated calcium entry, originally described as a reversible inhibitor of the gastric H⁺/K⁺-ATPase (Leach et al., 1992), which is often used as a biochemical tool to define functional TRPC channels. However, it should be noted here that SK&F-96356 also blocks voltage-gated T-type Ca²⁺ channels (Singh et al., 2010).

On the basis of these observations, one can argue that TRPC3 and TRPC6 might be part of the pacemaker activity in the SA node. The evidence for this is, however, very circumstantial. Clearly, we need better tools (e.g., selective TRPC3 and TRPC6 antagonists) to scrutinize this concept. By contrast, the involvement of TRPM4 in cardiac conduction anomalies is well documented (see the above section on TRP channelopathies; Abriel et al., 2012).

A number of TRP channels (including TRPC1, TRPC5, TRPC6, TRPV1, TRPV2, TRPP1, TRPP2, and TRPM4) have been detected in human cardiomyocytes, of which particular attention was paid to TRPC3 and TRPC6 as attractive targets in cardiac hypertrophy (Fig. 32; recently reviewed in Watanabe et al., 2013). Generally speaking, cardiac hypertrophy develops when the heart works against increased resistance (e.g., valvular stenosis or hypertension). Among the neurohumoral factors that may trigger the intracellular signaling cascade in cardiomyocytes leading to hypertrophy, previous studies focused on calcineurin (Fig. 32) (see Wang et al., 2014b). Indeed, in the mouse, overexpression of calcineurin induces massive heart hypertrophy (Molkentin et al., 1998). Calcineurin dephosphorylates NFAT; NFAT then translocates into the nucleus where it activates the hypertrophic response genes (Fig. 32; reviewed in Watanabe et al., 2013). Although most authorities agree that NFAT is the shared downstream target for both mechanical stress and humoral factors (e.g., angiotensin II and endothelin-1) to cause cardiac hypertrophy, opinions vary as to the molecular link between NFAT and the hypertrophic signals.

There is emerging evidence to implicate TRPC3 and TRPC6 in cardiac hypertrophy (see Eder and Molkentin, 2011; Watanabe et al., 2013). Genetic overexpression of Gαq proteins in the mouse causes heart failure and elevated TRPC3 and TRPC6 expression (Hirose et al., 2011). TRPC3 is also upregulated in several animal models of cardiac hypertrophy (see Watanabe et al., 2013).
TRPC6 expression is increased in response to pressure overload (Fig. 32; Kuwahara et al., 2006). Silencing of TRPC6 by siRNA blocked the hypertrophic signal elicited by either angiotensin II or endothelin-1. Conversely, TRPC6 transgenic mice displayed striking cardiomyopathy secondary to NFAT activation. Interestingly, the TRPC6 gene promoter region contains two conserved NFAT consensus sites, creating the framework for a positive feedback loop in which TRPC6 activation stimulates NFAT which, in turn, further enhances TRPC6 activity. Somewhat confusingly, gene silencing of TRPC1 and TRPC3 mimicked the effect of TRPC6 knockdown on cardiac hypertrophy (Ohba et al., 2007). The expression of TRPC1 is increased in the myocardium of rats with isoproterenol-induced cardiac hypertrophy (Chen et al., 2013d). Furthermore, TRPC1-null mice did not develop maladaptive cardiac hypertrophy when subjected to hemodynamic stress (Seth et al., 2009). One might speculate that TRPC1, TRPC3, and TRPC6 form a heteromultimer in the (mouse) heart, and the blockade of any of these three constituents is sufficient to disrupt the signaling cascade that activates NFAT. Indeed, only combined genetic deletion or pharmacological blockade of TRPC3 and TRPC6 channels protected against pressure overload-induced cardiac hypertrophy (Seo et al., 2014).

Klotho (named after the youngest of the three Fates in Greek mythology who was responsible for spinning the thread of human life) is a membrane protein related to β-glucuronidases. Klotho-deficient mice show premature aging (reviewed in Nabeshima, 2002). Of relevance to our discussion, these mice (although they do not show baseline cardiac abnormalities) respond to hemodynamic stress by developing exaggerated pathologic cardiac hypertrophy (Xie et al., 2012). This hypertrophy is absent in the TRPC6(−/−) animals. By contrast, transgenic mice with heart-specific TRPC6 overexpression develop spontaneous cardiac hypertrophy. Finally, soluble Klotho inhibits TRPC6-mediated currents in cardiomyocytes. Taken together, these findings imply that TRPC6 mediates the cardioprotective action of Klotho and thus strengthen the case for targeting TRPC6 in patients with cardiac hypertrophy.

TRPM4 (Guinamard et al., 2006), TRPV1 (Thilo et al., 2010), and TRPV2 (Iwata et al., 2013) may also be involved in the development of cardiac hypertrophy. In spontaneously hypertensive rats, increased levels of TRPM4 were reported (Guinamard et al., 2006). Interestingly, TRPM4-null mice are also hypertensive secondary to increased catecholamine secretion (Mathar et al., 2010). TRPV1 was likewise increased in the heart of mice suffering from heart failure secondary to hypertrophic cardiomyopathy (Thilo et al., 2010). Cardiac TRPV1 mRNA in these animals showed good correlation to ventricular thickness. The TRPV1(−/−) animals revealed less cardiac hypertrophy compared with the wild type when subjected to pressure overload by thoracic aortic constriction (Buckley and Stokes, 2011). The authors of this article speculated that TRPV1 antagonists may be repurposed clinically as antihypertrophic agents. Indeed, BCTC has recently been shown to be cardioprotective in a preclinical model of heart failure (Horton et al., 2013). Since BCTC is not selective for TRPV1, among others, it blocks TRPV4 and TRPM8 channels; this experiment needs to be replicated by a selective TRPV1 antagonist. As a cautionary note, postschismic cardiac recovery was impaired in the TRPV1-null mice (Wang et al., 2005). Finally, TRPV2 was found to be increased in the sarcolemna of patients with dilated cardiomyopathy (Iwata et al., 2013). The TRPV2 inhibitor tranilast slowed down the progression of dilated cardiomyopathy in both hamster (β-sarcoglycan–deficient animals) and mouse (transgenic animals expressing sialyltransferase) models of the human disease.

In TRPM2-null mice, myocardial necrosis and contractile dysfunction was ameliorated after left main coronary artery ligation/reperfusion injury (Hiroi et al., 2013). Myeloperoxidase staining revealed much fewer neutrophils in the infarcted area. These findings imply an important role for neutrophil TRM2 channels in myocardial ischemia/reperfusion injury. Of note, in a second study, no difference in infarcted area was noted between wild-type and TRPM2-knockout mice (Miller et al., 2013) and, surprisingly, the knockout animals even showed worse cardiac function after injury. The TRPM4 inhibitor 9-phenanthrol also protected rat hearts from ischemia/reperfusion damage (Wang et al., 2013b).

2. Transient Receptor Potential Channels and Hypertension. Hypertension is a chronic medical condition in which the pressure in the arteries is elevated (currently defined as >140/90 mm Hg). Hypertension affects one in four American adults. Most hypertension cases are "essential" (as opposed to secondary hypertension in which a well defined hypertensive factor such as renovascular occlusion can be identified) and may be attributed to an unhealthy lifestyle. Indeed, lifestyle changes (e.g., restriction of salt intake and increased physical activity) may restore normal blood pressure in some individuals. In a much simplified manner, blood pressure is determined by the strength of the heart stroke, the volume of the circulating blood, and the elasticity (diameter) of the blood vessels. Indeed, β-blockers (to weaken heart stroke), diuretics (to reduce blood volume), and angiotensin-converting enzyme inhibitors (vessel relaxants) are all clinically useful antihypertensive drugs. Below we focus on TRP channels that are implicated in setting the vascular tone by regulating the activity (constriction state) of vascular smooth muscle cells (Fig. 39).

Six members of the mammalian TRPC family are expressed in vascular smooth muscle cells (see
vascular tone (constriction versus relaxation). TRPC1/TRPC6 and TRPV4 exert opposite effects on TRPV4 knockout mice (Earley et al., 2009). If so, mediated by TRPV4 because it was absent in the relaxation (Earley et al., 2005). This effect is clearly donoric acid metabolites (e.g., 11,12-epoxyeicosatrienoic acid) activation of TRPV4 by endothelium-derived arachidonic acid metabolites (e.g., 11,12-epoxyeicosatrienoic acid) causes hyperpolarization and resultant vasorelaxation (Earley et al., 2005). This effect is clearly mediated by TRPV4 because it was absent in the TRPV4 knockout mice (Earley et al., 2009). If so, TRPC1/TRPC6 and TRPV4 exert opposite effects on vascular tone (constriction versus relaxation).

The recognition of the important role that Mg2+ plays in hypertension (in the clinics, intravenous magnesium was used to reduce blood pressure in patients with severe hypertension) has brought TRPM6 and TRPM7 (two TRP channels involved in Mg2+ homeostasis) into attention, although to date there is no convincing evidence to implicate TRM6 and/or TRPM7 in the pathobiology of high blood pressure (see Watanabe et al., 2013). As mentioned above, TRPM4-null mice develop hypertension secondary to increased catecholamine secretion (Mathar et al., 2010) and TRPM4 has been implicated in the Bayliss effect (see Vennekens, 2011).

3. Transient Receptor Potential Channels and Atherosclerosis. Atherosclerosis, defined as the narrowing of the lumen of an artery due to lipid and collagen deposition, is the leading cause of coronary artery disease/AMI, stroke, and peripheral vascular disease. Despite decades of intensive research, the pathogenesis of atherosclerosis remains only partially understood. The prevalent theory postulates a central role for endothelial damage (possibly initiated in some patients by sustained, low-grade inflammation) that paves the way for plaque (lipid, calcium, and collagen deposition in the intima) formation. These plaques may either limit blood flow (e.g., peripheral vascular disease) or dislodge and travel (embolize) to distant locations. An example of the latter phenomenon with potentially devastating consequences is ischemic stroke caused by carotid artery disease. Smoking, hypertension, and hypercholesterolemia are all known risk factors for developing atherosclerosis, probably by damaging endothelial cells one way or another. Monocytes then adhere to the site of endothelial damage and penetrate into the intima of the vessel. Finally, vascular smooth muscle cells get recruited, after a phenotypic switch from contractile to proliferative.

TRP channels have been implicated in the major steps (endothelial damage/dysfunction, monocyte adhesion/penetration, and vascular smooth muscle phenotypic switch) leading to atherosclerosis (reviewed in Kwan et al., 2007; Liu et al., 2008; Tano et al., 2012).

Ossabaw island pigs (established from feral swine who had adapted to the unique conditions in this island on the Georgia coast) have insular dwarfism and develop early onset T2DM and atherosclerosis when fed an atherogenic diet (Etheron, 1980; Sturek et al., 2007; Neeb et al., 2010). They also show high TRPC1, TRPC3, TRPC5, and TRPC6 expression (compared with lean pigs) that is proportional to the increase in serum cholesterol levels (Hu et al., 2009a). Strikingly, physical exercise reverses this increase in TRPC1 mRNA and protein levels (Edwards et al., 2010). This is the first (and thus far only) example of a TRP channel being regulated by physical activity.

Of TRP channels expressed in endothelial cells (TRPC1, TRPC3–TRPC7, TRPV1, TRPV4, and TRPM7), TRPC3 has attracted the most attention as a potential therapeutic target in atherosclerosis based on its abundance (relative to other TRP channels) in coronary artery endothelial cells of human origin (HCAECs) (see Smedlund et al., 2012). Unfortunately, results obtained with HCAECs should be interpreted with caution since TRP channel expression in these cells varies dramatically with passage number and assay conditions. Parenthetically, similar considerations apply to other cultured cells such as urothelial cells and odontoblasts in which conflicting TRP channels expressions were reported by different laboratories. Since TRPC3 is believed to be constitutively active, its upregulated expression may have profound effects on Ca2+ homeostasis (see Vazquez et al., 2004). Oxidative stress may provide an important contribution to endothelial damage. Using a dominant negative N-terminal fragment of TRPC3, preliminary evidence was obtained
that TRPC3 (maybe in combination with TRPC4) may be part of the redox-sensitive channel that mediates oxidative stress in endothelial cells (Balzer et al., 1999). In HCAECs, proinflammatory mediators (e.g., ATP) induce the expression of VCAM-1 that, in turn, attracts leukocytes to adhere to the endothelium (Smedlund et al., 2010). There is emerging evidence that TRPC3 is an obligatory component of the signaling cascade that connects the P2Y2 receptor (that recognizes ATP) to VCAM-1 (see Smedlund et al., 2012). A role for TRPC3 in endothelial NO release was also postulated (Huang et al., 2011). Of note, TRPC1 was implicated in mediating the endothelial damage caused by oxidized phospholipids but the relevance of this observation was questioned by the down-regulation of TRPC1 to almost undetectable levels in HCAECs under proinflammatory conditions (reviewed in Smedlund et al., 2012).

The increasing recognition of atherosclerosis as a low-grade, maladaptive inflammatory disease has brought attention to monocytes/macrophages as potential therapeutic targets. Atherosclerotic lesions are now believed to have a microenvironment that either promotes healing or, conversely, accelerates lesion growth and plaque instability. A decisive lesional factor may be efferocytosis, the ability of resident macrophages to clear pathogenic monocytes. There is preliminary evidence to implicate TRPC3 in efferocytosis (“burying the dead cells”). Bone marrow–derived macrophages obtained from TRPC3(−/−) mice showed decreased survival and impaired efferocytosis (Tano et al., 2011, 2014). If so, TRPC3 may play an important role not only in disease initiation (endothelial damage) but also in disease progression (see Tano et al., 2012).

Accumulation of apolipoprotein (Apo) B–containing lipoproteins in the subintima is thought to be a major triggering event in atherosclerosis. These lipoproteins attract monocytes from the circulation that, in turn, differentiate into lesional foam cells. The atherosclerotic plaque will progress if the formation of foam cells (set by monocyte influx and differentiation into macrophages) outpaces their clearance (due to apoptosis and efferocytosis). TRPC3 was found to be upregulated in monocytes isolated from hypertensive rats compared with normotensive control subjects (Zhao et al., 2012b). Importantly, a similar increase in TRPC3 was detected in monocytes obtained from the blood of patients with essential hypertension and/or T2DM. When challenged with the chemotactic peptide formyl-Met-Leu-Phe, these monocytes showed accelerated in vitro migration in correlation with their TRPC3 expression. Furthermore, monocytic TRPC3 mRNA strongly correlated with the IL-1 and TNF transcripts. Finally, monocytes isolated from TRPC3(−/−) animals exhibited impaired efferocytotic activity.

In ApoE-deficient mice transplanted with the bone marrow of TRPC3-null animals and kept on a high-fat diet, atherosclerotic plaques occurred later and were smaller with a reduced necrotic core compared with control subjects (Tano et al., 2014).

Taken together, these findings imply that TRPC3 may be upregulated in monocytes of patients with risk factors for atherosclerosis (e.g., essential hypertension and/or T2DM) and this upregulated TRPC3 expression may promote monocyte migration/foam cell formation and, at the same time, block efferocytosis. The potential role of TRPV2 and TRPM2 in monocyte functions is mentioned elsewhere (section III.B.2).

A phenotypic switch between the contractile and proliferative phenotype of vascular smooth muscle cells is believed to contribute to the narrowing of the vascular lumen during atherosclerosis. This molecular switch is a potential therapeutic target. Angiotensin II induces vascular smooth muscle proliferation via TRPC1 activation (Saleh et al., 2006). In a vascular injury model, TRPC1 appeared to mediate the switch from the contractile to the proliferative phenotype (Golovina et al., 2001; Kumar et al., 2006). This is in keeping with the postulated role of TRPC1 in vascular graft restenosis (Kumar et al., 2006), maladaptive cardiac hypertrophy (Ohba et al., 2007), and IPAH (Zhang et al., 2007). More recently, the TRPC3 antagonist Pyr3 was reported to inhibit smooth muscle proliferation and prevent stent-induced remodeling (Koenig et al., 2013).

Other potential TRP channels that may operate this phenotypic switch include TRPM3, TRPM7, TRPV1, and TRPV4 (Yang et al., 2006). TRPM3 is present both in proliferating and contractile vascular smooth muscle cells with low-level constitutive activity (Naylor et al., 2010). In freshly isolated aorta smooth muscle cells, activation of TRPM3 stimulated contractile responses. Loading the experimental system with cholesterol to generate foam cells suppressed TRPM3 activity to undetectable levels. Smooth muscle cells isolated from the ascending aorta also express functional TRPM7 channels (Baldoli et al., 2013). In these cells, angiotensin II increased TRPM7 expression via the Pyk2-ERK1/2-Erk1 signaling pathway (Inoue and Xiong, 2009). TRPM7 silencing with siRNA attenuated cell proliferation after angiotensin II administration. As expected, TRPM7 silencing also mimicked the effects of Mg2+ deficiency on human microvascular endothelial cells (HMVEC) (Baldoli and Maier, 2012). Pulmonary artery smooth muscle cells also express TRPV1 and TRPV4, TRPV1 and TRPV4 agonists (capsaicin and 4α-PDD, respectively) induced migratory responses in these cells in a manner that was inhibited by both capsazepine (a first-generation TRPV1 antagonist) (Wang et al., 2008a) and the TRPV4 blocker HC-067047 (Martin et al., 2012).

Of note, TRPV1 was implicated as the target mediating the protective effect of evodiamine on the development of atherosclerosis (Wei et al., 2013). In
ApoE knockout mice, long-term administration of evodiamine attenuates hyperlipidemia, reduces the size of atherosclerotic lesions, and alleviates fatty liver disease. Evodiamine was shown to function as a TRPV1 agonist (Pearce et al., 2004). Indeed, genetic deletion of TRPV1 abrogated the protective effect of evodiamine against atherosclerosis. Interestingly, double-knockout (ApoE/TRPV1-null) mice showed severe atherosclerosis and fatty liver disease (Wei et al., 2013).

Endothelial damage also stimulates the formation of thrombi. There is a growing body of evidence supporting a role for abnormal Ca$^{2+}$ signaling by TRP channels in the pathogenesis of platelet dysfunction (reviewed in Dionisio et al., 2012; Mahaut-Smith, 2013). Indeed, TRPC6 knockout mice showed prolonged bleeding time after tail injury, as well as an increased time for occlusion in the carotid artery injury thrombosis model (Paez Espinoza et al., 2012). These findings suggest that TRPC6 may be a new therapeutic target for managing thrombotic disorders. Elevated TRPC3 (Zbidi et al., 2009) and TRPC6 (Liu et al., 2008) expression was reported in platelets isolated from the blood of patients with T2DM. Apparently, this effect is secondary to the increase in blood glucose and is mediated by the PI3K pathway.

As an example of puzzling (and troubling) species-related differences in TRP channel expression, functional TRPV1 was detected in human, but not in mouse, platelets (Sage et al., 2013). Capsaicin evoked 5-hydroxytryptamine release from human platelets in a manner that was blocked by the TRPV1 antagonist 5-iodoresiniferatoxin (I-RTX) (Harper et al., 2009). It was speculated that TRPV1 might be a useful target to explore in patients with unstable coronary plaques (Li and Huang, 2011).

4. Transient Receptor Potential Channels and Cardiac Fibrosis. Cardiac fibrosis is not a distinct entity; rather, it is a common pathologic pathway for various diseases (e.g., AMI and chronic ischemic heart disease, inflammatory and hypertrophic cardiomyopathies, and aging-associated heart disease) that damages the myocardium (reviewed in Yue et al., 2013). The damaged myocardium is then replaced by connective tissue synthesized by fibroblasts and myofibroblasts. This is a problem because cardiac fibrosis interferes with electrical conduction and impairs the pumping function of the heart. Cardiac fibrosis is subdivided into reparative (e.g., after AMI) and reactive types, although this distinction is somewhat arbitrary.

Fibroblasts are abundant and quiescent in the normal heart. By contrast, myofibroblasts are rare in the healthy heart but become enriched during disease. In response to pathogenic stimuli (e.g., myocardial injury, oxidative stress, and/or mechanical stretch), fibroblasts get activated and a subset differentiates into myofibroblasts (see Yue et al., 2013). In turn, activated fibroblasts and myofibroblasts excessively synthesize and deposit extracellular matrix proteins. There is increasing evidence (summarized in Fig. 33) that TRP channels are involved in the activation and differentiation of cardiac fibroblasts (see Yue et al., 2013). This is important because existing therapeutic options to halt (or reverse) cardiac fibrosis are unsatisfactory.

There are at least three major signaling pathways involved in the biochemical cascade of fibrogenesis, namely the renin-angiotensin system, the TGFβ signaling pathway, and the oxidative stress pathway (Fig. 33). Cultured human cardiac fibroblasts express a number of TRP channels, including TRPC1, TRPC4, TRPC6, TRPV2, and TRPV4, as well as TRPM7 (Nishida et al., 2007; Rose et al., 2007; Du et al., 2010). In addition, mouse cardiac fibroblasts also express TRPC3, TRPM4, and TRPM6. This expression pattern is based on RT-PCR experiments and many observations lack functional correlate. However, there is indirect evidence that cardiac fibroblasts express abundant functional TRPC3 and TRPM7 channels. It was speculated that TRPM7 is a target for C-type natriuretic peptide (Rose et al., 2007). TRPM7 is also a downstream target for the TGFβ signaling pathway (Du et al., 2010). Indeed, knockdown of TRPM7 by shRNA inhibits TGFβ-induced fibroblast proliferation and collagen production (Du et al., 2010). Conversely, TRPM7 is upregulated in TGFβ-stimulated cardiac fibroblasts.

From cardiac fibroblasts, TRPM2-like and TRPV4-like currents were also recorded. The TRPM2-like current was evoked by hypoxia and was eliminated in the presence of gene knockdown by TRPM2 siRNA (Takahashi et al., 2012). On the basis of these observations, it was speculated that TRPM2 may be involved in the pathogenesis of hypoxia (ischemia)-induced cardiac fibrosis (reviewed in Yue et al., 2013). Using the TRPV4 agonist 4a-PDD, a TRPV4-like current can be measured in fibroblasts (Hatano et al., 2009). This is interesting because TRPV4 may be activated by mechanical stretch, a known etiological factor in the pathogenesis of cardiac fibrosis.

E. Transient Receptor Potential Channels in Obesity and Metabolic Disorders

In the United States, the prevalence of diabetes has reached epidemic proportions. Almost 26 million Americans (7% of the population) have diabetes and the prevalence of T2DM exceeds 20% in elderly individuals. Furthermore, an estimated 65% of American adults are overweight or obese. The prevalence of obesity is approaching 20% in the adolescent population, which is even more worrisome. The Healthy People 2010 Goal aimed at an obesity prevalence of 15%; as yet, no state has met this target.

Obesity is an increasingly significant medical problem in the developing world as well. For example, an
estimated 40% of individuals living in urban areas in India are overweight or obese. This is important because this rampant obesity is thought to bear at least partial responsibility for the rapidly increasing prevalence of T2DM as well as heart disease and possibly cancer worldwide. In women, being obese is a well established risk factor for breast and endometrioid adenocarcinoma.

The combined cost of obesity and diabetes is staggering. In 2010, the United States spent an estimated $146 billion on diabetes- and obesity-linked illnesses, accounting for approximately 6% of the $2.6 trillion healthcare expenditure. Obese Americans spend $1400 more annually on health care than their normal weight compatriots, and the estimated lifetime cost to Medicare of treating obesity-related diseases exceeds $160,000. If the current trend continues, obesity- and T2DM-related healthcare spending may reach $1 trillion in 10 years, potentially bankrupting Medicare. Clearly, there is a dire need for novel therapeutic interventions in weight loss and blood glucose control.

There is no shortage in speculation as to what is responsible for the worldwide explosion in obesity rates. There can be no dispute that we are living in an “obesogenic” environment. Meal and sugary drink sizes have become larger and we are bombarded with ads promoting food overconsumption (as memorialized in the documentary “Supersize Me!”). On the other side of the calorie equation is the reduced energy expenditure due to a sedentary lifestyle. Evolution may also bear some responsibility for this trend. At times of food shortages, slow metabolism (effective calory utilization) may promote survival. When food is plentiful, these biochemical traits may promote rapid weight gain. Ossabaw island pigs (as discussed above, used as a model to study the potential link between TRP channels and atherosclerosis) are a good example of the phenomenon. These pigs have adapted to the harsh environment of the island (they have a “thrifty phenotype”) and they gain weight rapidly when they are allowed to eat ad libitum.

A much debated question is the role of food additives in altering metabolism and promoting obesity. High-fructose corn syrup and “high-intensity” non-nutritive sweeteners have been used with increasing frequency over the years, mirroring the trend in obesity. Paradoxically, rats fed diets containing saccharin and acesulfame potassium show increased food intake, weight gain, and adiposity compared with control subjects. It was speculated that these non-nutritive sweeteners might activate sweet taste receptors in the gut, dissociating the chemosensory signal from caloric consequences, and setting off countercompensatory processes. If this also hold true in men, diet soda might not be “diet” at all.

Although sensible lifestyle changes (a combination of diet and exercise) can no doubt lead to weight loss, it is easier said than done. Sadly, compulsive overeating (popularly referred to as food addiction) can be difficult to overcome without the help of therapeutic intervention. Despite breakthrough discoveries in our understanding of the control of ingestive behavior (e.g., the role of leptins and their receptors), the success of drug therapy for obesity to date has been modest at best, beset with problems associated with adverse effects. A recent disappointment was the cannabinoid receptor 1 (CB1) inverse agonist rimonabant that caused depression and suicidal thoughts in many patients (reviewed in Di Marzo and Szallasi, 2008; Di Marzo et al., 2008).

1. Therapeutic Intervention for Appetite Control and Weight Loss: Central versus Peripheral Transient Receptor Potential Targets.

   a. Vanilloid transient receptor potential 1. Connoisseurs of hot spicy food are intimately familiar with the predominant pharmacological actions of capsaicin from personal experience: it induces profuse perspiration (known as gustatory sweating) as well as a hot, burning sensation in the tongue and oral mucosa that dissipates upon repeated challenge (reviewed in Szallasi and Blumberg, 1999). Evolutionary selective pressure seems to have maximized the pungency of capsaicin. It was speculated that the compound’s pungency is able to deter ambulatory animals from eating chili pepper fruits, favoring those plants whose seeds were dispersed widely by birds (see Szallasi and Blumberg, 1999). Indeed, the avian TRPV1 receptor is not activated by capsaicin (Jordt and Julius, 2002), and hence birds are undeterred from ingesting chili pepper fruits and can excrete the pepper seeds large distances away. This forms the basis of the development of hot pepper–flavored “squirrel-free” bird feed (Szallasi and Blumberg, 1999). It is still a mystery, however, why the same pungency that repels squirrels is perceived as pleasurable by many human beings.

   Several lines of evidence have established a link of TRPV1 to the regulation of body weight, although the exact nature of this link (whether TRPV1 agonism or blockade protects against obesity) remains controversial. There is anecdotal evidence that consumption of hot, spicy food is associated with a lower prevalence of obesity (see Szallasi and Blumberg, 1999). In accord, rats kept on a diet containing 0.014% capsaicin showed no change in calory intake but a significant (approximately 30%) reduction visceral fat weight compared with control subjects fed standard chow (Kawada et al., 1986). In long-term (120 days) feeding experiments, dietary capsaicin prevented obesity in wild-type, but not TRPV1 knockout, mice assigned to a high-fat diet (Zhang et al., 2007b). Dietary capsaicin also suppressed appetite and weigh gain in rabbits (Yu et al., 2012). A meta-analysis of 20 trials involving 563 participants also found a modest benefit (50 kcal per day increase in energy expenditure) for daily capsaicin
consumption (Ludy et al., 2012). This forms the experimental foundation of using Cheyenne pepper capsules as dietary supplements for weight management. It was speculated that capsaicin may suppress appetite and increase energy expenditure. Indeed, capsaicin was reported to increase O_2 consumption and thermogenesis in rat skeletal muscle (Cameron-Smith et al., 1990). In healthy volunteers, dietary capsaicin boosted thermogenesis by 50% with no effect on satiety (Clegg et al., 2013). Interestingly, non-pungent capsaicin analogs (e.g., evodiamine from the fruit of *Evodia rutaecarpa*; see Fig. 5 for structure) also boosted energy consumption and prevented weight gain both in mice kept on a high-fat diet (Kobayashi et al., 2001) and in human volunteers (Snitker et al., 2009).

Rats with chemically ablated (by high-dose systemic capsaicin) sensory neurons overeat when fed chow supplemented with glucose; however, on a fat-containing diet, they consume amounts similar to controls (Ritter and Taylor, 1989) after an initial overconsumption (Chavez et al., 1997). Somehow paradoxically, when kept on a high-fat diet, TRPV1 knockout mice accumulate less visceral (intra-abdominal) adipose tissue than their wild-type littermates (Figs. 34 and 35) despite the equivalent food consumption and dietary lipid uptake (Motter and Ahern, 2008). These animals also appear to be protected from fatty liver disease (Fig. 35). If so, TRPV1 antagonists may represent a novel strategy for intervention in patients with nonalcoholic steatohepatitis. Unfortunately, recent articles present a very different picture. Dietary capsaicin was found to increase hepatic uncoupling protein (UCP)-2 expression and reduce fat accumulation in hepatocytes in wild-type, but not in TRPV1-null, mice (Li et al., 2012, 2013). Activation of TRPV1 by dietary capsaicin in hepatocytes also improved the liver function tests (Tani et al., 2004; Li et al., 2013).

It was suggested that the resistance to weight gain in TRPV1 knockout mice was due to an increased thermogenic capacity. In rodents, chemical ablation by neonatal capsaicin treatment of TRPV1-expressing nerves yielded conflicting reports (reviewed in Szallasi and Blumberg, 1999). Some authors (e.g., van de Wall et al., 2006) found no change in food consumption, body weight, and energy balance in the capsaicin-treated animals (attributed to compensatory mechanisms), whereas others described lower weight predominantly due to atrophied BAT and a marked (approximately 40%) decrease in thermogenic response to infused norepinephrine after neonatal capsaicin administration (Cui et al., 1990). Adding to the confusion, capsaicin-treated animals did not lose weight after weight loss surgery (intragastric implantation of silicone bubbles) (Northway et al., 1992). On the basis of the latter finding, it was postulated that TRPV1 is an important mediator of satiety. Indeed, N-OEA, a fatty acid metabolite that suppresses appetite (see Thabuis et al., 2008; Sarro-Ramirez et al., 2013), functions as a TRPV1 agonist (Ahern, 2003; Almási et al., 2008).

A recent study found no difference in weight gain between TRPV1 knockout and wild-type mice kept on a high-fat diet (Marshall et al., 2013). These seemingly contradictory findings may be reconciled by a recent study in which the phenotype of TRPV1 knockout mouse was 1) dependent on the age of the animals and 2) was also heavily influenced by environmental variables (diet, room temperature, etc) that can impact thermoregulation (Wanner et al., 2011). For instance, young TRPV1 knockout mice exhibited increased locomotor activity, which could account for increased energy expenditure and protect against gaining weight. As the genetically TRPV1-deficient mice age, their motor activity declines and body weight concomitantly increases. Parenthetically, aging also seems to reverse the role of TRPV1 in systemic inflammation from anti-inflammatory to proinflammatory (Wanner et al., 2012). If these observations hold true in humans, young individuals could benefit from TRPV1 blockers for weight management, whereas older individuals may want to supplement their diet with TRPV1 agonists. Of note, the loss-of-function TRPV1 variant V585I appears to confer some protection against diet-induced obesity (Zhang et al., 2007b).

An interesting (and controversial) hypothesis postulates a central role for TRPV1-expressing adipocytes in regulating body weight and questions the feasibility of targeting TRPV1 for weight loss in individuals who are already obese (see Saito and Yoneshiro, 2013). By a combination of immunoblotting and RT-PCR, TRPV1 was detected in the visceral adipose tissue of mice and humans, with decreased expression in both obese humans and mice (ob/ob and db/db) relative to lean control subjects (Zhang et al., 2007b). In mice on a high-fat or high-sucrose diet, supplementation of food with the TRPV1 agonist monoacylglycerol increased mitochondrial UCP1 expression in BAT and prevented

![Fig. 34. TRPV1-null mice stay lean on a high-fat diet compared with their wild-type (WT) littermates. Reprinted with permission from Motter and Ahern (2008).](image-url)
white fat accumulation (Iwasaki et al., 2011). In preadipocyte 3T3-L1 cells, a model of adipocyte differentiation, capsaicin evoked Ca2+ influx and prevented adipogenesis (Zhang et al., 2007b). Conversely, knockdown of TRPV1 by RNA interference restored adipogenesis. Capsaicin responses were attenuated in fat cells isolated from obese individuals (Zhang et al., 2007b). Combined, these results suggest that capsaicin may prevent weight gain via UCP1 upregulation in subjects with normal body weight, but this beneficial effect may be lost in obese individuals due to down-regulation/loss of TRPV1 receptors on adipocytes.

In rodents, BAT is a major determinant of energy expenditure through thermic responses. BAT is rich in sensory afferents. Chemical ablation by capsaicin of TRPV1-expressing afferents renders young rats resistant to age-related obesity (Melnyk and Himms-Hagen, 1995). Of note, a major neuronal mediator of this effect on BAT appears to be CGRP. CGRP was shown to stimulate thermogenesis in BAT, and disruption of CGRP gene in mice resulted in protection against the metabolic effects and weight gain caused by a high-fat diet (Walker et al., 2010). As discussed below, CGRP has also been linked to T2DM, a frequent complication of obesity. For example, in the Zucker rat, elevated circulating CGRP levels were detected prior to the onset of obesity and insulin resistance, presumably as a result of increased sensory neuron activity (Gram et al., 2005). CGRP is, however, probably not the only important player in the sensory neuron-adipose tissue interaction. Capsaicin-sensitive nerves coexpress SP with CGRP, and mice whose NK1Rs were deleted by genetic recombination show protection against diet-induced weight gain comparable to that seen in the CGRP knockout animals (Karagiannides et al., 2011). This protective effect was replicated by NK1R antagonists (Karagiannides et al., 2008; Ramalho et al., 2013). It is worth mentioning here that obesity is a recognized risk factor for migraine and it was speculated that CGRP may be the molecular link between these two disorders (Recober and Goadsby, 2010).

An interesting (and somewhat neglected) weight management strategy is the use TRPV1 agonists to reduce energy consumption by altering (speeding up) gastrointestinal motility. Because the rate of gastric emptying directly impacts nutrient uptake, facilitating gastric motility by pharmacologic agents could have a positive impact on satiety. Indeed, there is good evidence that capsaicin can increase the rate of gastric motility in humans (Gonzalez et al., 1998; Debreceni et al., 1999). This also implies a therapeutic potential for TRPV1 agonists in the pharmacotherapy of gastroparesis (Barquist et al., 1996).

b. Vanilloid transient receptor potential 4. A pivotal connection was recently established between TRPV4 and UCP1 (also known as thermogenin), a key modulator of nonshivering thermogenesis in BAT (Ye et al., 2012). In BAT, UCP1 is constitutively active. Mitochondrial UCPs divert electrons to minimize ROS production and generate heat. The naked mole rat, which lives in subterranean burrows with constant ambient temperature and therefore has little need for heat generation, has a unique UCP1 that maintains...
ATP synthesis with essentially no ROS production (reviewed in Rodriguez et al., 2011; Lewis et al., 2013). These rats are the longest-living rodents (up to 32 years), at least when kept at the zoo (zoo.sandiegozoo.org/animals/naked-mole-rat). Because adult humans have virtually no BAT, until recently, the role of UCP1 in body weight regulation was unclear (Kozak and Annunciado-Koza, 2008). In 2012, “beige adipocytes” (UCP1-positive fat cells) were demonstrated in human white adipose as the thermogenic equivalent of murine BAT (Wu et al., 2012). The white to beige adipose tissue molecular switch is operated by irisin (Boström et al., 2012), the “magic exercise hormone” encoded by the “human exercise gene” (Kelly, 2012; Timmons et al., 2012). During strenuous physical exercise, skeletal muscle produces irisin (via AMP kinase) as the membrane cleavage product of FNDC5. In turn, irisin facilitates thermogenesis by upregulating UCP1 and thereby transforming white fat cells into beige (reviewed in Elbelt et al., 2013). Of note, irisin is already popularized as a “miracle weight loss pill” (www.mensfitness.com/nutrition/supplements/miracle-weight-loss-pill-irisin-allows-for-easy-workouts).

The transcription of UCP1 is under the control of peroxisome proliferator–activated receptor-γ coactivator 1α (PGC1α), originally identified as a cold-inducible factor in BAT. PGC1α was postulated to play a pivotal role both in obesity and T2DM (reviewed in Attie and Kendzierski, 2003). Indeed, PGC1α gene polymorphism has been linked to T2DM in Asian Indians (Vimaleswaran et al., 2005). PGC1α activators improve exercise endurance and promote weight loss in mice (Luo et al., 2012b). The UCP1 gene has a peroxisome proliferator–activated receptor-γ (PPARγ) responsive element in the enhancer region (Barbera et al., 2001). PPARγ is a known regulator of adipogenesis. The knockout mice do not deposit adipose tissue even if kept on a high-fat diet (Jones et al., 2005b). The variant allele G482S of PPARγC1a confers an increased risk of obesity in elderly men (Ridderstråle et al., 2006). Targeted PGC1α overexpression upregulates UCP1 and protects against obesity (see Liang and Ward, 2006). Conversely, PGC1α knockout mice gain weight and show early exhaustion during physical exercise. In aging mice, white adipose tissue (WAT) is redistributed from the subcutis to the viscera with a concomitant loss of UCP1 (Rogers and Smith, 2012). This phenomenon might explain why older individuals find it difficult to lose weight and are prone to develop insulin resistance (see Rogers and Smith, 2012). Loss-of-function polymorphism in the PGC1α gene was recently linked to obesity and T2DM (Oberkofler et al., 2004). Mitofusin 2 is the downstream target of PGC1α. Taken together, these findings highlight PGC1α as a promising target for weight control.

Most recently, a chemical screen identified TRPV4 as a negative modulator of PGC1α in adipocytes (Fig. 36; Ye et al., 2012). The TRPV4 antagonist GSK205 changed the phenotype of 3T3 F442A adipocytes (by upregulating factors that increase thermal responsiveness) and improved glucose tolerance in healthy mice. Moreover, the TRPV4 knockout mice were protected from obesity when kept on a high-fat diet despite the similar food intake and physical activity compared with their wild-type littermates. Importantly, GSK205 increased both PGC1α and UCP1 activity in obese mice. Unfortunately, a subsequent independent study reported the opposite phenotype for TRPV4 knockout mice. In this study, the knockout mice became obese on a high-fat diet and, even worse, developed severe OA (O’Conor et al., 2013). Parenthetically, in a different study, bone mass was increased by approximately 20% in male TRPV4-null mice compared with sex-matched wild-type animals (van der Eerden et al., 2013). As we saw above, similarly discrepant observations were published in the TRPV1 knockouts. One might argue that both the genetic background of the knockout animals (e.g., B6 mice are prone to weight gain, whereas B129 animals are not) and uncontrolled environmental factors can influence the apparent phenotype.

c. Ankyrin transient receptor potential 1. In mice, the TRPA1 agonist cinnamaldehyde was reported to increase UCP1 expression in BAT (Tamura et al., 2012). Cinnamaldehyde also reduced visceral adipose tissue deposition in mice fed a high-fat or high-sucrose diet although it had no effect on total calorie intake. If so, TRPA1 agonists may promote beige fat formation and increased energy utilization in humans. The TRPA1 agonist methyl syringate (a pungent ingredient in the castor oil tree Kalopanax) was reported to suppress food intake and gastric emptying (Kim et al., 2013). TRPA1 also appears to be expressed on enterochromaffin cells that contain CCK (Nozawa et al., 2009). Upon TRPA1 activation, these cells may release...
CCK that, in turn, may accelerate gastric emptying and promote satiety. In vitro, TRPA is activated by PUFA (Motter and Ahern, 2012). In feeding studies, the wild-type animals consumed much less PUFA-containing chow than did TRPA1-null mice. The typical US diet contains approximately 8% calorie-equivalent PUFA. In mice, prenatal PUFA exposure leads to adult obesity. On the basis of these studies, one might speculate that TRPA1 agonists can induce taste aversion to PUFAs. In relation to dietary TRPA1 agonist consumption (although not obesity, the topic of our discussion), it was suggested that TRPA1 agonism promotes longevity (at least in the worm, *C. elegans*) (Xiao et al., 2013), leading to the headline “Age Slowly by Eating Sushi Outside!” (www.express.co.uk/news/health/378380/age-slowly-by-eating-sushi-outside).

d. Melastatin transient receptor potential 5. The key role of TRPM5 in taste signaling is well documented (reviewed in Sprous and Palmer, 2010; Medler, 2011; Palmer and Lunn, 2013). Generally speaking, the “taste” sensation arises from recognition of a “tasty” ligand by a cognate taste receptor. These taste receptors are expressed in highly specialized sensory cells in the tongue. TRPM5 is a downstream target in type 2 taste receptor cells for GPCR cognate taste ligands and propagates the taste signal. TRPM5 activation by its cognate agonist, the sweet, bitter, and umami (savory) (see Sprous and Palmer, 2010; Palmer and Lunn, 2013). Indeed, genetic ablation of TRPM5 severely impairs the ability of mice to detect sweet, umami, and bitter, as well as “fat” (Liu et al., 2011a), taste (Zhang et al., 2003). When stimulated, these taste receptors initiate a second-messenger signaling cascade that culminates in the release of intracellular Ca²⁺. In turn, the elevated intracellular Ca²⁺ opens TRPM5 to allow Na⁺ enter the taste cells and propagate the taste signal.

A possible strategy for weight control is to make food less palatable (less tasty) by blocking TRPM5 in taste cells (reviewed in Sprous and Palmer, 2010; Palmer and Lunn, 2013). As proof of concept, TRPM5 knockout mice gain less weight than their wild-type littermates when kept on a carbohydrate-rich diet (Glendinning et al., 2012). In a different study, however, the TRPM5-deficient mice developed a robust preference for sucrose (probably based on the calory content since these animals cannot taste sweet), questioning the feasibility of this approach (de Araujo et al., 2008).

Quinine (a TRPM5 blocker) reduces weight gain in mice kept on standard chow (Cettour-Rose et al., 2013). This “antihedonistic” approach, however, may not be popular with the general population. Alternatively, TRPM5 may be stimulated by positive allosteric modulators in the hope that people will eat less from “supersweet” desserts (see Palmer and Lunn, 2013). Moreover, positive allosteric modulators may maintain the sweetness of sugary beverages with reduced calorie content. This is important because regular sodas are thought to be a main cause of obesity and, as discussed above, diet sodas may cause paradoxical weight gain.

TRPM5 is also expressed in a well-defined subset of enterodocrine cells, the so-called L cells (Kokrashvili et al., 2009). These cells secrete the incretin hormone GLP-1 that, in turn, influences satiety via three mechanisms: 1) it suppresses food consumption; 2) it facilitates gastric emptying, and 3) it promotes insulin release in response to glucose (reviewed in Silva and Bloom, 2012). Moreover, in response to glucose, TRPM5-expressing L cells release peptide YY, which is believed to suppress appetite in humans. Some gastric and duodenal L cells in humans also coexpress TRPM5 with gustducin (see Liman, 2010). Gustducin functions as a bitter taste receptor in the tongue. The biologic function of gustducin in gastric and duodenal L cells is unclear, but it was hypothesized that these TRPM5⁺/gustducin⁺ cells protect the body from toxins by evoking vomitus when stimulated. If this model holds true, it might be a fine line to synthesize such TRPM5 agonists that control appetite by releasing GLP-1 and peptide YY but do not cause vomiting as an on-target side effect. Perhaps surprisingly, the number of TRPM5⁺ cells is increased in the stomach of obese individuals (Widmayer et al., 2012). It is unclear whether this is a disease-causing or compensatory mechanism.

Most people would consider fat as tasteless; yet TRPM5-deficient mice lose their ability to distinguish LA-containing solutions from physiologic saline (Liu et al., 2011a). Moreover, TRPM5 mediates CCK release from enterochromaffin cells (I cells) in small intestinal tissue in response to LA (Shah et al., 2012). CCK is a peptide hormone that (as its name implies) stimulates gallbladder contractions. CCK also suppresses hunger. TRPM5 knockdown by siRNA blocks LA-induced CCK release by at least 60%.

TRPM5-like immunoreactivity was also detected in brush cells lining the gastrointestinal tract, particularly in the ileum and colon (Bezençon et al., 2008). Brush cells share some morphologic characteristics with taste cells. The functions of brush cells are not well understood, but their highly differentiated apical villi positioned toward the intestinal lumen suggest that they detect the presence of dietary nutrients. The presence of TRPM5 in brush cells is confirmed in genetically engineered mice in which the expression of TRPM5 was coupled to green fluorescent protein to visualize the distribution of TRPM5 protein. In brush cells, TRPM5 seems to be colocalized with the opiates β-endorphin and Met-enkephalin. In wild-type mice, the duodenum released β-endorphin into the lumen in response to hyperosmotic solutions. This response was, however, attenuated in the duodenum of TRPM knockout mice, indicating that TRPM5 was required for optimal β-endorphin release. Opiates are known to
influence intestinal motility, thus it can be speculated that TRPM5 affects nutrient processing in the small and large intestine by controlling β-endorphin and Met-enkephalin release.

**e. Melastatin transient receptor potential 8.** The TRPM8 agonist menthol increases locomotor activity and prevents diet-induced weight gain in wild-type, but not TRPM8 knockout, mice (Ma et al., 2012a). In humans, the TRPM8 variant L250L has been linked to truncal obesity. Menthol upregulates upstream transcription factor-1, although it is not clear whether this is mediated by TRPM8. Upstream transcription factor-1, a member of the helix-loop-helix leucine zipper family, is involved in the regulation of several genes that affect glucose and lipid metabolism.

**f. Canonical Transient Receptor Potentials.** Mature adipocytes were reported to express TRPC1 and TRPC5 in a way so that they negatively regulate adiponectin (Sukumar et al., 2012). Adiponectin is a “fat hormone” that is believed to play an important role in the pathogenesis of atherosclerosis. Indeed, plasma levels of adiponectin are inversely correlated with body fat. Transgenic mice overexpressing adiponectin show increased energy expenditure and stay lean. If TRPC5 blockade increases plasma adiponectin levels by eliminating the negative control, it may be beneficial in patients with metabolic syndrome/T2DM secondary to obesity. TRPC5 channels are also overexpressed in a porcine model of metabolic syndrome (Hu et al., 2009a).

Given the rather ubiquitous presence of TRPCs in contractile cells, the reports of TRPC expression in gastrointestinal smooth muscle are hardly unexpected (reviewed in Guibert et al., 2011; Holzer, 2011a,b). Furthermore, TRPC4 was detected in the interstitial cells of Cajal (Walker et al., 2002). These cells are a part of the pacemaker apparatus of the gastrointestinal tract, responsible for regulating smooth muscle contraction, and TRPC4 has been proposed as a candidate for the nonselective cation channel that is central to the pacemaker activity. The presence of these TRPC channels within the interstitial cells suggests a potential common control point for the cells involved in enteric motor neurotransmission and gastrointestinal tract peristalsis.

2. **Obesity as a Low-Grade Inflammatory Disease: Implications for Transient Receptor Potential Channel Targeting.** It was proposed that obesity is in fact a low-grade chronic inflammatory disorder (Johnson et al., 2012). Indeed, obesity is associated with macrophage accumulation in adipose tissue (Weisberg et al., 2003). In humans, the density of CD68+ macrophages in subcutaneous adipose tissue correlates with the body mass index. Obese mice reveal a proinflammatory gene expression profile (Xu et al., 2003). It was hypothesized that “obese” adipose tissue (secondary to this molecular switch) starts producing the chemokine macrophage chemotactic protein-1 (MCP1) that, in turn, attracts M1 macrophages to infiltrate the fat (see Johnson et al., 2012). M1 macrophages, in turn, secrete TNFα, which disrupts normal insulin signaling. Indeed, obese individuals have high plasma TNFα levels that precede the development of T2DM (Mishima et al., 2001). Of note, inflammation induces the upregulation of TRPV2 in sensory neurons (Shimosato et al., 2005). TRPV2 might also play some role in weight control since the TRPV2 knockout mice show slightly reduced body weight (Park et al., 2011).

3. **Targeting Transient Receptor Potential Channels in Metabolic Disorders and Diabetes.** Type 1 diabetes mellitus (T1DM) is an autoimmune disease that results from the destruction of insulin-secreting pancreatic β cells by autoreactive cytotoxic T cells. By contrast, T2DM is a consequence of insulin resistance (cells do not respond to insulin properly even if it is present at normal plasma levels) and is closely associated with obesity and a sedentary lifestyle. Of note, β cells will try to compensate for this insulin resistance by increasing insulin secretion (compensatory hyperinsulinemia) that may, eventually, lead to β cell exhaustion (reviewed and Suri and Szallasi, 2008). Thus, patients with T2DM may also develop insulin dependence and become similar to those with T1DM.

The nonobese diabetic (NOD) mouse is an attractive model to study human T1DM because it shares many of its salient features, including spontaneous onset and expression of disease-susceptible MHC molecules (reviewed in Suri and Szallasi, 2008). Indeed, human and mouse class II MHC molecules, the function of which is to present peptide antigens to CD4+ helper T cells, share a number of genetic, structural, and functional properties.

In 2006, a crucial role for TRPV1-expressing sensory afferents was demonstrated in the pathogenesis of T1DM (Razavi et al., 2006). In the mouse, neurogenic inflammation is clearly involved in maintaining islet inflammation (“insulitis”) and β cell death. With the recently recognized importance of adipose tissue-infiltrating lymphocytes in T2DM, it was postulated that sensory innervation of the fat may play an analogous role to pancreatic afferents (see Tsui et al., 2011). This unifying neuroimmune model blurs the line between T1DM and T2DM and places TRPV1 in a central position in the pharmacotherapy of both (see Tsui et al., 2007, 2011; Suri and Szalasi, 2008). Moreover, a number of functional TRP channels were shown to be expressed on pancreatic β cells in which they are believed to play a key role in the regulation of insulin secretion, opening up new avenues for the development of novel antidiabetic drugs (see Uchida and Tominaga, 2011; Colson et al., 2011, 2013).

4. **Targeting Transient Receptor Potential Channels in Sensory Afferents in the Pharmacotherapy of Diabetes.**
**a. A role for vanilloid transient receptor potential 1 in autoimmune diabetes.** In NOD mice, TRPV1 maps to the idd4 locus, a unique hypofunctional (hyposecretory) mutant resulting from two amino acid substitu-tes. High-dose capsaicin administered to neonatal NOD mice to ablate TRPV1-expressing neurons abrogated islet-specific autoimmunity (and thus protected the animals from the signs of autoimmune diabetes) despite the infiltration of other tissues (e.g., salivary glands) by autoreactive T cells (Razavi et al., 2006). In other words, the absence of TRPV1 protected the pancreatic islets without eliminating the diabetogenic T cell population.

After neonatal capsaicin treatment, there is a permanent loss of SP-like immunoreactivity in sensory neurons (see Szallas and Blumberg, 1999). Since SP is a well established mediator of neurogenic inflammation (see Szallas and Blumberg, 1999), it can be easily visualized how loss of SP may protect pancreatic islets from insulitis. Unexpectedly, SP administered directly into the pancreas of newly diabetic NOD mice was shown to inhibit T cell proliferative responses and restore normoglycemia (Razavi et al., 2006). To reconcile these conflicting observations, it was argued that SP has a dual action on insulitis. A relatively low level and sustained SP release promotes neurogenic pancreatitis, whereas higher, bolus-like SP doses inhibit (non-neurogenic) T cell responses (see Suri and Szallas, 2008). Unfortunately, this model is at variance with some of the literature (see below) that suggests a tonic action for SP in T cell responses.

The contribution of sensory nerve fibers to immune regulation is long recognized (reviewed in Cortright and Szallas, 2009) although its significance is still underappreciated. Rats whose TRPV1-expressing sensory neurons were destroyed by neonatal capsaicin administration show reduced ability to mount a primary antibody response to sheep red blood cell antigens. Of interest to this section, T cell proliferation in response to mitogens or IL-2 was also attenuated in capsaicin-treated rats, and this effect was reversed by SP administration (Santoni et al., 1996). The mechanisms underlying this altered immunoregulation remain to be understood. A subset of thymic T cells was reported to express TRPV1 (Wenning et al., 2011); therefore, the possibility that capsaicin may kill lymphocytes directly via TRPV1 activation cannot be discounted (Farfariello et al., 2012). Indeed, neonatal capsaicin treatment was shown to impair T cell maturation and induce apoptosis (Santoni et al., 2000). This possibility notwithstanding, a more likely explanation is depletion by capsaicin of neuronal SP since exogenous SP is able to fully restore antibody response to sheep red blood cell antigens and prevent capsaicin-induced apoptosis in T cells (Santoni et al., 1996, 2004). Thymocytes express NK1Rs (Santoni et al., 1999). Indeed, SP augments the proliferation of CD4+ T cells in the presence of mitogens, including human T cells (Payan et al., 1984); this effect is abolished in the presence of the NK1R blocker SR140333 [(S)-1-2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl][ethyl-4-phenyl] azonia-bicyclo[2.2.2]octane] (Santoni et al., 1999). These pharmacological actions are in accord with the presence of mRNA for NK1R in human T cells (see Bost, 2004). SP was reported to be present in human T cells with upregulated expression in the presence of HIV infection (Ho et al., 2002), which adds another layer to this complexity.

Unfortunately, no clear picture is emerging regarding the role of TRPV1 in neuroimmune regulation and dysfunction. As we saw above, genetic deletion of TRPV1 protects NOD mice against T1DM (Razavi et al., 2006). Likewise, capsaicin-induced depletion of sensory afferents protects SCID mice against T cell-mediated colitis (Gad et al., 2009). By contrast, TRPV1 knockout mice show 1 enhanced Th2-biased immune responses in the airways after ovalbumin sensitization (Mori et al., 2011) and 2 reveal diminished protection against experimental autoimmune hepatitis (Hegde et al., 2011), as well as autoimmune encephalitis (Paltser et al., 2013). In patients with MS, the missense rs877610 TRPV1 SNP conferred a higher risk for progressive disease. On the basis of these findings, it was postulated that TRPV1 is a critical disease modifier and potential therapeutic target in patients with MS (Paltser et al., 2013).

The participation of TRPV1 in atopic dermatitis is even more confusing. Although TRPV1(−/−) mice are less susceptible to dermatitis (Bánvölygi et al., 2005; Usuda et al., 2012), TRPV1 antagonists relieve only the pruritus with no effect on the underlying inflammation in mice (Lim and Park, 2012). In humans, topical capsaicin ameliorates allokinesis (itch induced by usually nonpruritic stimuli) in healthy volunteers but not in patients with atopic eczema (Weisshaar et al., 1998). A primary drawback of many of these studies is the lack of a relationship between an altered immune response and antigen specificity. Immunomodulation at the level of antigen-specific cellular responses is clearly more desirable than the global dampening of the immune response. Unfortunately, manipulation by TRPV1 antagonists of neuroimmune interactions amounts to global dampening. On the basis of the available literature, it is impossible to predict the net effect of TRPV1 blockade on autoimmune diseases. If the mouse data hold true for humans, TRPV1 antagonists will improve T1DM, IBD, and rheumatoid arthritis but may worsen asthma and autoimmune hepatitis.

In Ashkenazi Jews, T1DM was significantly associated with the rs222747 (M3151) genotype. Individuals who are homozygous for this allelic TRPV1 variant were found to have a 67% increase in the odds for developing the disease (Sadeh et al., 2013).
b. Vanilloid transient receptor potential 1 and type 2 diabetes mellitus: is type 2 diabetes mellitus a low-grade inflammatory disorder? Given the high prevalence of T2DM, surprisingly little is known about the etiology of this rampart disorder and the available pharmacotherapy is mostly symptomatic. In brief, T2DM is a long-term disorder that results from the inability of cells to react to circulating insulin (insulin resistance). This is perceived by the body as insufficient insulin production and triggers compensatory mechanisms to secrete more insulin (hyperinsulinemia). Hyperglycemia ensues when the pancreas is unable to keep up with the increased insulin demand. Eventually, β cells are exhausted and the patient becomes insulin resistant. Most patients with T2DM are obese, are hypertensive, and have dyslipidemia. Collectively, these related metabolic disorders are often referred to as “syndrome X.” Despite recent advances in treating diabetic complications (cardiovascular disease, kidney failure, retinopathy, neuropathy, etc), T2DM remains the fifth leading cause of death in the industrialized world. There is an increasing recognition that TRP channels are involved in the development of metabolic syndrome (reviewed in Colson et al., 2013).

Low-grade inflammation appears to precede the onset of T2DM and is believed to play a role in the development of insulin resistance (see Jin and Flavell, 2013). Indeed, markers of chronic low-grade inflammation (e.g., C-reactive protein [CRP]) are persistently high in patients with T2DM. Many anti-diabetic drugs used in the management of patients with T2DM (e.g., the PPARY agonists rosiglitazone and pioglitazone) possess anti-inflammatory activity and reduce CRP (Hanefeld et al., 2011). In fact, blood glucose decreases in tandem with CRP after pioglitazone treatment.

In an effort to link obesity and T2DM to sensory afferents and adipose inflammation, it was postulated that hypercaloric diets create a permissive milieu for T cells and bone marrow–derived macrophages to infiltrate the visceral fat (see Bouloumié et al., 2008). There is good evidence that the number of fat-resident macrophages positively correlates with the body mass (Weisberg et al., 2003; Lê et al., 2011). It is not clear what attracts the macrophages in the adipose tissue but a correlation among calprotectin, macrophage infiltration, and obesity was reported (Catalán et al., 2011). Macrophage infiltration appears to be the primary event that attracts the T cells to the fat (Morris et al., 2012). In lean adipose tissue, macrophages are sparse and scattered (Weisberg et al., 2003). By contrast, macrophages in obese individuals are abundant and form crown-like structures around the large adipocytes. These fat-resident macrophages can produce cytokines such as TNFα (Xu et al., 2003) that, in turn, may disrupt the insulin signaling cascade, leading to insulin resistance. According to this hypothesis, our traditional thinking of WAT is incorrect. WAT is not simply a fat (energy) depot; rather, it is a complicated endocrine organ involved in the regulation of glucose and insulin homeostasis (reviewed in Adamczak and Wiecek, 2013). For example, lean adipocytes secrete adiponectin, a fat hormone that plays an important role in physiologic glucose and lipid metabolism in insulin-sensitive cells. This WAT-driven biochemical cascade can malfunction in the presence of nutrient excess, creating a prodiabetic environment. If so, understanding the signals that cause this malfunction may lead to novel, disease-modifying pharmacological intervention in patients with T2DM.

In rodent models of T2DM, there is increasing evidence to imply an important, multifaceted role for TRPV1 in the development and maintenance of insulin resistance (see Suri and Szallasi, 2008; Tsui et al., 2011; Zsombok, 2013). Trpv1−/− mice are resistant to diet-induced obesity and associated impaired glucose tolerance (Motter and Ahern, 2008), at least when they grow older (Wanner et al., 2011). Importantly, chemical ablation by capsaicin of TRPV1-expressing sensory afferents (Gram et al., 2007) and pharmacological blockade of TRPV1 by antagonists such as BCTC (Tanaka et al., 2011) recapitulate the phenotype of Trpv1−/− mice. Of note, in a different study, BCTC was proposed to keep mice lean by inhibiting TRPV4 channels (Ye et al., 2012). TRPV1-positive sensory neurons possess high-affinity insulin receptors (Baiou et al., 2007). Insulin was suggested to facilitate the redistribution of TRPV1 from intracellular organelles to the plasma membrane and thereby sensitize the neurons to TRPV1 agonists (see Bishop and Premkumar, 2013). This may be an important molecular mechanism underlying the development of diabetic neuropathic pain (Bishnoi et al., 2011; Miura et al., 2011). Of note, insulin-secreting 1E cells express functional TRPV1 (Jabin Fägelsköld et al., 2012), hindering the interpretation of results obtained with TRPV1 ligands.

Upon stimulation, TRPV1-expressing afferents release SP and CGRP. The role of these neuropeptides in pain transmission and neurogenic inflammation was detailed elsewhere. Perhaps surprisingly, adipocytes express both CGRP receptors and SP (NK1) receptors, making them amenable to sensory neuronal control (Karagiannides et al., 2011). Furthermore, macrophages possess a SP recognition site that is different from NK1Rs (Hartung et al., 1986). Thus, SP released from adipose tissue afferents may attract macrophages both directly (by interacting at macrophage SP receptors) and indirectly (by facilitating the release of macrophage-attracting chemokines from adipocytes). Obese adipocytes have a proinflammatory phenotype: They secrete factors such as the MCP1 that attracts macrophages. In support of the key role that fat-infiltrating macrophages play in the pathogenesis of
T2DM, MCP1-deficient mice are resistant to diet-induced diabetes (see Johnson et al., 2012). Indeed, MCP1 is an emerging target for weight loss and T2DM (see Panee, 2012).

In pancreatic islets, CGRP was reported to inhibit insulin release (Kogire et al., 1991). Conversely, after glucose challenge, attenuated insulin secretion was noted in the SP-deficient mice. These findings imply that CGRP and SP may play opposing roles in the pancreas, in which CGRP antagonizes insulin release and SP stimulates insulin release (see Suri and Szallasi, 2008). Indeed, persistently high circulating CGRP levels were detected in obese humans with insulin resistance. The participation of SP in the pathogenesis of T2DM is, however, less clear. The phenotype of SP-null mice is confusing. These animals are more insulin sensitive than their wild-type littermates but their glucose tolerance is paradoxically impaired (Karagiannides et al., 2011). Adding to this complexity, SP also has a proinflammatory action in the pancreas. Indeed, recent evidence suggests that SP is critical for the development of chronic pancreatitis (reviewed in Di Sebastiano et al., 2004).

The opposing effects of SP and CGRP in inflammatory disorders (and neuroimmune interactions) are somewhat puzzling, yet not unprecedented. For example, in biopsies taken from the large intestine of patients with IBD, elevated SP and reduced CGRP levels were documented (see Engel et al., 2011a,b, 2012). In accord, SP(−/−) mice were protected in oxazolone colitis, whereas CGRP-null animals exhibited exaggerated inflammatory responses. These observations imply that after a simultaneous loss of SP and CGRP, the SP-null phenotype will predominate. TRPV1 is also elevated in the colonic mucosa of patients with IBD and a positive correlation between TRPV1 and pain was noted (Keszthelyi et al., 2013). Furthermore, there is good evidence that deletion of TRPV1 by genetic manipulation (Szitter et al., 2010) or neonatal capsaicin treatment (Kihara et al., 2003) is protective in colitis models. Confusingly, in adult rats, capsaicin treatment achieved the opposite effect: It worsened experimental colitis (McCafferty et al., 1997). However, a higher incidence of colonic adenomas was recently reported in TRPV1(−/−) mice, leading to the controversial proposal that neurogenic inflammation is, in fact, protective against colorectal carcinogenesis, at least in patients with IBD (Vinuesa et al., 2012).

Clearly, there is a delicate (and currently only partially understood) balance between TRPV1-positive sensory afferents and glucose homeostasis in which a sustained, TRPV1-dependent neuropeptide release may exert a tonic action on β cells (see Suri and Szallasi, 2008). Both increased and decreased neuropeptide release seem to impair glucose tolerance. Because the gamut of evidence points to the therapeutic potential of TRPV1 blockade in diabetic models, one might argue that overactive sensory afferents make an early and pivotal contribution to T2DM pathobiology by fueling islet cell inflammation (via SP release) and antagonizing insulin actions (via CGRP). It is not clear how these afferents “sense” the prodiabetic environment. One possible link is by Toll-like receptors. TRPV1-positive trigeminal ganglion and DRG neurons are known to express Toll-like receptor-4 (Diogenes et al., 2011), which might be activated by fatty acids in the presence of nutrient excess. Another possible connection is oxidative stress. Sensory C fiber afferents of the pancreas coexpress TRPV1 with TRPA1, an oxidative stress sensor. As discussed above, insulin itself (which is released in excess during the early phase of T2DM) may directly increase SP and CGRP release from sensory nerves by interacting at neuronal insulin receptors. Thus, a pathogenic positive feedback loop is created in which SP promotes insulitis and inflammatory mediators enhance neuropeptide release.

In diabetic obese (ob/ob) mice, the TRPV1 antagonist BCTC reduced fasting glucose, triglyceride, and insulin levels (Tanaka et al., 2011). Furthermore, BCTC increased insulin secretion in response to glucose in the oral glucose tolerance test. Thus, BCTC has a dual beneficial action: it acts both as insulin secretagogue and sensitizer. This is important because most per os antidiabetic agents function either as insulin secretagogues or sensitizers, but not both. Thus, TRPV1 antagonists may represent a novel class of antidiabetic drugs. As an added benefit, TRPV1 antagonists may also help patients lose weight, ameliorate diabetic neuropathic pain, and protect against cardiovascular complications. Of note, in preclinical studies and phase I/II clinical trials, blood glucose levels remained within normal limits in the TRPV1 antagonist-treated groups.

5. Targeting Transient Receptor Potential Channels in β Cells for Controlling Insulin Release. Mouse and rat primary β cells and/or insulin-secreting cell lines express a variety of TRP channels belonging to the vanilloid (TRPV2 and TRPV4), ankyrin (TRPA1), canonical (TRPC1, TRPC4, and TRPC6), and melastatin (TRPM2–TRPM5) subfamilies (reviewed in Jacobson and Philipson, 2007; Colso et al., 2013). In human islets, TRP channel expression appears to be restricted to four members of the melastatin family: TRPM2, TRPM4, TRPM5, and TRPM6. We focus on these channels below, but we first briefly review the molecular mechanism of insulin secretion.

Insulin secretion is a complex process driven by electrical activity and oscillations in intracellular Ca2+ concentration in pancreatic β cells (see Colso et al., 2013). The main driver of insulin secretion is plasma glucose. Glucose enters the β cells via the high Km glucose transporter-2. Glucose metabolism produces ATP, which, in turn, closes ATP-sensitive K+ channels.
This increases the input resistance, allowing a small inward current, the molecular identity of which is still elusive, to generate depolarization. A complex interplay between ion channels (e.g., ATP-sensitive K+ channels and voltage-dependent Ca2+ channels) results in a unique pattern of depolarizations in which bursts of action potentials are interrupted by hyperpolarized intervals. The resultant oscillatory increase in intracellular Ca2+ levels causes exocytosis of insulin-containing vesicles. In principle, Cl− efflux or cation influx could provide the depolarizing current that drives the β cells toward the initiation of electrical activity. TRP channels are interesting candidates for this background depolarizing current (see Qian et al., 2002).

a. Melastatin transient receptor potential 2. TRPM2 is natively expressed and forms a functional channel in β cells (see Togashi et al., 2006). There is good evidence that TRPM2 contributes to insulin release in response to glucose and incretin hormones (Togashi et al., 2006; Uchida and Tominaga, 2011). Knockdown of TRPM2 by siRNA reduces insulin release evoked by forskolin (an activator of adenyl cyclase) and exendin-4 (a GLP-1 receptor agonist) (Togashi et al., 2006). Furthermore, 2-APB inhibits exendin-4-evoked insulin release from rat pancreatic islets (Togashi et al., 2008). In the Trpm2(−/−) mouse, insulin secretion induced by glucose and GLP-1 was impaired, whereas the response to tolbutamide, a KATP channel inhibitor, was unchanged (Uchida et al., 2011). Indeed, Trpm2(−/−) mice show hypoinsulinemia and resultant hyperglycemia/impaired glucose tolerance, a diabetic phenotype. However, no correlation was detected between TRPM2 variants and the risk for developing T2DM (Romero et al., 2010).

Unexpectedly, glucose-stimulated insulin secretion evoked under conditions of diazoxide and high K+ (designated to “clamp” intracellular Ca2+ and inactivate the KATP channel-mediated pathways) was lost in Trpm2-deficient islets (Uchida et al., 2011). Since the intracellular Ca2+ levels under these conditions was not different between wild-type and knockout islets, these data suggest that TRPM2 mediates insulin secretion in a way that is independent of its role as a Ca2+ entry channel.

Of note, TRPM2 has been reported to play a role in intracellular Ca2+ release in pancreatic β cells (Lange et al., 2009). Internally applied ADPR gives rise to a single Ca2+ transient in both insulin-secreting 1 cells and primary mouse β cells, which was completely abolished in Trpm2(−/−) primary mouse β cells. Moreover, TRPM2 colocalizes with lysosome-associated membrane protein-1, a specific marker for lysosomes. On the basis of these results, it might be postulated that ADPR-dependent TRPM2-mediated Ca2+ release occurs predominantly from a lysosomal store. Clearly, further experiments are needed to clarify whether this lysosomal TRPM2 is involved in regulating insulin release.

As discussed elsewhere (e.g., Miller and Zhang, 2011), TRPM2 is a death channel operated by ROS during oxidative stress. Apoptosis of β cells is a prominent feature of diabetes (see Rhodes, 2005). In rat insulinoma cells, activation of TRPM2 by H2O2 initiates Ca2+ influx and resultant cell death (Ishii et al., 2006). Conversely, insulinoma cells with suppressed TRPM2 expression are protected from H2O2-induced cell death (Lange et al., 2009).

b. Melastatin transient receptor potential 4. TRPM4 is abundantly expressed in a number of insulinoma cell lines where it is involved in the control of insulin release (Cheng et al., 2007a). Indeed, in these neoplastic cells, blockade of TRPM4 was shown to decrease the magnitude of the Ca2+ signal and to inhibit insulin release in response to glucose, arginine-vasopressin (a Gs-coupled receptor agonist in β cells), and glibenclamide (Cheng et al., 2007a; Marigo et al., 2009). However, the role of TRPM4 in physiologic insulin secretion remains unclear. Although TRPM4 expression was detected both in human β cells and mouse pancreatic islets, studies on Trpm4(−/−) mice revealed no difference in glucose-induced insulin secretion compared with pancreatic islets obtained from wild-type animals (Vennekens et al., 2007). Moreover, the TRPM4 knockout mice did not exhibit any sign of impaired glucose tolerance. These data suggest that TRPM4 is probably not involved in the signal mechanism after glucose stimulation. By contrast, TRPM4 appears to be involved in glucagon secretion by the pancreatic α-cell line aTC1-6 (Nelson et al., 2011). It is noteworthy that TRPM4 was reported to form a heteromer with Sur1 (Woo et al., 2013). Sur1 gene polymorphism may be partially responsible for individual differences in response to sulfonylurea antidiabetic drugs (reviewed in Glamočlija and Jevrić-Cašević, 2010). It is unclear what role (if any) Sur1/TRPM4 heteromers play in T2DM.

c. Melastatin transient receptor potential 5 and melastatin transient receptor potential 6. TRPM5 is abundantly expressed in pancreatic β cells where it is highly colocalized with insulin (Colsoul et al., 2010). In rodent β cells, a Ca2+-activated nonselective monovalent cation channel was detected that was largely reduced in Trpm5(−/−) mice. Normal islets respond to glucose stimulation with three distinct types of oscillations: slow, mixed, and fast. Islets obtained from Trpm5(−/−) animals show a curious phenotype in that the fast glucose-induced oscillations in Vm and Ca2+ are markedly reduced. This implies that TRPM5 contributes to the slow depolarization in the slow interburst interval of the glucose-induced electrical activity, thereby shortening the interburst interval and leading to faster glucose-induced oscillations in Vm and Ca2+.

In remains unclear why TRPM5 is only functionally
relevant in the fast-oscillating subpopulation of the islets.

It was speculated that TRPM5-mediated depolarization is coupled to the glycolytic rate of the cell (seeColsoul et al., 2011, 2013). According to the dual oscillator model, fast oscillations are characterized by a high glycolytic rate and a resulting high ATP production. Under these conditions, TRPM5 should be able to depolarize \( V_m \) in the interburst interval since the hyperpolarizing \( K_{ATP} \) current is largely inactive (seeColsoul et al., 2013). This is in contrast with the situation in slow oscillating islets in which the oscillating glycolytic rate and the resulting high activity of \( K_{ATP} \) in the interburst interval will render TRPM5 insufficient to depolarize \( V_m \). Of note, fast Ca\(^{2+}\) oscillations are more efficient than the slow ones in triggering insulin release. In accord, glucose-induced insulin release was reduced in isolated pancreatic islets from \( Trpm5^{−/−} \) mice (Brixel et al., 2010). Moreover, \( Trpm5^{−/−} \) mice display low plasma insulin levels and impaired glucose tolerance during oral and intraperitoneal glucose tolerance tests, consistent with a prediabetic phenotype caused by \( β \) cell dysfunction. Of note, TRPM5 expression is negatively correlated with blood glucose concentrations in the small intestine from patients with diabetes (Young et al., 2009). An association was recently found between TRPM5 variants and prediabetic phenotypes in individuals who are at risk for developing T2DM (seeColsoul et al., 2013).

Low serum Mg\(^{2+}\) (<1.6 mg/dl) confers increased risk for T2DM as well as other diseases (reviewed inWälti et al., 2003; Rosanoff et al., 2012). TRPM6 is a gatekeeper of human Mg\(^{2+}\) metabolism (seeMontell, 2003). Insulin was reported to enhance surface expression of TRPM6 in human \( β \) cells. Two TRPM6 variants (V1393I and K1584E) were recently described in women with gestational DM (Nair et al., 2012).

d. Melastatin transient receptor potential 3, vanilloid transient receptor potential 2, vanilloid transient receptor potential 4, and ankyrin transient receptor potential potential 1. TRPM3 is expressed in mouse pancreatic islets (Wagner et al., 2008). However, the TRPM3 blocker mafenamic acid did not block glucose-induced Ca\(^{2+}\) increase (Klose et al., 2011), indicating that TRPM3 is not involved in physiologic insulin release. TRPM3 was recently proposed to constitute a regulated Zn\(^{2+}\) entry pathway in pancreatic \( β \) cells (Wagner et al., 2010). Zinc is important for insulin release. Since Zn\(^{2+}\) ions are coreleased with insulin, pancreatic \( β \) cells have to replenish their Zn\(^{2+}\) stores. Indeed, Zn\(^{2+}\) deficiency has been linked to impaired insulin synthesis and the development of T2DM (seeChausmer, 1998).

TRPV2 is expressed in murine pancreatic \( β \) cells (Hisanaga et al., 2009). Under serum-free conditions, TRPV2-like immunoreactivity is localized to intracellular compartments. Addition of serum induces translocation of TRPV2 to the plasma membrane (Kanzaki et al., 1999; Hisanaga et al., 2009). Genetic inactivation (knockdown) of the insulin receptor attenuated insulin-induced translocation of TRPV2 (Hisanaga et al., 2009). Interestingly, inhibition of TRPV2 reduced glucose-induced insulin secretion. These data indicate that insulin released from \( β \) cells may create a positive feedback loop by recruiting TRPV2 to the plasma membrane and thereby accelerating insulin secretion (seeUchida and Tominaga, 2011).

TRPV4 is expressed both in insulinoma cell lines and mouse primary \( β \) cells (Casas et al., 2008). Knockdown of \( Trpv4 \) by siRNA treatment protected MIN6 insulinoma cells against human islet amyloid polypeptide (hIAP)–induced Ca\(^{2+}\) elevation and resultant apoptosis. hIAPP, the main component of amyloid, is associated with the loss of pancreatic \( β \) cells during T2DM (Lorenzo et al., 1994). It was speculated that TRPV4 is the main sensor for detecting the physical changes in the plasma membrane induced by hIAPP aggregation.

Finally, TRPA1 is abundantly expressed in freshly isolated rat pancreatic \( β \) cells (Cao et al., 2013a). Activation of TRPA1 in \( β \) cells by mustard oil and 4-hydroxy-2-nonenal evokes insulin release. Interestingly, the TRPA1 antagonist HC-030031 inhibited glucose-induced insulin release, implying a role for TRPA1 in physiologic insulin control (Leibiger et al., 2002). For the sake of completeness, it should be mentioned here that TRPM8\(^{−/−} \) mice were reported to exhibit prolonged hypoglycemia in response to insulin (McCoy et al., 2013a). It was suggested that TRPM8-expressing afferents may control insulin clearance in the liver. In diabetic Han Chinese patients, TRPC1 polymorphism was linked to diabetic nephropathy (Chen et al., 2013c).

F. Transient Receptor Potential Channels in the Brain as Novel Targets for Neurologic and Psychiatric Disorders

Despite the plethora of reports on TRP channel expression in the brain (reviewed inReboreda, 2012; Vennekens et al., 2012), our understanding of the participation of these channels in physiologic functions and disease states is still rudimentary. However, there is no shortage of speculations linking TRP channels to the whole spectrum of neurologic and psychiatric diseases from the common (e.g., stroke, anxiety disorders, and AD) to the esoteric (e.g., Western Pacific ALS-G/dementia complex). On the basis of a combination of immunostaining and molecular studies, many TRP channels appear to be expressed by brain tissue, some highly and broadly (e.g., TRPC3 and TRPC5) and others at low levels in a few nuclei (e.g., TRPV1). The function of these channels remains to be elucidated, but there is evidence that multiple TRPs may
Contribute to neuronal excitability and neurotransmitter signaling in the brain. It was speculated that TRP channels (when they “go bad”) may be involved in the development and maintenance of addiction (see Wescott et al., 2013).

1. Vanilloid Transient Receptor Potential 1 and Canonical Transient Receptor Potential 5 in Anxiety Disorders. Ongoing controversy surrounds the expression and role of TRPV1 in the brain. Early studies described widespread TRPV1 expression (albeit at much lower levels than in sensory neurons) throughout the whole neuroaxis of the rat, using a combination of immunohistochemical, molecular (RT-PCR), and [3H]RTX binding studies (Sasamura et al., 1998; Mezey et al., 2000; Szabó et al., 2002; Roberts et al., 2004; Tóth et al., 2005; Cristino et al., 2006). Neonatal capsaicin treatment was reported to induce widespread neurodegenerative changes in the rat brain (reviewed in Szallasi and Blumberg, 1999). Indeed, no TRPV1 was detected by [3H]RTX autoradiography in the brain of these animals when they reached adult age (Roberts et al., 2004). TRPV1 expression was also absent in the brain of TRPV1(−/−) mice.

In basal ganglia, TRPV1 was highly colocalized with tyrosine hydroxylase, suggesting that TRPV1 might be involved in the pathogenesis of Parkinson disease (Mezey et al., 2000). In the mouse, unilateral injection of the neurotoxin 6-hydroxydopamine into the striatum is known to cause locomotor disturbances (e.g., shuffling gait and short steps) that are characteristic of Parkinson disease (Metz et al., 2005). In these animals, the TRPV1 agonist O-arachidonoylthanolamine was found to reduce L-DOPA-induced dyskinesia and this beneficial effect was absent in mice whose TRPV1 receptors were eliminated by high-dose capsaicin treatment (González-Aparicio and Moratalla, 2014).

Importantly, neurons in brain slices and/or neurons obtained from brain nuclei and kept in culture showed Ca2+ fluxes in response to capsaicin that were prevented by the first-generation TRPV1 antagonist capsazepine (Sasamura et al., 1998; Marinelli et al., 2002; Gibson et al., 2008). In the amygdala, capsaicin affects LTP via TRPV1 activation (Zschenderlein et al., 2011). Rodents, whose brain TRPV1 was eliminated by genetic recombination (Caterina et al., 1997) or neonatal capsaicin treatment (see Szallasi and Blumberg, 1999), showed no obvious deficits in behavioral assays. This is surprising since the brain of TRPV1(−/−) mice was reported to show multiple electrophysiological anomalies (e.g., reduced tetanus-induced LTP after electrical stimulation (Brown et al., 2013), whereas the TRPV1 knock-in animals (Cre-loxP–based conditional expression) showed stereotyped behavior in response to capsaicin (Arenkiel et al., 2008). Furthermore, TRPV1 was reported to facilitate glutamate release in the striatum (Musella et al., 2009), and to control synaptic plasticity in the superior colliculus (Maione et al., 2009a).

In addition to neurons, TRPV1 expression was also described in astrocytes (Doly et al., 2004; Verhkratsky et al., 2013). Indeed, the selective TRPV1 agonist RTX was reported to induce Fos expression in astrocytes in wild-type, but not TRPV1 knockout, mice (Mannari et al., 2013). It was speculated that astrocytic TRPV1 might be involved in the pathogenesis of neurodegenerative disorders (Ho et al., 2012).

The TRPV1-null mice adapt more easily than their wild-type littermates to aversive light and they explore freely the open arm of the elevated maze, indicative of reduced unconditional fear response (Marsch et al., 2007). Furthermore, they exhibit less freezing in auditory fear conditioning assays. These findings imply a therapeutic potential for TRPV1 antagonists as novel anxiolytic agents (see Moreira et al., 2012).

Anxiety is a common affective (mood) disorder, treated by minor tranquilizers and anxiolytic drugs. Although these agents are effective, they are associated with common side effects (e.g., sedation, impotence, and headaches), have an abuse potential, and can be even lethal if overdosed. “Atypical” anxiolytic agents such as buspirone are not sedative but can cause paradox nervousness and asomnia. It is clear that there is a great need for novel anxiolytic drugs with improved efficacy and safety.

Cannabinoids exert a well-documented biphasic action on mood, anxiety, and fear, both in humans (relaxation and euphoria at low doses, followed by panic and anxiety—a “bad trip”—at high doses) and experimental animals (as studied in the unconditioned fear test) (see Di Marzo et al., 2008). The cannabinoid CB1 receptor is among the most abundant GPCRs in the brain. The anxiolytic effect of tetrahydrocannabinol (the active ingredient in marijuana) is well known, and the anandamide transport inhibitor AM404 [N-(4-hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide] (which increases the concentrations of bioavailable anandamide, an “endocannabinoid” in the brain, in the vicinity of cannabinoid CB1 receptors) mimics the anxiolytic effect of cannabis (see Di Marzo et al., 2008). In keeping with these findings, the CB1 receptor knockout mice display increased anxiety behavior under emotional stress, as well as sustained fear response (reviewed in Valverde et al., 2005). On the basis of these observations, it was postulated that the endocannabinoid system suppresses the fear response. This model begs the following question: what target is responsible for the paradox anxiogenic effect of cannabinoids?

Capsaicin microinjected into the periaqueductal gray matter, a midbrain structure strongly implicated in anxiety, evoked anxiety-like behavior in mice subjected to the elevated maze test (Mascarenhas et al., 2013). So, what is the endovanilloid that regulates brain TRPV1? Anandamide (at concentrations much higher than those required to active CB1 receptors) was
reported to activate TRPV1 (reviewed in Di Marzo et al., 2002). Although it is unlikely that anandamide in the brain can reach sufficiently high concentrations to activate TRPV1, it might do so in concert with other related brain-derived endocannabinoids such as NADA (Huang et al., 2002). It was speculated that cannabino-
oids are anxiolytic by interacting with presynaptic CB1 receptors but can exert the opposite (anxiogenic) effect when they activate postsynaptic TRPV1 receptors (see Moreira et al., 2012). In fact, CB1 and TRPV1 were reported to be colocalized in the hippocampus (Cristino et al., 2006). Thus, TRPV1 may mediate the “dark side” of endocannabinoids (Cernak et al., 2004).

A recent work relying on a powerful combination of reporter mice, in situ hybridization, electrophysiological recordings, and Ca$^{2+}$ imaging suggests that TRPV1 expression is restricted to very few brain regions, most notably the caudate nucleus of the hypothalamus, as well as endothelial cells (Cavanaugh et al., 2011). At present, it is unclear how one can reconcile these strikingly different findings. Clearly, additional work will need to be done to explain the differences that have been noted between wild-type and TRPV1(-/-) mice.

In a much broader context, this raises the following uncomfortable question: when do we target experimental artifacts, especially when findings are unexpected and contradict entrenched dogmas? In the TRPV1 field, reports of functional TRPV1 channels in non-
neuronal tissues (e.g., urothelium, skin, and lymphoid tissues) were even more unexpected than the finding of TRPV1 in the brain. We would be curious to see what reporter mice would reveal in nonneuronal TRPV1 expression patterns.

TRPC5 is robustly expressed in the hippocampus and amygdala (Strübing et al., 2001; Chung et al., 2006). In the hippocampus of young rats, TRPC5 is expressed on the growth cone where it interacts with stathmin-2 (Greka et al., 2003). Inhibition of TRPC5 activity by a dominant negative variant resulted in longer neuritis. The semaphorin 3A (a
activity by a dominant negative variant resulted in shorter neuritis with reduced arborization. Together, these data indicate that TRPC3 channels play an important role not only in synaptic transmission in cerebellar Purkinje cells, but also in their survival.
Identification of selective pharmacologic agents will be useful in determining whether TRPC3 plays a broader role in ataxias.

Staggerer mutant (sg/sg) mice were named after their unsteady (staggering) gait. They also show muscle hypotonia and tremor and die prematurely. These mice have an underdeveloped cerebellar cortex with a reduced number of abnormal Purkinje cells. The genetic defect that is responsible for the phenotype of these mice is the loss of the retinoid-related orphan receptor-α gene. Human spinalcerebellar ataxia type-1 is associated with retinoid-related orphan receptor-α dysfunction that manifests in the absence of mGluR-mediated slow EPSPs. It is noteworthy that TRPC3 is a downstream target for mGluR signaling, and the expression of TRPC3 is markedly reduced in the sg/sg mice (Mitsumura et al., 2011).

Paradoxically, TRPC3 knockout mice also exhibit an ataxia phenotype. Similar to the Staggerer mutant mice, these animals show absent mGluR-mediated slow EPSPs. However, unlike in the Staggerer mice, the Purkinje cell morphology is normal. To reconcile these findings, it was postulated that cerebellar TRPC3 is constitutively active and the ion flux is partially controlled by channel trafficking (Trebak, 2010). In Moonwalker mice, the hyperactive TRPC3 channel causes Purkinje cell death due to Na⁺ and Ca²⁺ overload. In the Staggerer and Moonwalker mice, the hyperactive TRPC3 causes Purkinje cell death due to Na⁺ and Ca²⁺ overload. In the Staggerer and Moonwalker mice, the hyperactive TRPC3 causes Purkinje cell death due to Na⁺ and Ca²⁺ overload. In the Staggerer and Moonwalker mice, the hyperactive TRPC3 causes Purkinje cell death due to Na⁺ and Ca²⁺ overload.

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Of note, TRPC3 is a downstream target for BDNF (Li et al., 2005b; Jia et al., 2007). It was speculated that TRPC3 might be involved in the pathogenesis of Rett syndrome (a neurodevelopmental disorders that mainly affects girls) (Amaral et al., 2007) and bipolar disorder (Roedding et al., 2013). Almost all cases of Rett syndrome are attributable to a mutation in the methyl CpG-binding protein (MECP2) gene. The link (if any) between this mutation and TRPC3 is unclear.

3. Melastatin Transient Receptor Potential 2, Melastatin Transient Receptor Potential 7, and Canonical Transient Receptor Potential 3: Potential Targets in Stroke Patients. In the United States, stroke is a leading cause of disability and the second leading cause of death (over 1 million strokes are responsible for close to 200,000 death). In the brain, TRPM2 is expressed by neurons and microglia. In keeping with the concept that TRPM2 functions as a redox sensor (Jiang et al., 2010; Naziroglu, 2011a), TRPM2(−/−) mice show protection against various pathologies related to oxidative stress, including the focal ischemia model of stroke. In stroke models, TRPM2 expression is increased (Fonfria et al., 2006a), and knockdown of TRPM2 by shRNA enhances blood flow and reduces stroke volume after experimental stroke in male (but not in female) mice (Jia et al., 2011), which is a puzzling example of sex-related differences in TRP channel functions. In the hippocampus, TRPM2 appears to be involved in NMDA-mediated metaplasticity (Xie et al., 2011).

TRPM2 (Verma et al., 2012), TRPM4 (Loh et al., 2014), and TRPM7 have been implicated in ischemic brain injury (reviewed in Aarts and Tymianski, 2005; Bae and Sun, 2013). In vivo siRNA-mediated silencing of TRPM4 reduces brain infarct volume by half and facilitates functional recovery in rats with middle cerebral artery occlusion (Loh et al., 2014). Knockdown of TRPM7 also reduces cell death due to oxygen-glucose deprivation in isolated neuronal cultures, implying a role for TRPM7 antagonists in the treatment of stroke. Indeed, lentiviral delivery of TRPM7 shRNA prevents ischemic neuronal cell death after experimental stroke in the rat (Sun et al., 2009). However, the expectations toward TRPM7 as a therapeutic target in stroke have been tempered by the lack of association between TRPM7 variants and the risk for suffering ischemic stroke (Romero et al., 2009).

Rupture of blood vessels in the brain, with the subsequent accumulation of blood in the parenchyma, causes accumulation of blood-derived factors, induces excessive inflammatory changes, and is involved in the progression of intracerebral hemorrhage. Thrombin leaks into the brain parenchyma and induces brain injury and astrogliosis. Thrombin typically upregulates TRPC3, which in turn contributes to pathologic astrogliosis (Shirakawa et al., 2010). Administration of the specific TRPC3 blocker Pyr3 in a mouse model reduced neurologic deficits, neuronal injury, brain edema, and perihematomal accumulation of astrocytes and ameliorated intracerebral hemorrhage brain injury (Munakata et al., 2013). Therefore, TRPC3 is a potential therapeutic target for the treatment of hemorrhagic brain injury (Munakata et al., 2013). The TRPC6 activator hyperforin attenuated brain infarct volume in rats with middle cerebral artery occlusion in a CREB-mediated fashion (Lin et al., 2013). Interestingly, TRPC6 also seems to link neuroprotectin D1 (an endogenous compound that limits ischemic brain injury) to CREB activation via the Ras/MEK/ERK pathway (Yao et al., 2013).

4. Transient Receptor Potential Channels in Neurodegenerative Disorders and Mental Retardation. AD is a devastating neurodegenerative disorder that affects an estimated 2.5 to 5.1 million Americans (a firm diagnosis of AD can only be made by postmortem examination of the brain), placing severe emotional burden on the affected families and draining the financial resources of the healthcare system. Sadly, there is no effective treatment of AD. A histologic hallmark of AD is β-amyloid deposition in the
TRP Channels as Drug Targets

striatum. TRPM2 is expressed in the rat striatum, where it is activated under oxidative stress by ROS (Hill et al., 2006). In AD models, striatal TRPM2 (a neuronal death channel) is upregulated, and knockdown of this channels by siRNA was reported to ameliorate β-amyloid–induced neurotoxicity (Fonfria et al., 2005). These findings offer a glimmer of hope that TRPM2 antagonists may halt (or at least slow down) the relentless progression of AD.

Astrocytic TRPA1 was recently implicated in the pathogenesis of AD. Indeed, the TRPA1 agonist acrolein, a highly electrophilic α,β-unsaturated aldehyde generated during the combustion of organic materials (Kehrer and Biswal, 2000), induces an AD-like condition in the rat (Huang et al., 2013). It was speculated that TRPA1 channels regulate astrocytic resting Ca^{2+} levels and inhibitory synapse efficacy via GAT-3 (GABA transporter-3) (Shigetomi et al., 2012). Hippocampal LTP, a form of synaptic plasticity involved in learning and memory formation, depends on the release of n-serine from astrocytes (Henneberger et al., 2010), which, in turn, is regulated by TRPA1 (Shigetomi et al., 2013). The astrocytic hypothesis of AD states that astrocytes convert into a chronically activated phenotype. Switching off these astrocytes by genetic manipulation stops cognitive decline in preclinical AD models (Furman et al., 2012). One might argue that TRPA1 antagonists could be beneficial in patients with AD by blocking astrocytic TRPA1. It is noteworthy that in cerebral arteries of transgenic mice that overexpress a mutated form of the human amyloid precursor protein (APP mice) abnormal responses to acetylcholine were observed, attributed to TRPV4 dysfunction in the endothelium (Zhang et al., 2013b).

TRPC5 is located in the locus for nonsyndromic, X-linked mental retardation (Sossey-Alaoui et al., 1999). TRPC5 is robustly expressed in the hippocampus (Strübing et al., 2001). As discussed above, the phenotype of the TRPC5-null mouse in behavioral assays is controversial (no phenotype in one study and impaired gait and motor coordination in another). It was proposed that TRPC5 plays a key role in normal neurodevelopment; abnormal TRPC5 activity might lead to autism, mental retardation, and other brain disorders (reviewed in Reboreda, 2012). Of note, TRPC6 has been linked to normal exploratory behavior in the mouse (Beis et al., 2011).

Loss-of-function TRPM2 (P1018L) and TRPM7 (T1482I) mutants have been linked to PD-G and Western Pacific ALS-G (Hermosura et al., 2005, 2008). The mutant TRPM7 has normal kinase activity but shows increased sensitivity to Mg^{2+} blockade. The mutation by itself is not sufficient to produce the disease: It needs coexistent environmental factors. In Guam, the drinking water is extremely low in Mg^{2+} and the diet of natives used to include fruit bat as a delicacy. Fruit bats feast on cycad nuts containing β-methyl-amino-alanin, a known neurotoxin (see Banack and Murch, 2009). The human striatum expresses a novel, N-terminal truncated TRPM2 variant, the biologic function of which is unclear (Uemura et al., 2005).

5. Transient Receptor Potential Channels and Neuroregeneration

As detailed above, TRP channels (especially TRPC5, but also TRPC1, TRPC6, TRPV1, and TRPV4) are thought to play a crucial role in the brain in the regulation of synaptic plasticity, neuronal activity, and neurogenesis, as well as growth cone guidance. More in-depth studies on the brain architecture in developing TRPC5 mice, as well as experiments looking at receptor-activated neurite extension in brain slices should help clarify the relative contribution of TRPC5 to axonal pathfinding (see for detailed reviews, Nilius, 2012; Vennekens et al., 2012).

G. Transient Receptor Potential Channels in Cancer

The “war on cancer” initiated by President Nixon in 1971 is one of the most overused slogans in medical research. If this is a war, it is one with no clear victory in sight and the opening of a new front in cancer research that may offer a glimmer of hope is most welcome progress. Although molecular biology has substantially advanced our understanding of cancer biology, certain cancers (e.g., metastatic melanoma and pancreatic adenocarcinoma) are still as deadly as ever. Indeed, with over half million victims annually, cancer remains a leading cause of death in the United States, second only to heart disease. The increasingly confusing and controversial literature on TRP channels and cancer should be read with these sobering thoughts in mind.

In a much simplified manner, cancer can be thought of as an uncontrolled growth of tumor cells with seemingly limitless replicative potential that is either due to enhanced proliferation (e.g., Burkitt lymphoma) or resistance to apoptotic death (e.g., follicular lymphoma overexpressing the antiapoptotic protein BCL-2). Carcinogenesis appears to be a multistep process in which mutations that confer selective growth advantage accumulate, allowing the progeny of mutated cells to outgrow their normal counterparts. A critical step in this process is the acquisition by cancerous cells of the potential to invade surrounding tissues and metastasize to distant locations. When it happens, it transforms a disease that is potentially curable by surgical excision into one that requires systemic chemotherapy and may prove deadly.

Now it is well recognized that tumor cells can produce factors to create a microenvironment that promotes their growth. For example, many cancers depend on VEGF production for acquiring and maintaining adequate blood supply (from a clinical point of view, reviewed in Hicklin and Ellis, 2005). Indeed, bevacizumab (Avastin; Genentech, South San Francisco, CA), a humanized anti-VEGF antibody, is broadly used
in the management of glioblastoma and metastatic colonic adenocarcinoma to starve these cancers from O$_2$ and nutrients (www.avastin.com). It is noteworthy that a number of TRP channels (e.g., TRPV4 as well as TRPC1 and TRPC6) seem to play an important role in neoangiogenesis. Indeed, TRPV4 is expressed in the vascular endothelium (see Nilius et al., 2003) and has been implicated as a potential therapeutic target in ocular neovascularization disorders (e.g., diabetic retinopathy). TRPC1 is essential for VEGF-induced angiogenesis in the zebrafish (Yu et al., 2010). In U87 glioma cells, a role for TRPC1 in hypoxia-induced VEGF expression was reported (Wang et al., 2009). Furthermore, TRPC6 has been implicated as an important downstream target in VEGF-induced angiogenesis (Pocock et al., 2004; Ge et al., 2009). Indeed, in HMVECs, overexpression of a dominant negative mutant of TRPC6 was shown to inhibit migration, sprouting, and proliferation in response to VEGF administration (Hamdollah Zadeh et al., 2008). Conversely, overexpression of a wild-type TRPC6 construct enhanced the proliferation and migration of HMVECs. It is tempting to speculate that targeting vascular TRP channels (in particular TRPC6) may aid in depriving cancers from their blood supply.

Although no clear picture has yet emerged, there is increasing evidence that certain TRP channels may play opposing roles (oncogenic versus tumor suppressor) during carcinogenesis (reviewed in Prevarskaya et al., 2010; Lehen’kyi and Prevarskaya, 2011; Liberati et al., 2013; Morelli et al., 2013). To resolve some of the discrepancies in the literature, one might even postulate that this effect is cell type dependent; that is, a TRP channel that is oncogenic in one cell type may be conversely tumor suppressor in another. If this hypothesis holds true, TRPV2 could be a dramatic example of this phenomenon. It is virtually absent in non-neoplastic prostate tissue but is upregulated in metastatic, castration-resistant prostatic adenocarcinoma (Monet et al., 2010). When transplanted into nude mice, the growth of the xenograft is inhibited by knockdown of the Trpv2 gene. By contrast, TRPV2 is highly expressed in normal, but not in neoplastic (glioblastoma), brain tissue (Morelli et al., 2012). Overexpression of TRPV2 inhibits glioblastoma growth in a mouse xenograft model and promotes differentiation toward a more mature glial phenotype (Morelli et al., 2012; Nabissi et al., 2013). In other words, TRPV2 may function as an oncogene in the prostate and a tumor suppressor gene in the brain. Another puzzling example is TRPV1. TRPV1 expression was reported in normal urothelial tissue (Lazzeri et al., 2004) with a decrease in urothelial dysplasia and a loss of expression in invasive urothelial carcinoma (Lazzeri et al., 2005). On the basis of these findings, it was postulated that TRPV1 plays an antioncogenic role in the bladder (Santoni and Farfariello, 2011). By contrast, TRPV1 appears to be highly expressed in glioblastoma and only weakly expressed (or absent) in surrounding normal brain (Stock et al., 2012). This is a potential problem for drug development as an agent that may inhibit the growth of one kind of tumor might conversely promote the formation of a different kind of malignancy. This behavior is, however, not unprecedented and did not prevent the use of other chemotherapeutic agents. For example, the use of alkylating agents has been linked to the development of secondary hematologic malignancies (high-grade myelodysplastic syndrome and/or acute myeloid leukemia) in some patients. For such a deadly disease such as glioblastoma (see below), the possibility that the patient may develop another, late malignancy after a TRP channel-targeting treatment is of a lesser concern.

Given the broad expression of TRP channels in normal tissues, the finding of altered TRP channel expression (including TRPV1, TRPV2, TRPV6, TRPC3, TRPC5, TRPC6, TRPM1–TRPM4, TRPM7, and TRPM8) in various cancers was hardly unexpected (reviewed in Prevarskaya et al., 2010; Lehen’kyi and Prevarskaya, 2011; Liberati et al., 2013; Morelli et al., 2013). For most TRP channels and cancers, it is unclear whether the observed changes are therapeutically relevant (i.e., involved in the proliferation and migration of tumor cells and/or in their resistance to chemotherapeutic agents) or simply an epiphenomenon of cancer progression. A notable exception is TRPM8. Increased TRPM8 expression was detected in aggressive prostatic adenocarcinoma (Tsavaler et al., 2001; Zhang and Barritt, 2006; Gkika et al., 2010; Gkika and Prevarskaya, 2011). In vitro, menthol, a TRPM8 agonist, inhibits proliferation of prostate cancer cells (Wang et al., 2012b). D-3263 [3-(2-amino-ethyl)-1(R)-(2(S)-isopropyl-5(R)-methyl cyclohexanecarbonyl)-5-methoxy-1,3-dihydro-benzimidazol-2-one hydrochloride], a TRPM8 agonist, was recently advanced to a phase I clinical trial (ClinicalTrials.gov identifier NCT00839631) in patients with metastatic prostatic carcinoma with a strategy to kill TRPM8-expressing tumor cells by Ca$^{2+}$ and Na$^+$ overload through TRPM8 activation. Below are a few selected cancers in which, at least in our opinion, aberrant TRP channels expression may have important clinical ramifications.

1. Glioblastoma Multiforme. Glioblastoma multiforme (GBM) is a highly aggressive (World Health Organization grade IV) and deadly form of brain cancer (malignant glioma) with a 3-month median survival time from the diagnosis without treatment (Fig. 37). Even with standard treatment regimens, the life expectancy of patients with GBM is less than a year, with only 2% of patients living longer than 3 years after diagnosis. Although some investigational treatment modalities (e.g., vaccine and gene therapy) showed initial promise, the medium survival has showed only very modest (approximately 3-month)
improvement during the past 3 decades. GBM is a greatly infiltrative tumor (not amenable to complete surgical removal with clean margins) that is also highly resistant to chemotherapy and/or radiation therapy. A number of recent observations in the TRP channel field offer a new glimmer of hope for patients with GBM.

As mentioned above, TRPV2 is highly expressed in normal brain tissue (mostly in astrocytes); indeed, TRPV2 was originally cloned as a vanilloid receptor 1-like protein from rat brain (Caterina et al., 1999). Although the physiologic role of TRPV2 in normal brain functions remains enigmatic, emerging evidence implicates an important role for TRPV2 in GBM tumorigenesis. In U87MG glioma cells (a human primary GBM cell line), silencing of TRPV2 by siRNA impairs proliferation growth by stimulating cell cycle (upregulated primary GBM cell line), silencing of TRPV2 by siRNA tumorigenesis. In U87MG glioma cells (a human brain functions remains enigmatic, emerging evidence implicates an important role for TRPV2 in GBM tumorigenesis. In U87MG glioma cells (a human primary GBM cell line), silencing of TRPV2 by siRNA impairs proliferation growth by stimulating cell cycle (upregulated cyclin E1 and CDK2 and overexpressed Raf-1 and BCL-xl) and protecting tumor cells from apoptosis (via down-regulation of Fas/CD95 and caspase-8 expression) (Nabissi et al., 2010). In keeping with these in vitro findings, TRPV2 siRNA accelerated GBM progression in a mouse xenograft model (Morelli et al., 2010). Importantly, the reserve is also true: 1) overexpression of TRPV2 in glioma cells decreases viability by increasing proapoptotic Fas/CD95 expression, and 2) reduces the tumor volume and mitotic rate when GBM is xenografted into nude mice. Intriguingly, the residual tumor shows evidence of differentiation toward a more mature (i.e., lower grade) astrocytic phenotype. Provided that TRPV2 can be overexpressed in human GBM via gene therapy, the above observations may represent the first real breakthrough in GBM research because the prognosis of grade 2 (or even grade 3) glioma (5-year survival of 27% and 65%, respectively) is far superior to that of GBM (practically no patient survives for 5 years).

In contrast with TRPV2, which is highly expressed throughout the whole neuroaxis, the expression of TRPV1 in normal brain is controversial. The conflicting reports are discussed elsewhere in this review. Here it suffices to mention that a high level of TRPV1 expression was reported in GBM (but not in surrounding non-neoplastic brain tissue) and it was speculated that TRPV1 agonism may be used for therapeutic purposes (Stock et al., 2012). Indeed, TRPV1 agonists induced apoptosis in glioma cells in vitro via a subdivision of the endoplasmic reticulum stress pathway that is controlled by activating transcription factor-3. On the basis of their observations, the authors argue that endovanilloids (endogenous brain-derived TRPV1 agonists) released from neural precursors cells patrol the juvenile brain by eliminating TRPV1-expressing glioma cells. When this endogenous defense mechanism is impaired (neural precursors cells decline with age and eventually disappear), high-grade gliomas may arise. These TRPV1-expressing cancers may be killed (or at least controlled) by either stimulating endovanilloid release or TRPV1 agonist therapy. Indeed, arvanil, a synthetic TRPV1 agonist given systemically (via intraperitoneal injections), prolonged survival of SCID mice injected with GBM cells. This is an elegant theory with potential clinical ramifications. Although it is rather doubtful that patients will tolerate systemic TRPV1 agonist (arvanil or other) injections, intrathecal administration represents a viable alternative. Indeed, intrathecal RTX (an ultrapotent TRPV1 agonist) injections have been tried both in preclinical models (e.g., dogs with osteosarcoma) and a small number of patients to relieve cancer pain with tolerable side effects.

The high level of expression of TRPC1 (Bomben and Sontheimer, 2010) and TRPC6 (Chigurupati et al., 2010; Ding et al., 2010) both in glioma cell lines and patient-derived GBM samples is well documented. These channels are interesting in that they appear to be involved in multiple steps of GBM tumorigenesis. The potential role of TRPC1 and TRPC6 in VEGF-induced neoangiogenesis was previously mentioned in this work. Bevacizumab is one of the few therapeutic options in GBM, and one might speculate that TRPC1 and/or TRPC6 blockade may boost the therapeutic efficacy of bevacizumab. It is worth mentioning that knockdown of TRPC6 (by overexpression of a negative dominant mutant or via siRNA interference) in GBM cells reduced tumor volume in a murine xenograft model and increased survival in the intracranial mouse model (Ding et al., 2010).

2. Breast Cancer. With the exception of non-melanoma skin cancers, breast carcinoma is the most common type of cancer among American women. In fact, one in eight women (approximately 12%) in the United States will develop invasive breast carcinoma during their lifetime. The American Cancer Society estimates that about 230,000 new cases of invasive breast carcinoma will be diagnosed in 2013 and close to 40,000 women will die from it. Although death rates from breast cancer have been declining since the late 1980s (attributed to a combination of early detection by screening and advances in therapy such as herceptin), breast carcinoma remains the second leading cause of cancer death among women, exceeded only by lung.
cancer. Breast cancer is a heterogeneous group of diseases, including both relatively indolent cancers (e.g., tubular carcinoma and mucinous carcinoma) that almost never metastasize and highly aggressive cancers (e.g., such as the so-called triple negative carcinoma that is resistant to both tamoxifen and hereceptin therapy because it does not express estrogen receptors and is also negative for Her2/neu overexpression).

Overexpression of a number of TRP channels (including TRPC1, TRPC5, TRPC6, TRPM7, TRPM8, and TRPV6) was described in breast carcinomas compared with normal breast tissue (reviewed in Ouadid-Ahidouch et al., 2013). Of these channels, TRPC5, TRPM7, and TRPV6 appear to be the most interesting. TRPC5 was shown to render breast cancer cell lines resistant to chemotherapy (adriamycin) through NFAT isoform c3-mediated P-glycoprotein upregulation, enabling the cancer cells to pump cytotoxic drugs out of the cells (Ma et al., 2012b). TRPM7 seems to be exclusively expressed in high-grade [Scarff-Bloom-Richardson (SBR) grade 3/3] breast carcinomas (Guilbert et al., 2009; Dhenin-Duthille et al., 2011). In a mouse xenograft model, TRPM7 expression is required for metastasis formation (Middelbeek et al., 2012). If so, it is hardly surprising that a high level of TRPM7 expression is an independent adverse prognostic factor in patients with breast cancer (see Ouadid-Ahidouch et al., 2013). Interestingly, it was speculated that this risk can be minimized by dietary Mg2+ supplementation (Sahmoun and Singh, 2010). Microarray analysis of breast cancer samples identified TRPV6 overexpression as a feature of estrogen receptor-negative carcinomas (see Bolanz et al., 2008). Similar to TRPM7, TRPV6 overexpression portends an adverse prognostic significance in breast cancer. In MCF7 breast cancer cells, tamoxifen inhibits TRPV6 activity in a way that is independent of estrogen receptor activation (Bolanz et al., 2008). On the basis of these findings, it was proposed that TRPV6 may be a viable therapeutic target in estrogen receptor-negative breast cancer (see Peters et al., 2012).

3. Prostatic Adenocarcinoma. With an estimated 240,000 new cases and 30,000 deaths in 2013, prostate cancer remains the most common cancer among American men (aside from nonmelanoma skin cancers). Prostate-specific antigen (PSA) screening dramatically increased prostate cancer detection but resulted in disappointingly little reduction in death. Indeed, most prostate cancers are indolent and patients die with, and not from, their disease. Identifying the cancers that will spread and kill patients via a simple laboratory marker is the ultimate goal in prostate cancer research. Gleason grading works well in representative tumor samples but often has limited value in limited specimens (e.g., minute focus of adenocarcinoma in a core biopsy). Until such a marker is identified, many men will continue undergoing unnecessary prostatectomy.

Compared with non-neoplastic prostate, increased expression of TRPV1–TRPV4, TRPV6, TRPC1, TRPC3, TRPC4, TRPC6, and TRPM8 was reported in prostatic adenocarcinoma (reviewed in Gkika and Prevarskaya, 2011). The significance of TRPV1 expression in prostate carcinoma cells (Sanchez et al., 2005) is unclear since the TRPV1 agonist capsaicin was reported to stimulate the growth of androgen-responsive cancer cells derived from lymph node metastasis of prostate carcinoma (Malagarie-Cazenave et al., 2009), a most puzzling observation since TRPV1 agonism is usually advocated to kill TRPV1-expressing cancerous cells. Indeed, capsaicin induces apoptosis in the PC-3 prostate cancer cell line (Sánchez et al., 2006). TRPV2 and TRPV3 are more interesting since they are predominantly expressed in castration-resistant carcinomas. Silencing of TRPV2 by siRNA reduced tumor volume and increased survival in mice with xenografted prostate cancers (Monet et al., 2010).

In contrast with vanilloid and canonical TRP channels whose function in physiologic prostate function is essentially unknown, TRPM8 functions as an androgen-responsive element that is involved in protein secretion (in acinar cells) and proliferation (in smooth muscle) (see Zhang and Barritt, 2006). In PC3 cells, TRPM8 is overexpressed in both the plasma membrane and the endoplasmic reticulum. Interestingly, PSA can stimulate the redistribution of TRPM8 from the endoplasmic reticulum to the plasma membrane (Gkika et al., 2010). It was speculated that plasma membrane-bound TRPM8 plays a protective role against prostate cancer progression, whereas the shift toward the endoplasmic reticulum indicates cancer progression. The experimental findings are, however, confusing. In PC3 cells, PSA treatment (presumably by enhancing TRPM8 activity in the plasma membrane) reduced cell motility and proliferation, implying a therapeutic value for TRPM8 agonism in the treatment of prostate cancer (Gkika et al., 2010). However, in another study, it were the TRPM8 antagonists AMTB and JNJ41876666 that reduced the proliferation rate of prostate cancer cells, whereas the TRPM8 agonist icilin was without any effect (Valero et al., 2012). Moreover, TRPM8 silencing induced apoptosis in lymph node metastasis-derived prostate carcinoma cells (Thebault et al., 2005). This finding was interpreted to imply that TRPM8 expression is required for the survival and growth of metastatic prostate carcinoma.

TRPM8 expression appears to correlate with the grade of prostate cancer: the higher the grade, the higher the expression. It is, however, unclear whether TRPM8 expression is an independent adverse prognostic factor in biopsies. It also remains to be seen whether serum TRPM8 (with or without PSA) can be used as a marker to screen individuals for possible high-grade prostate carcinoma. Of note, serum TRPM8 mRNA was advocated as a useful diagnostic test to identify patients with metastatic prostate cancer (Bai et al., 2010).
4. Ovarian, Pancreatic, and Gastric Adenocarcinoma. 
Ovarian, pancreatic, and gastric adenocarcinomas are deadly diseases that are often discovered in an advanced stage when they are no longer amenable to complete surgical removal. In fact, the 5-year survival rate in ovarian carcinoma drops from 90% (cancer still confined to the ovary) to 30% or less when the cancer has spread outside the pelvis. Pancreatic cancer has an extremely poor prognosis, with a 6% 5-year survival rate with all stages combined. The stage IV gastric cancer survival rate is also dismal (<10%). Clearly, there is a large, unmet need in the medical management of late stage (widely metastatic) ovarian, pancreatic, and gastric carcinomas.

An increase in mRNA levels encoding TRPC1 and TRPC3–TRPC5 was recently described in ovarian adenocarcinoma cells (Zeng et al., 2013), correlating with the grade of the carcinoma. A novel spliced isoform of TRPC1 with exon 9 deletion was also detected (Zeng et al., 2013). Transfection of cancer cells with siRNA targeting these TRPC channel genes (Zeng et al., 2013), or application of TRPC channel-specific blocking antibodies (Zeng et al., 2013), reduced the proliferation of cancer cells in vitro. A second, independent study also demonstrated a therapeutic potential for silencing TRPC3 in the ovarian carcinoma cell line SKOV3 (Yang et al., 2009). It remains to be seen whether these in vitro findings also hold true for in vivo tumours.

In pancreatic ductal adenocarcinoma, TRPM7 (Rybarczyk et al., 2012) and TRPM8 (Yee et al., 2012a) have been identified as potential therapeutic targets. Compared with non-neoplastic pancreatic tissue, TRPM7 is highly expressed (13-fold) in pancreatic cancer. Importantly, an inverse correlation was found between patient survival and TRPM7 expression. In BxPC3 cells, silencing of TRPM7 by siRNA interference inhibited Mg$^{2+}$ fluorescence and blocked the migration, but (disappointingly) not the proliferation, of cancer cells (Rybarczyk et al., 2012). On a more positive note, in a second independent study, the combination of anti-TRPM7 siRNA and gemcitabine produced enhanced tumor cell killing compared with gemcitabine alone (Yee et al., 2012b). A subset of patients with pancreatic carcinoma aberrantly expresses TRPM8 (Yee et al., 2012a). Targeted inhibition of TRPM8 in these patients may increase survival.

In gastric carcinoma, TRPC6 and TRPM7 are being investigated as potential therapeutic targets. Expression of TRPC6 is greatly elevated in human gastric carcinoma compared with non-neoplastic gastric epithelium (Cai et al., 2009). Silencing of TRPC6 was reported to inhibit the formation of gastric carcinomas in nude mice. Human gastric adenocarcinoma cells also express abundant TRPM7. In these cells, anti-TRPM7 siRNA inhibits growth and promotes apoptosis (Kim et al., 2008c).

5. Melanoma and Nonmelanoma Skin Cancers.
Malignant melanoma is a potentially deadly form of skin cancer. The incidence of melanoma has been steadily increasing over the past 3 decades, attributed to increased sun (UV) exposure. In 2013, an estimated 77,000 new cases of melanoma will be diagnosed and 10,000 patients will die of the disease. Of note, survivors of melanoma are about nine times more likely to develop another melanoma than the general population, indicative of genetic predisposition. Whereas localized melanoma portends a good prognosis (if removed surgically with clean margins), metastatic melanoma (especially the BRAF mutation negative cases) remains a deadly disease with few treatment options. Nonmelanoma skin cancer is the most common form of cancer in the United States, with one in five Americans developing skin cancer in the course of a lifetime.

The melastatin subfamily of TRP channels was named after a protein (now called TRPM1) that is highly expressed in benign nevi, including Spitz nevi (Erickson et al., 2009), with reduced expression in primary melanoma and absence of expression in metastatic melanoma (Duncan et al., 1998). These observations led to the suggestion that the TRPM1 gene is a tumor suppressor. In melanocytes, TRPM1 expression appears to be regulated by MITF. Indeed, the promoter region of the TRPM1 gene has a MITF-recognition site. Interestingly, TRPM2 has two isoforms (TRPM2-AS and TRPM2-TE) that are upregulated in melanoma (see Guo et al., 2012). These isoforms are thought to function as antisense transcripts that negatively regulate TRPM2.

Indeed, overexpression of wild-type TRPM2 and knockdown of TRPM2-TE exert the same effect: increased susceptibility of melanoma cells to apoptosis (reviewed in Guo et al., 2012). Although not strictly related to melanoma, it is worth mentioning here that an anti-TRPM1 autoantibody was described in patients with occult melanoma and paraneoplastic syndrome, namely melanoma-associated retinopathy (Dhingra et al., 2011; Dalal et al., 2013).

Local excision is curative in most patients with BCC or SQCC of the skin. However, both BCC and SQCC have subtypes (e.g., morphea-like BCC and basaloid SQCC) that are difficult to manage surgically. Multifocal-superficial BCC may involve large areas in sun-exposed skin. Some of these areas (e.g., eyelid) allow limited surgical intervention. BCC is thought to represent a maturation defect in keratinocyte differentiation (the carcinoma cells resemble the normal basal layer of the epidermis, hence the name BCC). TRPC1/TRPC4 was recently implicated in the Ca$^{2+}$ signaling that regulates keratinocyte differentiation (Beck et al., 2008). One might speculate that creams containing TRPC1 and TRPC4 agonists may restore normal differentiation in the epidermis and thereby prevent the arising of further BCC. Invasive SQCC often arises in a background of dysplasia attributed to sun damage (called actinic keratosis) that can be quite extensive. The role of TRPV1 in skin carcinogenesis is controversial. The TRPV1 antagonist AMG9810 [(2E)-N-(2,3-dihydro-1,4-benzodioxin-6-yl)-3-[4-(1,1-dimethylethyl)phenyl]-2-propenamide] was
reported to promote skin tumor (papilloma) formation in mouse skin (Li et al., 2011d). In similar experiments, TRPV1 knockout mice also showed an increased number of skin papillomas compared with their wild-type littermates (Bode et al., 2009). However, mice in which TRPV1 was chemically deleted by neonatal RTX administration showed no increase in skin papilloma formation (A. Szallasi and P.M. Blumberg, unpublished observations). Regardless of the interpretation of these conflicting results, TRPV1 appears to be overexpressed in SQCC and thus may represent a therapeutic target for tumor cell killing (Marincsák et al., 2009). It would be interesting to see whether a topical cream containing capsaicin or RTX can eliminate actinic keratosis. Of note, capsaicin appears to kill oral SQCC cells in culture independent of TRPV1 (Gonzales et al., 2014).

In ex vivo human skin explants, terpenoids restored normal keratinocyte differentiation in the sun-damaged areas (actinic keratosis, a precursor for SQCC) by activating TRPC6 (Woelfle et al., 2010). In head and neck SQCC, TRPC6 is highly expressed and plays a role in carcinogenesis as evidenced from knockdown of Trpc6 in head and neck SQCC-derived cell lines. siRNA-induced knockdown of Trpc6 expression in head and neck SQCC-derived cells dramatically inhibited tumor invasiveness. Thus, TRPC6 is likely to be a promising therapeutic target in the treatment of head and neck SQCC (Bernaldo de Quirós et al., 2013). Broadly speaking, head and neck SQCC comprises at least two distinct subsets of cancer, one driven by human papillomavirus and the other related to smoking. It is unclear whether TRPC6 overexpression characterizes either or both cancers. Moreover, TRPC6 overexpression appears to be an adverse prognostic marker is esophageal SQCC (Zhang et al., 2013d).

**H. Transient Receptor Potential Channels as Therapeutic Targets in Dermatology**

The skin is the largest organ of the human body. For the average adult, the skin has a surface area of 1.5–2 m² and weighs 3–4 kg. Functions of the human skin are extremely diverse and often underappreciated. For example, the skin serves as a sensory organ and mechanical barrier that connects us to, and separates us from, our environment. Moreover, the skin is an immunologic organ. It also participates in water and electrolyte regulation and in maintaining normal body temperature. Finally, the skin is an important organ of sensuality and psychologic well-being. Indeed, in the United States alone, women spend over $7 billion on cosmetics and beauty products and another $2 billion on laser hair removal, facelifts, and other cosmetic procedures such as microdermabrasion.

The skin is divided into three layers: the epidermis (with the skin appendages), the dermis, and the subcutaneous adipose tissue. Skin cells (keratinocytes, melanocytes, and hair follicle cells, as well as endothelial cells, fibroblasts, Langerhans cells, adipose cells, and smooth muscle cells) express a number of TRP channels that are implicated in a variety of key cutaneous functions, including skin-derived pruritus, proliferation, differentiation, apoptosis, and inflammatory processes (summarized in Fig. 38; reviewed in Denda and Tsutsumi, 2011; Moran et al., 2011; Valdes-Rodriguez et al., 2013). The role of TRP channels in itch and skin cancer was discussed elsewhere in this review. Here we focus on dermatological disorders (e.g., alopecia/hirsutism, rosacea, atopic dermatitis/eczema, and psoriasis) in which targeting TRP channels may have therapeutic value.

The growth of hair is a cyclic process that involves three phases: the growth phase (anagen), the transition phase (catagen), and the resting phase (telogen). The physiologic regulation of hair growth cycle is not completely understood but IGF-1 appears to play a critical role in promoting hair growth (reviewed in Su et al., 1999). Indeed, finasteride (Propecia; Merck, Whitehouse Station, NJ) is believed to stimulate hair growth by upregulating IGF-1 in dermal papillae. The target for IGF-1 is a receptor tyrosine kinase that supports cell growth (mitogen) and survival (antiapoptotic) in many cell types. Indeed, mice carrying null mutations in the Igf-1 and Igf-1r genes remain small (dwarfism) if they survive (many die shortly after birth) due to general organ hypoplasia, including the epidermis (Liu et al., 1993). Surprisingly, the heterozygous Igf1r(+/−) knockout mice live longer and have an otherwise unremarkable phenotype (Holzenberger et al., 2003).

In the hair follicle, IGF-1 is believed to maintain the anagen stage and postpone the catagen stage. In support of this concept, transgenic mice that overexpress IGF-1 in their skin have early, accelerated hair follicle development (Semenova et al., 2008). By contrast, proinflammatory cytokines (e.g., IL-1α, IL-1β, TNFα, and IFNγ) are thought to promote hair loss by inducing apoptosis in the hair bulb.

There is emerging evidence that TRPV1 may be involved in the regulation of human hair growth (Bodó et al., 2005). In organ-cultured human hair follicles, activation of TRPV1 by capsaicin markedly suppressed hair shaft elongation and induced apoptosis-driven catagen regression. Interestingly, this effect was accompanied by an alteration in gene expression profiles, promoting the intrafollicular production of cytokines (e.g., IL-1β and TNFα) that inhibit hair growth. Indeed, TRPV1-null mice exhibit a significant delay in hair follicle cycling compared with wild-type animals, supporting the concept that TRPV1 may exert negative control (i.e., a growth-inhibitory function) in mammalian skin (Biró et al., 2006). These observations imply a therapeutic value for topical TRPV1 agonist preparations in eliminating unwanted hair growth. One can
also argue that TRPV1 antagonist hair lotions may reverse hair loss.

Of note, TRPV1 is also expressed in human sebaceous glands as well as the immortalized SZ95 sebocyte cell line (Tóth et al., 2009). In these cells, activation of TRPV1 selectively inhibits both basal and arachidonic acid–induced lipid synthesis (a hallmark of sebocyte differentiation) and alters the expression profiles of multiple genes involved in cellular lipid homeostasis. If so, topical TRPV1 agonist application may improve acne vulgaris, possibly in combination with existing therapeutic options such as antibiotics, hormonal treatment, and retinoids. The involvement of TRPV1 in solar aging of the skin is controversial (Lan et al., 2013).

TRPV3 is abundantly expressed in the skin (Peier et al., 2002; Xu et al., 2002; Chung et al., 2003), including the hair follicles (Asakawa et al., 2006).
Indeed, TRPV3 knockout mice have a characteristic hair phenotype that includes wavy hair coat and curly whiskers (they also have a very thin stratum corneum) (Cheng et al., 2010a). Apparently, normal hair growth requires tightly controlled TRPV3 activity. For example, the constitutively active, gain-of-function trpv3 gene mutation (TRPV3<sup>G573S</sup>) is associated with a hairless phenotype both in DS-Nh mice and WBN/Kob-Ht rats (Asakawa et al., 2006; Xiao et al., 2008). In these animals, histologic analysis revealed impaired hair follicle morphogenesis and hair cycling (Imura et al., 2007). In rodents, TRPV3 is thought to form a signalplex with TNFα and the EGFR that regulates hair morphogenesis. In human hair follicle organ cultures, activation of TRPV3 also inhibited hair shaft elongation and promoted apoptosis-driven catagen regression (Borbíró et al., 2011). Combined, these observations suggest that TRPV3 agonists may control unwanted hair growth (e.g., hirsutism); conversely, TRPV3 antagonists may promote hair growth in patients with alopecia (see Moran et al., 2011; Nilius and Bíró, 2013).

It is noteworthy that DS-Nh mice carrying the gain-of-function trpv3 gene mutation (TRPV3<sup>G573S</sup>) not only show hair loss but also develop a skin disorder that resembles human atopic dermatitis (Yoshioka et al., 2009). Moreover, TRPV3<sup>G573S</sup> transgenic mice that overexpress the constitutively active mutant TRPV3 in epidermal keratinocytes also spontaneously develop an atopic dermatitis-like skin disorder.

A widely held concept states that atopic dermatitis requires a breached skin barrier due to scratching and/ or impaired barrier formation. Interestingly, skin barrier formation is impaired both in TRPV3-null and gain-of-function mutant mice (Cheng et al., 2010a). It was postulated that TRPV3 forms a complex with ADAM17, EGFR, and TGFα to regulate terminal keratinocyte differentiation (reviewed in Nilius and Bíró, 2013). Apparently, both increased and decreased TRPV3 production interferes with this signaling pathway. Hairless rats fed a Mg<sup>2+</sup>-deficient diet develop dermatitis. It was speculated (Luo et al., 2012a) that TRPV3 in keratinocytes is under the tonic inhibitory control of Mg<sup>2+</sup>. Dietary Mg<sup>2+</sup> deficiency liberates TRPV3 from this negative control and results in a constitutively active channel. It is noteworthy that in many patients, pregnancy (i.e., often associated with subclinical Mg<sup>2+</sup> deficiency) aggravates atopic dermatitis. Of note, TRPV4 contributes to intercellular bridge formation between keratinocytes (Sokabe et al., 2010) and skin barrier is also compromised in TRPV4 knockout mice. Human keratinocytes reveal abundant TRPV4 expression (Facer et al., 2007). Administration of a topical TRPV4 agonist (GSK1016790A) protects skin barrier formation (Kida et al., 2012).

The prevalence of atopic dermatitis is increasing in American pediatric populations already approaching 20%. In the skin of NC/nga mice, a model of atopic dermatitis, an increased density of nerve fibers that coexpress GRP with TRPV1 was detected (Tominaga et al., 2009). Indeed, desensitization to capsaicin relieves scratching behavior in NC/nga mice. Moreover, chemical ablation of GRP/TRPV1 nerves by neonatal capsaicin administration prevents the development of atopic dermatitis–like symptoms in these animals (Mihara et al., 2004). Importantly, the TRPV1 antagonist PAC-14028 ameliorates the atopic dermatitis–like symptoms in the NC/nga mice (Lim and Park, 2012). These observations have paved the way to the phase I clinical trials with PAC-14028 in patients with atopic dermatitis. Of note, artemin is increased in atopic dermatitis skin lesions. In mice, intradermal artemin injections cause abnormal sensory nerve sprouting and thermal hyperalgesia. In transgenic mice, skin-expressed artemin is retrogradely transported into the sensory ganglia, where it increases TRPV1 expression (Murota et al., 2012). These findings give further credence to the PAC-14028 trials. Somewhat confusingly, in NC/Tnd mice, another model of human atopic dermatitis, the stimulation of TRPV1, but not the blockade, relieved the scratching behavior (Amagai et al., 2013).

Human keratinocytes in culture express various TRPC channels, including TRPC6. In psoriatic skin lesions, the expression of TRPC6 is markedly reduced (Leuner et al., 2011). Activation of TRPC5 by hyperforin partially restores the disturbed keratinocyte differentiation in psoriatic lesions (Müller et al., 2008). This implies a therapeutic potential for TRPC6 agonist creams in the pharmacotherapy of psoriasis. TRPV1 is another potential drug target in psoriasis. It was speculated that neuropeptides released from TRPV1-positive sensory nerve endings innervating the skin (in particular, SP) exert trophic functions on keratinocytes (reviewed in Szallasi and Blumberg, 1999). Overactivity of these nerves (manifesting in increased SP release) may impair keratinocyte differentiation and contribute to the pathogenesis of psoriasis (Divito et al., 2011). Indeed, on a largely empirical basis, topical capsaicin was tried in a small number of patients with psoriasis with conflicting reports as to clinical benefit (see Hautkappe et al., 1998).

Skin biopsies taken patients with rosacea reveal an increase in TRPV3-positive dermal (but not in epidermal) cells (Sulk et al., 2012). With an estimated 18 million Americans affected, rosacea (watery eyes and small visible blood vessel in facial skin) is a common but poorly understood disease. Although it is clinically indolent, rosacea may cause significant psychologic and social problems such as low self-esteem and avoidance of social interactions. There is no known cure for rosacea. If the TRPV3<sup>+</sup> cells in the dermis are involved in the pathogenesis of rosacea, they may represent the first real breakthrough for finding a cure.
I. Transient Receptor Potential Channels and Eye Diseases

Dry eye disease (also known as xerophthalmia or keratoconjunctivitis sicca) is a common condition, the prevalence of which is increasing with age, affecting 10% of the elderly population. The management of dry eye disease can be frustrating because artificial tear drops provide only temporary relief. Symptoms are often worse during the summer months when patients sleep in air-conditioned rooms with reduced humidity. Although dry eye disease is indolent, it has been associated with sleep disturbances/deprivation and resultant depression. In mice with allergic keratoconjunctivitis, the (nonselective) TRPV1 antagonist capsazepine reduced tearing and blinking (Acosta et al., 2013).

The mouse cornea is densely innervated by TRPM8-positive, cold-responsive afferents (Robbins et al., 2012). Indeed, in some studies, the vast majority (up to 90%) of corneal afferents were activated by cold and/or menthol (Hirata and Oshinsky, 2012). Low concentrations of menthol increased basal lacrimation in wild-type, but not in TRPM8 knockout, mice (Robbins et al., 2012). Importantly, at concentrations at which it increased tear production, menthol did not evoke nociceptive behavior, nor did it interfere with protective eye reflexes. Taken together, these findings imply that a TRPM8 agonist-containing eye drop may be a novel therapeutic approach to stimulate tear production in patients with dry eye disease (Parra et al., 2010; Kurose and Meng, 2013).

Glaucoma is a group of diseases that can damage the optic nerve. The cause of the nerve damage is usually an increase in the fluid pressure inside the eye. Without treatment, first the peripheral (or side) vision is lost and then the straight ahead vision decreases until no vision remains. In fact, glaucoma is the leading cause of blindness in the United States. It was recently speculated that TRPC6, a hyperosmotic solution and pressure-sensitive channel, may be an appealing target in glaucoma patients who are not candidates for eye surgery (Fan et al., 2012). In Turkish patients, the TRPM5 polymorphism rs34551253 was reported to confer increased risk for developing open-angle glaucoma (Okumus et al., 2013).

Ocular neovascularization disorders (including proliferative retinopathy of prematurity, abnormal growth of blood vessels and associated vascular leakage in diabetic retinopathy, and exudative age-related macular degeneration) represent a leading cause of blindness worldwide. Angiogenesis is the physiologic process by which new blood vessels (capillaries) develop from preexisting ones. Aberrant growth of blood vessels (neovascularization) is believed to constitute the final, common pathway in severe retinopathies. The mechanisms that govern this pathologic neovascularization have not yet been fully elucidated. Since growth factors such as VEGF and platelet-derived growth factor guide angiogenic sprouting during physiologic angiogenesis, it is a reasonable assumption that aberrant neovascularization may be due to a “dysorchestrated” production of these growth factors. A shared feature of ocular neovascularization disorders is ischemia, which, in turn, was shown to upregulate VEGF production. Indeed, it was suggested that VEGF may be the predominant angiogenic stimulus in proliferative retinopathies, paving the way to clinical trials with VEGF antagonists. Although VEGF inhibitors (e.g., ranimizumab and bevacizumab) have no doubt revolutionized the care of patients with age-related macular degeneration, they appear to be less effective in other forms of ocular neovascularization disorders, indicating that VEGF is not the only agent that drives the pathology in these patients.

TRPV4 is highly expressed in the mouse and human retina, where it is involved in mediating Ca^{2+} currents (Gilliam and Wensel, 2011; Ryskamp et al., 2011). Importantly, retinal endothelial cells also express functional TRPV4 channels. Indeed, VEGF evokes Ca^{2+} currents in retinal endothelial cells that are blocked by the TRPV4 antagonists RN1734 [2,4-dichloro-N-(2-isopropylaminoethyl)benzenesulfonamide] and HC-067046. It was speculated that TRPV4 might function in retinal endothelial cells as a shared downstream target for VEGF and other stimuli of abnormal angiogenesis. If this hypothesis holds true, TRPV4 antagonist-containing eye drops may supplement (or even supplant) VEGF inhibitors in the medical management of patients with ocular neovascularization disorders.

IV. Conclusions

Many great success stories open with humble beginnings. The TRP channel story began in 1969 when Cosens and Manning identified a spontaneously formed Drosophila mutant that behaved as if it was blind under bright illumination (Cosens and Manning, 1969). Twenty years later, the gene responsible for this abnormal light response was identified and termed trp for “transient receptor potential” (Montell and Rubin, 1989). This seminal discovery paved the way to the isolation of the first mammalian homologs of the Drosophila TRP channels, the so-called TRPCs (Wes et al., 1995; Zhu et al., 1995). Since then, mammalian TRP channels have grown in number to form a unique (different from traditional ligand-gated ion channels) superfamly of 28 proteins that can be subdivided into six subfamilies: TRPA, TRPC, TRPM, TRPML, TRPP, and TRPV.

TRP channels are conserved during evolution and are expressed in most cell types, where they exert strong effects on cellular functions and signaling pathways. Not
surprisingly, mutations in genes encoding human TRP channels are responsible for hereditary diseases, which are referred to as TRP channelopathies. Diseases caused by gain-of-function mutations (e.g., TRPV3 in Olmsted syndrome) may be more amenable to therapeutic intervention because overactive channels could be targeted by small molecule antagonists. For diseases caused by loss-of-function mutations, reversal of the abnormal channel function is likely to require a less validated approach such as gene therapy.

The rapid progress in TRP channel research has brought the understanding of the pathogenic roles that these channels play in the development and maintenance of acquired diseases within reach. Whereas the initial emphasis was on “painful” TRP channels expressed on nociceptive neurons (Patapoutian et al., 2009), recent research has expanded TRP channel drug discovery efforts into new disease areas such as respiratory disorders (chronic cough, COPD, and asthma), chronic itch, obesity, diabetes, overactive bladder, anxiety, addiction, stroke, cardiac hypertrophy, and cancer (Fig. 39) (reviewed in Moran et al., 2011; Kaneko and Szallasi, 2013). This is an exciting development since many of these disorders represent large, unmet medical needs.

After the molecular cloning of the capsaicin receptor TRPV1 in 1997 (Caterina et al., 1997), it took less than a decade to develop the first TRPV1 antagonists to be tried in the clinics as novel analgesic drugs (Szallasi et al., 2007). Concentrated efforts by approximately 50 pharmaceutical companies have yielded a plethora (>1000) of patents and compounds, none of which has progressed thus far beyond stage 2 of clinical development due to lack of clinical efficacy and/or on-target adverse effects, most important, hyperthermia and burns secondary to impaired noxious heat pain sensation (reviewed in Moran et al., 2011; Kort and Kym, 2012; Szallasi and Sheta, 2012; Brederson et al., 2013; Szolcsányi and Pintér, 2013). For other TRP channels, on-target side effects may represent an even bigger hurdle. For example, blockade of TRPM4 may be beneficial in the treatment of MS (Schattling et al., 2012) and anaphylaxis (Vennekens et al., 2007, but it may cause potentially fatal cardiac arrhythmias (Abriel et al., 2012). TRPV4 is an even more problematic drug target because both gain-of-function and loss-of-function TRPV4 mutations have been linked to human disease (Nilius and Voets, 2013). Indeed, systemic activation of endothelial TRPV4 by the agonist GSK1016790A caused catastrophic cardiovascular collapse (Willette et al., 2008).

To a limited degree, systemic side effects may be prevented by organ-specific drug delivery (e.g., topical creams for dermatological disorders and nebulizers for airway diseases). An innovative alternative strategy to circumvent side effects is by selectively targeting TRP channels in diseased tissues. For example, TRPV1 is sensitized by chemical messengers or signaling pathways during inflammation (Szallasi et al., 2007).
Augmented permeation of sensitized TRPV1 by the activity-dependent agonist Cap-ET allows the targeted delivery of the cationic anesthetic Na⁺ channel blocker QX-314, taming neurons with hyperactive TRPV1 but sparing normal nociception (Li et al., 2011a). Sensitization itself seems to depend on the interaction of TRPV1 with the scaffolding protein AKAP79 (Zhang et al., 2008d); indeed, molecules that interfere with this interaction exhibit analgesic potential (Btosh et al., 2013; Fischer et al., 2013).

There is increasing evidence for redundancy in TRP channel functions; therefore, selectively blocking a channel may not achieve the desired clinical effect. For example, Belvisi and colleagues (2011) recently suggested that clinically meaningful cough relief may require the simultaneous inhibition of TRPV1 and TRPA1 (Grace et al., 2012). This implies that a functionally integrated constellation of signaling molecules (in our example, TRPV1 and TRPA1 coexpressed in vagal afferents mediating cough) is a better target for effective intervention than a single signaling molecule, a concept referred to as “constellation pharmacology” (Teichert et al., 2012). Chronic neuropathic pain is an even better example. Although the loss-of-function TRPV1 variant I585V was reported to confer decreased risk for painful OA (Valdes et al., 2011), neither the selective TRPV1 antagonist AMG9810, nor the TRPA1 blocker HC-030031, ameliorated ongoing spontaneous pain in a model of advanced OA (Okun et al., 2012). Moreover, the TRPV1 antagonist ABT-116 failed to provide pain relief in client-owned dogs with hip OA (Malek et al., 2012), and a clinical trial with AZD1386 involving 241 patients with OA in seven different countries had to be terminated prematurely for lack of efficacy (Svensson et al., 2010; Miller et al., 2014). These disappointing results contrast with the powerful and lasting analgesic action of intrathecal or intrarticular RTX in dogs with severe OA (Iadarola and Gonnella, 2013). RTX silences the whole TRPV1-expressing nerve, providing the ultimate example of constellation pharmacology.

The more details we learn about TRP channels, the more questions arise. Most of our knowledge was obtained in knockout mice and rodent models of human disease. It is becoming increasingly clear that these models do not necessarily reflect the human disease as exemplified by the lack of analgesic effect of the TRPV1 antagonist AZD1386 in clinical trials enrolling patients with pain related to GERD (Krarup et al., 2013) or OA (Svensson et al., 2010). Yet, TRPV1 remains a viable therapeutic target. Intravesical RTX dramatically improved bladder function in patients with overactive bladder (Cruz et al., 1997), and intrathecal RTX provided permanent relief of intractable cancer pain (Iadarola and Mannes, 2011).

In summary, this is a rapidly evolving field. The benefits and side effects of drugs targeting TRP channels should be compared with currently available therapeutic options. It is important to temper our expectations and remember that not a single category of drugs will ever do the trick. Indeed, we must accept the need for drug combinations. For most indications, TRP channel drugs will not be “magic bullets,” but may be useful (although imperfect) parts of therapeutic regimens. The obstacles facing the development of clinically useful TRP channel drugs are real but are probably not insurmountable. Clearly, there is a dire need for effective translational research by getting the clinicians together with the basic researchers. With this review, we hope to facilitate this process. One is tempted to remember Churchill’s description of El-Alemein, when he stated “This is not the beginning of the end, but the end of the beginning” (www.brainyquote.com/quotes/quotes/w/winstonchu163144.html).

Appendix

Suggested Reading: Books on TRP Channels:


Novartis Foundation (2004) Mammalian TRP Channels as Molecular Targets, John Wiley & Sons Ltd, Chichester, UK.

Sherkheli A (2010) Trip through the Pharmacology of Hot- and Cold-Sensing TRP Channels: A Hope for Better Painkiller and Antiinflammatory Drugs, VDM Verlag Dr. Muller, Saarbruecken, Germany.


Most recently, differential methylation of the TRPA1 gene promoter has been linked to discordant heat pain sensitivity in humans (Bell et al., 2014), suggesting that not only TRP channel blockers may be superior to selective TRP channel activation on the primary bladder afferent activities of the rat. Neurourol Urodyn 26:354–357.

Another recently discovered TRP channel involved in anxiety is TRPC4 (Riccio et al., 2014). Of note, TRpc4 KO mice also show reduced cocaine self-administration (Rasmussen et al., 2013), implying a role for addictive behavior. Because TRPC4 blockade restores erectile function in diabetic rats (Sung et al., 2014), drugs targeting this channel seem to have a number of attractive indications, ranging from behavioral health to erectile dysfunction.

The small-molecule dual TRPC3 and TRPC6 antagonists GSK233255A and GSK2833503A prevented the development of cardiac hypertrophy in rodents subjected to pressure overload, although genetic deletion of TRpc3 or TRpc6 alone was not protective in the same model (Seo et al., 2014). This finding supports the notion that nonselective TRP channel blockers may be superior to selective ones in pharmacology.

Lastly, TRPC5 expression was identified as an adverse prognostic marker in breast cancer, predicting resistance to chemotherapy and poor survival (Ma et al., 2014).

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