The G Protein–Coupled Receptor–Transient Receptor Potential Channel Axis: Molecular Insights for Targeting Disorders of Sensation and Inflammation

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Abstract—Sensory nerves are equipped with receptors and ion channels that allow them to detect and respond to diverse chemical, mechanical, and thermal stimuli. These sensory proteins include G protein–coupled receptors (GPCRs) and transient receptor potential (TRP) ion channels. A subclass of peptidergic sensory nerves express GPCRs and TRP channels that detect noxious, irritant, and inflammatory stimuli. Activation of these nerves triggers protective mechanisms that lead to withdrawal from danger (pain), removal of irritants

ABBREVIATIONS: AA, arachidonic acid; AKAP, A-kinase anchoring protein; BCTC, N-(4-tert-butylphenyl)-4-(3-chloropyridin-2-yl)piperazine-1-carboxamide; BK, bradykinin; CaM, Ca2+-binding calmodulin protein; CB, cannabinoid receptor; CGRP, calcitonin gene-related peptide; COPD, chronic obstructive pulmonary disease; DAG, diacylglycerol; DRG, dorsal root ganglion; EET, epoxyeicosatrienoic acid; EP R, prostaglandin E2 receptor 1; ERK1/2, extracellular signal-related kinases (also known as MAPK1/2 or p44/p42); Gα, heterotrimeric GTP-binding protein α subunit; Gβγ, heterotrimeric G protein subunits β and γ; GPCR, G protein–coupled receptor; HC-030031, 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4-isopropylphenyl)acetamide; HNE, 4-hydroxynonenal; NHE, 4-hydroxytryptamine; IC50, 50% inhibitory concentration; IBD, inflammatory bowel disease; IB4+, isolectin B4+; IB D, inflammatory bowel disease; IBS, irritable bowel syndrome; IP3, inositol-1,4,5-trisphosphate; Mrgpr, M3G-related G protein–coupled receptor; NGF, nerve growth factor; PAR, protease-activated receptor; PDE, phosphodiesterase; PG, prostaglandin; PKA, cAMP-dependent protein kinase A; PKC, protein kinase C; PKD, protein kinase D (atypical PKC); PKP, phosphatidylinositol-4,5-bisphosphate; PIP, PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PIP2, phosphatidylinositol-4,5-bisphosphate; PIR, PIP2-binding regulator of TRP channels; PLA, phospholipase A; PLC, phospholipase C; SP, substance P; TG, trigeminal ganglion; TG5, bile acid receptor (GPBA receptor); TLR5, thymic stromal lymphopoietin; TRPA, transient receptor potential ankyrin; TRPC, transient receptor potential canonical; TRPM, transient receptor potential melastatin; TRPV, transient receptor potential vaniloid.
I. Introduction

Sensory nerves that innervate the skin and the major visceral organ systems play a vital role in protection. The cell bodies of these neurons in dorsal root ganglia (DRG) or trigeminal ganglia (TG) project nerve fibers to peripheral tissues and to the dorsal horn of the spinal cord. Their peripheral projections are equipped with many receptors and ion channels that allow them to detect and respond to diverse chemical, mechanical, and thermal stimuli. A subclass of these neurons expresses receptors and channels that are specialized for the detection of noxious, irritant, and inflammatory stimuli. The activation of these nerves triggers acute protective processes that lead to withdrawal from danger (pain), removal of irritants (itch, cough), and resolution of infection (neurogenic inflammation). These physiologic processes are essential for survival and are normally under tight control. However, dysregulation and disease can lead to chronic pain, pruritus, cough, and inflammation, which are poorly understood, difficult to treat, and are a major cause of suffering. A deeper understanding of these normal protective processes and of how they become dysregulated during diseases is required to develop more selective and effective therapies for these chronic conditions.

Dysregulated sensory responses to noxious, irritant, and inflammatory stimuli underlie diseases of global relevance and can be caused by chronic inflammatory and neuronal diseases. Inflammatory and neuropathic pain is characterized by heightened responses to painful stimuli (hyperalgesia), the perception of pain from normally non-noxious stimuli (allodynia), and by loss of inhibitory mechanisms that prevent central pain transmission (reviewed in Scholz and Woolf, 2007; Patapoutian et al., 2009). Pain is a major societal burden. It is estimated to afflict 1 in 5 people at some point during their lives, and pain is the leading public health problem in the United States, where 100 million people seek medical attention each year because of acute or chronic pain, with an enormous economic impact (Gaskin and Richard, 2012). The acute and protective process of itch can also become dysregulated and chronic (see reviews by Ikoma et al., 2006, and Bautista et al., 2014). Acute itch, such as that caused by an insect sting, is transient and can be readily treated with histamine receptor antagonists. However, dermal and metabolic diseases can lead to chronic pruritus that is resistant to antihistamine drugs and is poorly understood and difficult to treat. Neuropathic itch is caused by many neurologic disorders (Binder et al., 2008). For example, the pruritus of patients with cholestatic liver disease can be so profound and intractable that it is an indication for liver transplantation (European Association for the Study of Liver, 2009). Defects in the normally protective processes of acute inflammation can lead to the chronic inflammatory diseases that affect all organ systems, and which can also lead to chronic pain (see reviews by Galli et al., 2008, and Grace et al., 2014b).

G protein–coupled receptors (GPCRs) and transient receptor potential (TRP) ion channels are cell surface proteins of sensory nerves functioning at the “front line” for sensing noxious, irritating, and inflammatory stimuli (Fig. 1). With ~850 members in mammals, GPCRs are the largest family of signaling proteins. They are receptors for structurally diverse endogenous and exogenous ligands, participate in all pathophysiological processes, and are established therapeutic targets, with 30% of current drugs targeting GPCRs (Overington et al., 2006). More than 40 GPCR families contribute to pain processing and function as sensors on peripheral nerves to detect noxious, irritant, and inflammatory stimuli, including proteases, peptides, amines, and lipids (Stone and Molliver, 2009). Drug-screening efforts targeting nociceptive GPCRs, such as neurokinin or bradykinin receptors, have resulted in the identification of many antagonists that are effective in preclinical studies of disease and led to great expectations for new effective pain treatments. However, with the exception of histamine receptor antagonists, these drugs have generally been disappointing in clinical trials (Steinhoff et al., 2014). TRP channels comprise a small family of nonselective cation channels (28 members in mammals) that also participate in widespread pathophysiologic processes and are an emerging therapeutic target (Patapoutian et al., 2009; Brederson et al., 2013). TRP channels of sensory nerves directly sense endogenous and exogenous chemical, mechanical, and thermal stimuli. TRP channels are also major downstream effectors of GPCR signaling. This GPCR-TRP axis is vitally important for pain, itch, cough, and neurogenic inflammation (Basbaum et al., 2009; Bautista et al., 2014; Grace et al., 2014b). Signaling pathways that emanate from the GPCR superfamily converge on the small TRP family, leading to altered channel activity or expression. GPCR signaling events in the skin and the gastrointestinal and respiratory systems. We discuss the signaling mechanisms that underlie the GPCR-TRP axis and evaluate how new information about the structure of GPCRs and TRP channels provides insights into their functional interactions. We propose that a deeper understanding of the GPCR-TRP axis may facilitate the development of more selective and effective therapies to treat dysregulated processes that underlie chronic pain, itch, cough, and inflammation.
can generate mediators that stimulate TRP channels (i.e., channel activation) or that enhance their responses to TRP agonists (i.e., TRP sensitization). The net result is that TRP channels can amplify that effects of GPCRs and mediate their contributions to transmission of pain, itch, cough, and neurogenic inflammation (Clapham, 2003).

The discovery of functional cross-talk between GPCRs and TRP channels occurred concurrently with the characterization of the first TRP channels in *Drosophila melanogaster*. Known as receptor-operated channel theory, this mechanism describes how GPCRs stimulate cell signaling and lipid metabolism pathways, which converge on TRP channels to lower their activation threshold and initiate rapid ion flux across the cell surface or internal membranes (Montell, 2005b). In mammals, the GPCR-TRP axis is enriched by the coexpression of GPCRs and TRPs in select cell types (e.g., subpopulations of sensory neurons) and by an abundance of signaling intermediates (e.g., adaptor proteins, kinases, lipid metabolites) that functionally link GPCRs to TRPs (Pethő and Reeh, 2012). These intermediates afford

Fig. 1. Classification of GPCR-TRP channel interactions involved in major pain and sensory conditions. Acute activation of nociceptive or pruriceptive sensory neurons can trigger protective behavioral responses, such as avoidance behavior and irritant removal, including coughing and scratching. Dysregulation of these mechanisms can be caused by nerve damage, infection, or inflammation leading to heightened, chronic sensory, or pain states, including nociceptive pain, inflammatory pain, neuropathic pain, and itch. The associated disease states and their clinical settings, as well as the underlying mediators, are described. These states are stimulated by different cellular processes (e.g., infection) or exogenous agents (e.g., insect sting) located centrally or in tissues of the periphery, such as the skin and peripheral nerve terminals. Numerous TRP channels contribute to inflammatory states and can be sensitized by a host of GPCRs to reduce their activation threshold. This enhances normal nociceptive responses, leading to heightened pain (hyperalgesia) or allodynia. Associated references for GPCR-TRP channel sensitization can be found in Tables 1 and 2. CNS, central nervous system; PNS, peripheral nervous system (adapted with permission from Patapoutian et al., 2009).
a high degree of specificity within the GPCR-TRP signaling axis and explain how multiple GPCRs can converge upon a limited number of TRP channels to promote different physiologic outcomes (e.g., pain versus itch). These signaling molecules may also provide new targets for treatment of chronic pain, itch, cough, and inflammatory conditions. Furthermore, they may also increase the usefulness of established GPCR and TRP channel antagonists that are currently compromised by on-target side effects related to the widespread distribution of GPCRs and TRPs and their participation in diverse physiologic processes. A deeper understanding of the high-resolution structures of GPCRs and TRP channels, including the identification of protein and lipid binding sites and of key regulatory domains, may facilitate the development of drugs that target the GPCR-TRP axis in a cell type–specific manner, leading to improved treatments for disorders of sensation and inflammation.

Herein we discuss the importance of the GPCR-TRP axis in the detection of noxious, irritant, and inflammatory stimuli in the skin and gastrointestinal and respiratory systems and summarize the relevance of the axis to chronic pain, itch, cough, and inflammation. We discuss the signaling mechanisms that underlie the GPCR-TRP axis and evaluate how new information about the structure of GPCRs and TRP channels provides insights into functional interactions. We review the utility of antagonists of GPCRs and TRP channels for the treatment of chronic sensory and inflammatory disorders and assess whether targeting the GPCR-TRP axis represents a viable option for the treatment of chronic pain, itch, cough, and inflammation.

II. The G Protein–Coupled Receptor–Transient Receptor Potential Axis in Pain Transmission

A. G Protein–Coupled Receptors and Transient Receptor Potential Channels as Molecular Sensors of Nociceptive Neurons

Subsets of primary afferent neurons are the first cells in the pathway that sense noxious stimuli. These are first-order pseudo-unipolar neurons, with a single axon branch that projects both peripherally to target organs and centrally to second-order neurons in the dorsal horn of the spinal cord. Neurons within the DRG project to visceral organs and the periphery; afferents within the TG project to the head and face; vagal afferents of the nodose and jugular ganglia facilitate sensation in the head and visceral organs such as the lung and gastrointestinal tract. The peripheral projections of these neurons express multiple receptors and ion channels that allow them to detect and respond to diverse stimuli, providing critical sensory feedback upon exposure to innocuous or noxious substances and environments. There are two classes of high-threshold nociceptors that can centrally transmit information about noxious stimuli: the moderate-conducting, thinly myelinated Aδ fibers and the slow-conducting small diameter, unmyelinated C-fibers. C-fibers are the predominant form in the periphery and possess high noxious thresholds to generate pain responses of greater intensity and duration (Stucky et al., 2001). These nociceptors are commonly differentiated into two groups, those that are “nonpeptidergic” and bind the plant isolectin B4+ (IB4+) or the “peptidergic” neurons that express the nerve growth factor (NGF) TrkA tyrosine kinase receptor and the pronociceptive and proinflammatory neuropeptides substance P (SP), neuropekinin A, and calcitonin gene-related peptide (CGRP). In addition to afferent fiber size and nerve conductance velocity, the receptor/ion channel expression profile determines their sensory function and defines subtypes of sensory neurons as “polymodal nociceptors” that can respond to more than one type of stimulus (e.g., chemical, mechanical, thermal). For example, a subset of TrkA-positive C-fiber afferents express transient receptor potential ankyrin 1 (TRPA1) and transient receptor potential vanilloid 1 (TRPV1) channels to facilitate responses to noxious heat (>43°C) and noxious chemicals (Story et al., 2003; Kobayashi et al., 2005; Wilson et al., 2011). However, not all C-fiber excitation leads to nociception, because polymodal C-fibers can also express transient receptor potential melastatin 8 (TRPM8) for response to nonpainful cooling stimuli (Basaum et al., 2009).

TRP channels are sensors of the environment and integrators of receptor signaling in a variety of cell types. In primary neurons, relative to the ion currents of their voltage-gated cousins (L-type calcium, sodium, and potassium ion channels), TRP channels exhibit slower kinetics and initiate pain transmission via sustained ion flux, resulting in depolarizing membrane potentials of sufficient duration and magnitude to evoke action potentials. To ensure that nociceptors generate robust and frequent action potentials only in response to harmful stimuli, TRP channels possess high thresholds of activation, whereby ion conductance occurs in response to substantial changes in voltage, temperature, exogenous ligands, and intracellular lipids. Furthermore, the likelihood of a nociceptive TRP channel to become active is determined by its open probability and cell surface expression, which can be markedly influenced by channel agonists and sensitizing agents (Studer and McNaughton, 2010). GPCRs regulate kinases and the synthesis of lipid second messengers, both of which can control TRP activity. In this manner GPCRs influence multiple pathways to lower the TRP channel activation threshold (Ramsey et al., 2006). Once activated, TRP channel-mediated pain transmission occurs via conductance of ions on nerve terminals to cause localized membrane depolarization and stimulation of ion-sensitive and voltage-sensitive channels to produce generator potentials and elicit downstream nerve excitation. TRP channels also stimulate cation-sensitive
signaling pathways (e.g., Ca\(^{2+}\)-stimulated protein kinase C [PKC] activity) and transcriptional changes to promote expression and release of painful or proinflammatory peptides, such as SP and CGRP, from peptidergic neurons.

**B. The Evolutionarily Conserved G Protein–Coupled Receptor–Transient Receptor Potential Ion Channel Coupling Paradigm**

The seminal discovery of a *D. melanogaster* mutant phenotype that rapidly loses sensitivity to sustained light led to the identification of the founding TRP channel family members (Hardie and Minke, 1992; Phillips et al., 1992). Characterization of the signaling pathways in photoreceptors has contributed enormously to our understanding of GPCR signaling in mammalian systems, forming the basis of a central paradigm for receptor-operated TRP channel gating across species (Minke and Cook, 2002). In the plasma membrane of *Drosophila* photoreceptor cells, the receptor-operated TRP channel pathway requires GPCR stimulation and release of a heterotrimeric GTP-bound G protein complex, phospholipase C (PLC) activity, and metabolism of phospholipids and phosphatidylinositol-4,5-bisphosphate (PIP\(_2\)) to generate lipid second messengers, such as inositol-1,4,5-trisphosphate (IP\(_3\)), membrane-associated diacylglycerol (DAG) and arachidonic acid (AA). IP\(_3\) binds its receptor to release Ca\(^{2+}\) from intracellular stores, and DAG stimulates kinase activity. Although the mechanism is still not entirely clear, the activation threshold of TRP channels is lowered (i.e., sensitized) by the presence of Ca\(^{2+}\) ions and fatty acid second messengers to permit channel opening and produce a light-induced current. This process is desensitized by DAG-sensitive kinase phosphorylation of the TRP channel and the Ca\(^{2+}\)-sensitive calmodulin protein (CaM), which binds to the channel to prevent further ionic permeability (Fig. 2A) (Hardie and Raghu, 2001; Montell, 2005a). The GPCR-TRP functional relationship is also evident in the nematode *Caenorhabditis elegans*, where GPCRs and TRP channel homologs are critical for survival by conferring the ability to detect and respond to changes in temperature, noxious substances, pheromones, and osmotic conditions (Clapham et al., 2001; de Bono and Maricq, 2005; Xiao and Xu, 2009).

The regulatory components of vertebrate GPCR-TRP signaling axis are largely conserved in pain transmission pathways within mammalian sensory neurons. Phospholipid metabolism generates lipid precursors, such as DAG, that can activate protein kinases (e.g., DAG stimulates PKC), which phosphorylate TRPs. Moreover, phospholipids can be converted into polyunsaturated fatty acids, which are TRP channel ligands (Minke and Cook, 2002). TRP channels also exhibit independent sensory functions and can also regulate GPCRs, which is suggestive of functional cooperativity between these two classes of membrane proteins (Xiao and Xu, 2009). GPCRs and TRPs can be recruited to dynamic “supramolecular” complexes that facilitate functional interactions (Sheng and Sala, 2001). The discovery of multiple algesic or analgesic GPCRs and nociceptive TRP channels in sensory neurons and other cell types reveals a complex regulatory system that controls neuronal excitability and nonneuronal cell function to mediate nociceptive and inflammatory responses (Fig. 2B).

**C. G Protein–Coupled Receptors that Sense Noxious Stimuli and Initiate Neurogenic Inflammation**

GPCRs are structurally characterized by seven transmembrane domains, an extracellular N terminus, an intracellular C terminus, and three extracellular and three intracellular loops. They are receptors for diverse exogenous and endogenous ligands and are critically important for sensation (e.g., vision, taste, smell, pain, and itch) and pathophysiologic control. The peripheral projections of nociceptive neurons express a suite of GPCRs that allow them to “sample” the extracellular environment for noxious stimuli. These stimuli include proteases (e.g., mast cell tryptase), peptides (e.g., bradykinin from circulation), purines (e.g., ATP), cytokines/chemokines (e.g., interleukins), and lipids (e.g., prostaglandins), resulting in the activation of different classes of GPCRs. Each receptor is exquisitely selective for particular ligands and ligand binding results in diverse, cell- and tissue-dependent outcomes that can ultimately cause pain or inflammatory processes to occur (Nygaard et al., 2013). TRP channels amplify or sustain these painful processes, and GPCRs transduce signals to activate or sensitize TRP channels through several mechanisms. GPCR-stimulated second messenger kinases (e.g., cAMP-dependent protein kinase A [PKA], PKC) phosphorylate TRPs to reduce activation thresholds in response to endogenous channel agonists (Pethő and Reeh, 2012). GPCRs stimulate phospholipase activity to release tonic inhibitory effects of PIP\(_2\) on ion channels (Huang et al., 2010). Metabolic generation of lipids such as anandamide (N-arachidonoylthanolamine) or epoxyeicosatrienoic acids (EETs) are endogenous TRP channel ligands (Watanabe et al., 2003). Receptor tyrosine kinase receptors can promote TRP channel expression (Brederson et al., 2013) and plasma membrane insertion (Zhang et al., 2005) to enhance neuronal responsiveness to TRP stimuli. Together these processes stimulate the release of neuropeptides such as SP and CGRP from peripheral nociceptive endings of nociceptors to promote arteriolar vasodilation, plasma extravasation, and granulocyte infiltration in postcapillary venules (i.e., neurogenic inflammation). The stimulation of TRP channels of the peripheral nerve terminals also has an excitatory effect, resulting in the activation of voltage-sensitive, Ca\(^{2+}\)-activated and ATP-sensitive channels, which induce localized membrane depolarization and formation of generator potentials that are capable of propagating action potentials (Bourinet et al., 2014). Hence, GPCRs are entirely dependent upon ion channels for the central transduction of painful signals from...
the periphery and viscera. Several key “algesic” and “analgesic” GPCRs that control TRP channels are described below.

1. Algesic G Protein–Coupled Receptors. The generation and release of painful and inflammatory mediators from immune cells, epithelial tissue, and the circulation activate multiple different classes of GPCRs, and these are identified according to their activating ligand or stimulatory mechanism. Classes that mediate proinflammatory, nociceptive, or pruritic events include those stimulated by peptides (e.g., bradykinin and neurokinin receptors), lipids (e.g., prostaglandin receptors), amines (e.g., 5-hydroxytryptamine [5-HT] and histamine receptors), nucleotides (e.g., purinergic and adenosine receptors), and proteases (e.g., protease-activated receptors). Evidence indicates that each of these GPCR classes functionally interacts with TRP channels to induce painful, inflammatory, or pruritic cellular outcomes and an extensive summary of these pathways is provided (Tables 1 and 2). Key sensitizing receptors, such as the tyrosine kinase receptor TrkA, 5-HT, purinergic, and the prostaglandin-activated EP receptors, have been reviewed (Pethö and Reeh, 2012; McKelvey et al., 2013; Bannwarth and Kostine, 2014; Bautista et al., 2014). The best studied sensory GPCR-TRP channel regulatory pathways include the protease-activated receptor and bradykinin receptor families, and these are discussed in greater detail below. Intriguingly, the versatility of these receptors to respond to injury, inflammation, itch, and neuropathic disorders requires stimulation of multiple TRP channels through multiple signaling pathways.

a. Protease-activated receptors. The pathophysiologic roles of protease-activated receptors (PARs) have been extensively reviewed (Macfarlane et al., 2001; Noorbakhsh et al., 2003; Ossovskaya and Bunnett, 2004; Ramachandran et al., 2012). PARs are a family of 4 GPCRs (PAR1–4) that are widely expressed in the peripheral and central nervous systems as well as many other cell types, where they participate in hemostasis, inflammation, pain, and repair mechanisms. In contrast to most GPCRs that interact with soluble extracellular ligands, PAR ligands reside within the receptors themselves. Proteases cleave specific sites within the extracellular N-terminal domains of PAR1, PAR2, and PAR4 to reveal tethered ligand domains that bind to and activate cleaved receptors. PAR1, PAR2, and PAR4 are expressed by nociceptive neurons (Vellani et al., 2010), and PAR2 in particular can sensitize or excite sensory neurons to promote neurogenic inflammation, pain, and itch (Ramachandran et al., 2012). PARs couple to the major Ga subunits (Gaq/11, Gas, Gis/Gia, G12/13) to stimulate PLC and phospholipase A2 (PLA2) and activate PKA, PKC, and protein kinase D (atypical PKCδ). These, in turn, sensitize or activate TRP channels, including TRPV1, TRPA1, and TRPV4, to initiate pain and inflammation through peripheral and central mechanisms (Veldhuis and Bunnett, 2013). PARs can be cleaved by multiple proteases at different locations to expose different N-terminal sequences and stabilize distinct “biased” GPCR active conformations. Neutrophil elastase and the proinflammatory cysteine protease cathepsin S have shown PAR2 cleavage specificity at sites distinct from the canonical trypsin/tryptase site, resulting in activation of alternative G protein signaling pathways (Ramachandran et al., 2009; Zhao et al., 2014). Multiple PAR-activated pathways, including trypsin-stimulated Gaq protein, PLA2 activity, and tyrosine kinase signaling or cathepsin S–stimulated Gaq and PKA signaling may therefore converge on a single TRP channel effector (e.g., TRPV4) to cause inflammatory pain (Poole et al., 2013; Zhao et al., 2014). Furthermore, cleavage of the canonical PAR2 cleavage site induces rapid receptor phosphorylation and internalization, whereas cathepsin S cleavage does not, potentially influencing the duration and...
TABLE 1
Nociceptive, inflammatory, and pruritic pathways caused by direct stimulation

*PIP2* metabolism is considered to relieve inhibition of most TRP channels that regulate nociceptive responses and is therefore omitted, unless indicated to increase activity (Rohacs, 2007).

<table>
<thead>
<tr>
<th>Direct Mechanisms of Activation</th>
<th>Reference</th>
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<tr>
<td><strong>TRPV1</strong></td>
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<tr>
<td>Thermal</td>
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<tr>
<td>Noxious heat &gt;43°C</td>
<td>Tominaga et al., 1998</td>
</tr>
<tr>
<td>Chemical</td>
<td></td>
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<tr>
<td>Protons pH &lt;5.9</td>
<td>Tominaga et al., 1998</td>
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<tr>
<td>4-HNE</td>
<td>DelloStritto et al., 2014</td>
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<tr>
<td>Arachidonic acid/AEA</td>
<td>Smart et al., 2000; Sousa-Valente et al., 2014</td>
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<tr>
<td>ATP</td>
<td>Moriyama et al., 2003; Lishko et al., 2007; Ma et al., 2012</td>
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<tr>
<td>Leukotriene B4</td>
<td>Vigna et al., 2011</td>
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<tr>
<td>Oleoylthanolamide</td>
<td>Suardìaz et al., 2007</td>
</tr>
<tr>
<td>20-HETE</td>
<td>Wen et al., 2012</td>
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<tr>
<td>5-(S)-HETE, 12-(S)-HETE</td>
<td>Hwang et al., 2000</td>
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<tr>
<td>5-(S)-HPETE, 12-(S)-HPETE</td>
<td>Hwang et al., 2000</td>
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<tr>
<td>PGE2/ PGI2</td>
<td>Moriyama et al., 2005</td>
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<tr>
<td>LPA</td>
<td>Nieto-Posadas et al., 2012</td>
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<td>Capsaicin</td>
<td>Caterina et al., 1997</td>
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<td>Resiniferatoxin</td>
<td>Szallasi et al., 1999</td>
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<td>Piperine</td>
<td>McNamara et al., 2005</td>
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<td>Tarantula toxin (DxTx)</td>
<td>Bohlen et al., 2010</td>
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<td><strong>TRPV2</strong></td>
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<tr>
<td>Thermal</td>
<td></td>
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<td>Noxious heat &gt;52°C</td>
<td>Hu et al., 2004</td>
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<tr>
<td>Chemical</td>
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<tr>
<td>2-APB</td>
<td>Monet et al., 2009</td>
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<td>Arachidonic acid</td>
<td>Hu et al., 2005</td>
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<td>Lysophospholipids</td>
<td>Stokes et al., 2004</td>
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<td><strong>TRPV3</strong></td>
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<td>Thermal</td>
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<td>Warm &gt;32°C</td>
<td>Peier et al., 2002b</td>
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<tr>
<td>Chemical</td>
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<tr>
<td>Carvacrol, eugenol, thymol</td>
<td>Xu et al., 2006</td>
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<td>2-APB</td>
<td>Hu et al., 2006</td>
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<td>Arachidonic acid</td>
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<td>Monoterpenoids (camphor)</td>
<td>Vogt-Eisele et al., 2007</td>
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<tr>
<td>1,8-Cineole</td>
<td>Takaishi et al., 2012</td>
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<td><strong>TRPV4</strong></td>
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<tr>
<td>Thermal</td>
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<tr>
<td>Warm &gt;28°C</td>
<td>Güler et al., 2002; Todaka et al., 2004</td>
</tr>
<tr>
<td>Mechanical</td>
<td></td>
</tr>
<tr>
<td>Membrane stretch/swelling</td>
<td>Alessandri-Haber et al., 2003; Mochizuki et al., 2009</td>
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<tr>
<td>Cilia beating</td>
<td>Lorenzo et al., 2008</td>
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<tr>
<td>Chemical</td>
<td></td>
</tr>
<tr>
<td>Arachidonic acid, AEA, 5’,6’-EET, 8’,9’-EET</td>
<td>Watanabe et al., 2003</td>
</tr>
<tr>
<td><strong>TRPM2</strong></td>
<td></td>
</tr>
<tr>
<td>Chemical</td>
<td></td>
</tr>
<tr>
<td>Protons &lt;pH 6.7</td>
<td>Du et al., 2009</td>
</tr>
<tr>
<td>H2O2</td>
<td>Fonfria et al., 2004; Naziroglu et al., 2011</td>
</tr>
<tr>
<td>Adenine dinucleotides</td>
<td>Kraft et al., 2004; Grubisha et al., 2006; Du et al., 2009</td>
</tr>
<tr>
<td><strong>TRPM3</strong></td>
<td></td>
</tr>
<tr>
<td>Thermal</td>
<td></td>
</tr>
<tr>
<td>Heat &gt;37°C</td>
<td>Vriens et al., 2011</td>
</tr>
<tr>
<td>Chemical</td>
<td></td>
</tr>
<tr>
<td>Pregnenolone sulfate</td>
<td>Wagner et al., 2008</td>
</tr>
<tr>
<td>n-Erythro-sphingosine</td>
<td>Grimm et al., 2005</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>Vriens et al., 2014</td>
</tr>
<tr>
<td><strong>TRPM8</strong></td>
<td></td>
</tr>
<tr>
<td>Thermal</td>
<td></td>
</tr>
<tr>
<td>Cold 2–28°C</td>
<td>McKemy et al., 2002; Peier et al., 2002a</td>
</tr>
<tr>
<td>Chemical</td>
<td></td>
</tr>
<tr>
<td>PIP2</td>
<td>Rohács et al., 2005</td>
</tr>
<tr>
<td>Lysophospholipids</td>
<td>Andersson et al., 2007</td>
</tr>
<tr>
<td>Menthol, icilin</td>
<td>Peier et al., 2002a</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>Takaishi et al., 2012</td>
</tr>
<tr>
<td>Camphor</td>
<td>Selescu et al., 2013</td>
</tr>
<tr>
<td><strong>TRPA1</strong></td>
<td></td>
</tr>
<tr>
<td>Thermal</td>
<td></td>
</tr>
<tr>
<td>Noxious cold &lt;17°C</td>
<td>Andersson et al., 2011</td>
</tr>
<tr>
<td>Chemical</td>
<td></td>
</tr>
<tr>
<td>Cannabinoids</td>
<td>Trevisani et al., 2007; Taylor-Clark et al., 2008a</td>
</tr>
<tr>
<td>4-HNE</td>
<td>Sisignano et al., 2012</td>
</tr>
<tr>
<td>5’,6’-EET</td>
<td></td>
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</tbody>
</table>

(continued)
intensity of subsequent cell signaling events (DeFea et al., 2000; Zhao et al., 2014). The specificity for initiating distinct signaling and lipid metabolic pathways may be a requirement to promote distinct physiologic outcomes such as inflammatory pain and wound healing.

**b. Bradykinin receptors.** The contributions of bradykinin (BK) receptors (B1R and B2R) to pain, inflammation, and TRP channel activation has been the subject of multiple reviews (Pethö and Reeh, 2012; Regoli et al., 2012; Blaes and Girolami, 2013). Proteases of the kallikrein-kinogen system cleave propeptides to produce painful ligands of the kinin family. Blood-derived BK is a key mediator of nociception and can be differentially processed to evoke a variety of physiologic responses via B1R and B2R. These receptors contribute to chronic diseases of pain, inflammation, neurodegeneration, and cardiovascular systems and are closely associated with the renin-angiotensin system.

BK-dependent pain transmission is predominantly attributed to the B2R, which is coexpressed by peptidergic primary afferents with TRPV1, TRPA1, TRPM8, and TRPV4. B2R couples to Gαs proteins (Gαq, Gαo, Gαi1, and Gα12/13) on DRG nerve terminals to stimulate PKA, PKC, cyclooxygenases 1/2, lipoxygenase (e.g., 5- or 12-lipoxygenase) and phospholipases (PLC and PLA2) (Liebmann et al., 1996; Wang et al., 2008; Pethö and Reeh, 2012). This leads to increased production of inflammatory prostanooids and eicosanoids and rapid stimulation of ion channels to evoke action potentials resulting in pain, inflammation, or itch. TRPV1 and TRPA1 knockout mice show diminished nocifensive behaviors when stimulated with moderate BK doses (Katanosaka et al., 2008). This is supported by neuronal studies demonstrating that BK-dependent intracellular Ca2+ transients or action potentials are partially mediated by TRPV1 or TRPA1 (Bandell et al., 2004; Bautista et al., 2005). Normal nociceptor excitability is observed when 

**2. Analgesic G Protein–Coupled Receptors.** Some of the most effective strategies for counteracting nociception may be derived from endogenous analgesic systems within the body. Tissue insult and recruitment of immune cells counteract peripheral and central pain transmission via production of endogenous opioid and somatostatin peptides and the fatty acid cannabinoids. Activation of opioid receptors on peripheral sensory neurons reduces nerve excitability and suppresses neurotransmitter or neuropeptide release. There is also evidence for the analgesic opioid receptors (μ-, κ-, δ-ORs) and cannabinoids (CB1 and CB2) receptors to function synergistically in the inhibition of inflammatory or nociceptive pain (Anand et al., 2009). The opioid, cannabinoid, and somatostatin receptors stimulate Goi/o to inhibit adenyl cyclase–dependent cAMP production, resulting in down-regulation of PKA-dependent TRP channel activity and release of βγ trimeric subunits to inhibit N-, T-, and P/Q-type Ca channels (Williams et al., 2013). Together, such processes reduce nerve excitability and presynaptic neurotransmitter release (Pinter et al., 2006; Stein and Machelska, 2011; Williams et al., 2013).

The Mas-related G protein–coupled receptor family members (Mrg receptors) also contribute to analgesia in animal models of protracted pathologic pain. These receptors are selectively expressed on small diameter neurons and contribute to processing of pain and itch responses (Dong et al., 2001; Han et al., 2013). Several lines of evidence from wild-type or Mrg knockout mice indicate that these receptors are upregulated in response to sciatic nerve injury and provide an endogenous mechanism for the inhibition of mechanical, thermal, and inflammatory pain hypersensitivity (Guan et al., 2010; He et al., 2014). MrgC receptor–dependent mechanisms of analgesia are not entirely clear but may enhance μ-opioid receptor Goi/o signaling (Wang et al., 2013).

**D. Pain Transducing Transient Receptor Potential Channels**

The discovery of TRP channels, their expression profiles, and biophysical properties have been reviewed in detail (Ramsey et al., 2006; Hardie, 2007; Nilius et al., 2007; Wu et al., 2010). The mammalian TRP channels are tetrameric, nonselective cation permeable pores that are associated with intracellular and plasma membranes. There are 28 TRP genes in mammals that are divided into six TRP channel protein families: canonical (TRPC), melastatin
### Table 2
Nociceptive, inflammatory, and pruritic pathways caused by receptor-mediated TRP channel stimulation

<table>
<thead>
<tr>
<th>Receptor (Ligand)</th>
<th>Signaling Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPV1</td>
<td>B&lt;sub&gt;1&lt;/sub&gt;, B&lt;sub&gt;2&lt;/sub&gt; (bradykinin)</td>
<td>PKC, 12-LOX</td>
</tr>
<tr>
<td></td>
<td>H&lt;sub&gt;1&lt;/sub&gt;R, H&lt;sub&gt;2&lt;/sub&gt;R (histamine)</td>
<td>PLA&lt;sub&gt;2&lt;/sub&gt;, 12-LOX, PLC/PKC</td>
</tr>
<tr>
<td></td>
<td>PAR&lt;sub&gt;2&lt;/sub&gt; (trypsin, PAR&lt;sub&gt;2&lt;/sub&gt;-activating peptide)</td>
<td>PLC, PKA, PKC, PKD</td>
</tr>
<tr>
<td></td>
<td>NPR-C (atriuretic peptide C)</td>
<td>β&lt;sub&gt;y&lt;/sub&gt; G proteins/ PLCβ/PKC</td>
</tr>
<tr>
<td></td>
<td>Prokineticin PK1, PK2 (B&lt;sub&gt;0&lt;/sub&gt;v&lt;sub&gt;8&lt;/sub&gt;)</td>
<td>PKC, PKC, PI3K, p38, B2</td>
</tr>
<tr>
<td></td>
<td>TrkA (NGF)</td>
<td>β-Arrestin</td>
</tr>
<tr>
<td></td>
<td>μ-Opioid receptor (morphine, DAMGO)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>MrgA3 (chloroquine)</td>
<td>PLCβ, PKC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PKC, PKA, and AKAP</td>
</tr>
<tr>
<td></td>
<td>5-HT receptor (5-HT&lt;sub&gt;2&lt;/sub&gt;, 5-HT&lt;sub&gt;3&lt;/sub&gt;, 5-HT&lt;sub&gt;5&lt;/sub&gt;)</td>
<td>PKC, PKA, and AKAP</td>
</tr>
<tr>
<td></td>
<td>Prokineticin receptor (prolactin/estriadiol)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>TrkA (NGF), Ret/GFRα (GNDF), Mrg receptor</td>
<td>—</td>
</tr>
<tr>
<td>iplesK, phosphoinositide 3-kinase; PKD, protein kinase D.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
that nonselectively conduct cations (Ca\textsuperscript{2+}, Na\textsuperscript{+}), leading to rapid changes in intracellular cation concentrations. On sensory neurons, sustained ion flux regulates Ca\textsuperscript{2+}-sensitive proteins and cellular events and the stimulation of other voltage-gated channels to initiate generator potentials, neuronal excitation, and central transmission of sensory information. They possess several common features: 6 transmembrane domains, a pore-forming channel gating region between the 5th and 6th transmembrane domains, and intracellular N and C termini (Fig. 3). In the TRPV, TRPM, and TRPC families, the intracellular end of the 6th transmembrane domain participates in formation of the “lower gate” of the pore and contains a highly conserved helical sequence known as the “TRP domain,” which has been proposed to regulate channel tetramerization and interactions with phospholipids (Clapham, 2003). Highly structured ankyrin repeats at the N terminus participate in ligand binding, protein-protein interactions, and tetramerization (Lishko et al., 2007). Recently, the structure of TRPV1 was solved to 3.4 Å by electron cryomicroscopy and confirms these prior assessments (Liao et al., 2013) (see secton IIIA).

TRP channels can bind many endogenous lipids and exogenous natural or synthetic compounds. They are targets for kinases, signaling adaptors, such as the A-kinase anchoring protein (AKAP; described below), and trafficking proteins. The capacity of TRP channels to sense endogenous and environmental stimuli and to regulate ion fluxes illustrates their important roles as cellular sensors and signaling effectors. An extensive list of the thermal, mechanical, chemical, and signaling stimuli that can control TRP channel activity is provided in Table 1. Key features of proinflammatory, pruritogenic, or nociceptive TRP channels are summarized below.

1. Transient Receptor Potential Vanilloid 1. The vanilloid receptor subtype 1 or TRPV1 was the first mammalian TRP vanilloid ion channel to be cloned (Caterina et al., 1997). Small diameter, unmyelinated C-fibers and lightly myelinated Aδ fibers are major cellular sites of TRPV1 expression (Cavanaugh et al., 2008). Trpv1<sup>−/−</sup> mice respond poorly to noxious heat, acid, and vanilloid-evoked pain, and lack the ability to promote tissue swelling, neuropeptide release, and cytokine release after inflammation, illustrating the key role of this channel in nociception and neurogenic inflammation (Caterina et al., 2000; Keeble et al., 2005). TRPV1 also contributes to painful disorders, including diabetic-induced neuropathic pain, cancer pain, and inflammatory pain (Alawi and Keeble, 2010). Lipid derivatives that can potentiate or stimulate TRPV1 activity include prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) (Moriyama et al., 2005) and numerous polyunsaturated fatty acids (Matta et al., 2007). The arachidonic fatty acid derivative and CB\textsubscript{1} receptor agonist anandamide is a well studied example that functionally inhibits neuronal activation at low concentrations and activates TRPV1 at higher concentrations (Tognetto et al., 2001). Complementary studies on capsaicin-induced release of the painful neuropeptide substance P into rat spinal cords reveal that similar effects can be observed with pharmacological inhibition of CB\textsubscript{1} (Lever and Malcangio, 2002). Many GPCRs sensitize or activate TRPV1, including receptors for proteases, serotonin, histamine, tachykinins, and bradykinins (see Tables 1 and 2). However, not all GPCRs signal through the same cascades and the presence of auxiliary TRPV1-interacting scaffolding proteins, such as AKAP150 and arrestins, can potentially influence tissue-specific outcomes (see section III.D) (Schnizler et al., 2008; Jeske et al., 2009; Zhang et al., 2008).

2. Transient Receptor Potential Vanilloid 2. TRPV2 shares 50% sequence homology with TRPV1, is activated by high heat thresholds (52°C), and is expressed on small, medium, or large diameter primary afferent neurons, motor neurons of the spinal cord, and in osmosensing regions of the hypothalamus (Caterina et al., 1999; Lewinter et al., 2008; Nedungadi et al., 2012). TRPV2 responds to stretch, lysophospholipids, and cannabinoids, with the most potent being the nonpsychotropic compound cannabidiol. Activation of TRPV2 stimulates CGRP release from DRG sensory neurons, indicating a pronociceptive and proinflammatory role (Qin et al., 2008). More recently TRPV2 has also been implicated in nitric oxide production for the control of intestinal motility (Mihara et al., 2013).

3. Transient Receptor Potential Vanilloid 3. TRPV3 is a warm-sensing channel that is expressed by TRPV1-positive afferent neurons and in the skin, brain, colon, and epithelia of the nose, tongue, and cornea, where it contributes to sensory, nociceptive, inflammatory, and pruritic pathways (Xu et al., 2002; Moussaieff et al., 2008; Ueda et al., 2009; Nilius et al., 2014). A TRPV3-dependent Ca\textsuperscript{2+} flux is essential for skin barrier formation, the release of interleukin and PGE\textsubscript{2}, and nitric oxide production. Uniquely, TRPV3 is potentiated by repeated exposure to stimulating stimuli like warm temperatures (Peier et al., 2002b). Sensitization of TRPV3 by GPCR-mediated signaling has been demonstrated by stimulation of M1 acetylcholine receptor (in a heterologous expression system) and reveals the sensitization of TRPV3 activity occurs through PI\textsubscript{3} depletion and Ca\textsuperscript{2+} sensitivity (Doerner et al., 2011) and inflammatory...
fatty acids, such as AA, but not through lipoxygenase and epoxygenase-derived products of AA metabolism (Hu et al., 2006). PGE$_2$ can also sensitize TRPV3 responses in the skin to cause peripheral pain (Huang et al., 2008). TRPV3 is involved in cutaneous thermosensation and can convey sensory information from keratinocytes to sensory neurons via an ATP-release mechanism (Moqrich et al., 2005; Mandadi et al., 2009). TRPV3 also contributes to pruritus, atomic dermatitis, and loss of wound healing in dysregulated systems and is involved in epidermal growth factor receptor signaling and transglutaminase activity, which are essential for epidermal barrier integrity and defense (Asakawa et al., 2006; Xu et al., 2006; Imura et al., 2007; Cheng et al., 2010; Miyamoto et al., 2011; Nilius and Biro, 2013).

4. Transient Receptor Potential Vanilloid 4. TRPV4 is expressed by A$\delta$- and C-fibers and by nonneuronal cells (e.g., epithelia, endothelia, and skin) and is sensitive to membrane stretch or hypotonic conditions (Alessandri-Haber et al., 2003; Suzuki et al., 2003b; Hartmanngruber et al., 2007). Studies using selective TRPV4 agonists/antagonists and of trpv4$^{-/-}$ mice have demonstrated an important role for TRPV4 in thermal and mechanical hyperalgesia and in chronic inflammatory conditions, such as colitis, through transcriptional regulation and the release of cytokines, SP, and CGRP (Brierley et al., 2008; D’Aldebert et al., 2011). TRPV4 also regulates vasodilatation, ciliary beating frequency, wound healing, skin barrier formation, and bladder voiding (Liedtke and Friedman, 2003; Todaka et al., 2004; Alessandri-Haber et al., 2006; Lorenzo et al., 2008; Earley et al., 2009). TRPV4 activity is modulated by many GPCRs, including those for proteases, histamine, serotonin, and acetylcholine (Tables 1 and 2). GPCR-dependent phosphorylation is an essential mechanism for TRPV4 sensitization, yet TRPV4 is also highly sensitive to endogenous inflammatory lipid mediators, including anandamide and AA metabolites (e.g., 5,6-EET or 11,12-EET) (Watanabe et al., 2003; Earley et al., 2003). TRPV4 is also a key integrator of many signaling molecules. It can associate with PKA and PKC, which requires the scaffold A$\delta$-kinase anchoring protein AKAP79 (Fan et al., 2009), and interacts with Src kinase, integrins, cytoskeletal proteins, tight junction, and adaptor proteins (Suzuki et al., 2003a; Wegierski et al., 2006; Alessandri-Haber et al., 2008; Becker et al., 2009; Fan et al., 2009; Akazawa et al., 2013). Members of the PKC and CKII substrate in neurons adaptor protein family (PACSIN; also known as Syndapin 1) bind to an N-terminal proline-rich region of TRPV4 to regulate channel internalization and responses to hypotonicity (Fig. 3D) (Cuajungco et al., 2006; D’Hoedt et al., 2008). TRPV4 activity contributes to pain and proinflammatory processes through upregulation of edema (Vergnolle et al., 2010); epidermal, epithelial and endothelial cell permeability (Reiter et al., 2006; Willette et al., 2008; Sokabe et al., 2010); and chemokine/cytokine production (D’Aldebert et al., 2011). Thus, cell-specific auxiliary proteins that regulate the intracellular localization and function of TRPV4 are recognized as potential therapeutic targets for the regulation of TRPV4 in its capacity as a proinflammatory effector molecule (Garcia-Elias et al., 2013).

5. Transient Receptor Potential MelaStatin 2. The eight members of the melastatin subfamily have low-level sequence homology. They are grouped according to their lack of N-terminal ankyrin repeats, and three of the members possess an extended C-terminal tail that contains an active kinase domain (TRPM6 and TRPM7) or ADP ribose-binding phosphohydrolase homology domains (TRPM2). These channels have diverse roles in cold sensation, neuronal development, taste reception, T-cell regulation, glucose-induced insulin release, and regulation of mast and glial cells (Wu et al., 2010). TRPM2, TRPM3, and TRPM8 contribute to pain processing. TRPM2 is expressed in central and peripheral neurons, microglia, and neutrophils and is sensitive to oxidative or nitrosative stress, intracellular Ca$^{2+}$, and acidity (Kraft et al., 2004; Lange et al., 2008; Du et al., 2009; Naziroglu et al., 2011). Trpm2$^{-/-}$ mice have normal thermal or mechanical thresholds yet possess reduced nociceptive responses in inflammatory or neuropathic pain, most likely via TRPM2-dependent chemokine release from infiltrating peripheral macrophages, neutrophils, and spinal microglia (Yamamoto et al., 2008; Haraguchi et al., 2012; Isami et al., 2013).

6. Transient Receptor Potential Melastatin 3. TRPM3 has diverse roles in sensation and insulin secretion and is expressed in the kidney, testes, brain, and spinal cord (Lee et al., 2003). TRPM3 is also expressed on sensory neurons of DRG and TG, and trpm3$^{-/-}$ mice show deficiencies in heat sensation and development of inflammatory heat hyperalgesia (Vriens et al., 2011). Intriguingly, the cation permeation pathway in TRPM3 may function independently of the canonical central ion pore, allowing Na$^+$ influx and sensory neuronal excitation even after pharmacological inhibition of the central pore (Vriens et al., 2014). It remains to be determined if other TRP channels possess a similar secondary ion permeation pathway and how passage of both Ca$^{2+}$ and Na$^+$ may influence neuronal excitatory responses and other channels in a neuropathic pain setting. The potential for TRPM3 to be sensitized by GPCR signaling has been demonstrated with carbamol-induced muscarinic receptor signaling and sensitivity to Ca$^{2+}$ store depletion by thapsigargin (Lee et al., 2003). TRPM3 has also been observed to interact with Ca$^{2+}$-sensitive proteins CaM and S100b, which are proposed to compete for a PIP$_2$ binding site in the N terminus, yet the functional consequences of this remain unclear (Holendova et al., 2012).

7. Transient Receptor Potential Melastatin 8. TRPM8 is a well known thermosensitive ion channel for cold temperatures, responding within 8–28°C, and
also to cooling agents, such as menthol and icilin (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007; Gavva et al., 2008). Initially shown to be expressed in small-diameter TrkA-positive sensory neurons (Peier et al., 2002a), tissue analyses in mice engineered to express a green fluorescent protein reporter from the TRPM8 locus reveal channel expression in a unique population of neurons that do not express typical nociceptive markers such as IB4 or TRPV1 (Dhaka et al., 2008). This is in contrast to ~30–50% of acutely dissociated or cultured TRPM8-positive neurons that also functionally express TRPV1, suggesting species differences or artifacts related to neuronal isolation and culturing (Babes et al., 2004; Okazawa et al., 2004). Assessment of TRPM8 to neuronal isolation and culturing (Babes et al., 2004; Babes et al., 2007; Ramachandran et al., 2013; Shapovalov et al., 2013; Than et al., 2013). The use of selective antagonists have confirmed the involvement of TRPM8 in thermoregulation and augmented nerve excitability through voltage-gated Na⁺ channels (Gavva et al., 2012; Sarria et al., 2012). Furthermore, TRPM8 is an integrator of cell signaling pathways: it can bind G proteins and therefore play a role in initiating signaling pathways through mechanisms that are yet to be completely understood (Klasen et al., 2012). The regulation of TRPM8 by G protein signaling pathways involves typical PIP₂/phospholipase activity and noncanonical pathways. The PIP₂-binding protein Pirt (PIP-binding regulator of TRP channels) interacts with TRPM8 (and can also bind TRPV1), and the expression of Pirt enhances thermosensitivity (Tang et al., 2013). Several lines of evidence suggest that TRPM8 functions as an anti-inflammatory TRP channel. TRPM8 exerts an anti-inflammatory activity in the mouse colon via inhibition of neuropeptide release (Ramachandran et al., 2013), and bradykinin or histamine-induced inflammatory signaling can also lead to direct TRPM8 interactions with the Go₃q subunit, resulting in TRPM8 inhibition (Zhang et al., 2012; Li and Zhang, 2013).

8. Transient Receptor Potential Ankyrin 1. TRPA1 possesses 17 N-terminal ankyrin repeats (in human) and is sensitive to a wide range of noxious stimuli (Corey et al., 2004). It is expressed by TrkA- and TRPV1-positive C-fibers, and although TRPA1 sensitivity to noxious cold and mechanical stress has been debated (McKemy, 2005), functional studies and a familial TRPA1 gain-of-function mutation has associated TRPA1 currents with cold and chemical nociception (e.g., <16°C) or environmental/endogenous irritants (Kwan et al., 2006; Kremeyer et al., 2010). The N terminus contains key regulatory regions in the linker region between the ankyrin repeats and first transmembrane domain, including conserved N-terminal cysteine residues (Doerner et al., 2007). These sites are essential for TRPA1 to “sense” and covalently bind painful molecules that possess electrophilic chemical groups, such as noxious irritants in airways (cigarette smoke, tear gas), pungent chemicals in the digestive system (allicin from garlic, allyl isothiocyanate from mustard oil, cinnamaldehyde from cinnamon) and reactive oxygen species released by damaged tissue (Tables 1 and 2) (Bautista et al., 2006; Macpherson et al., 2007; Andre et al., 2008; Bron et al., 2008; Bessac et al., 2009; Taylor-Clark et al., 2009; Cordero-Morales et al., 2011). TRPA1 is highly sensitive to inflammatory fatty acids and highly reactive thiol-reactive species, such as 4-hydroxynonenol (4-HNE), prostaglandin metabolites [15-dPGJ(2), PGA(2), and PGA(1)], and hydrogen peroxide (Trevisani et al., 2007; Andersson et al., 2008; Materazzi et al., 2008). The role of TRPA1 in inflammatory pain was recently discussed in detail (Bautista et al., 2013). It is a key integrator of receptor signaling by all major painful and inflammatory GPCRs (proteases, bradykinin, prostaglandins, ATP, or histamine) to promote pain, plasma extravasation and edema, itch, and vasodilatation, and although multiple kinases sensitize TRPA1 channel gating, the phosphorylation status of TRPA1 is not well characterized (Bandell et al., 2004; Chen et al., 2011; Wilson et al., 2011; Brederson et al., 2013). Animal studies have linked TRPA1 activity to mechanical hyperalgesia, visceral pain, inflammatory bowel disease, and respiratory disease (Engel et al., 2011; Sisignano et al., 2012; Hughes et al., 2013; Kondo et al., 2013; Lin et al., 2013).

9. Transient Receptor Potential Canonical. The TRP canonical (TRPC) subfamily is divided into three subgroups by sequence homology and functional similarities: C1/C4/C5 and C3/C6/C7, with the rat pheromone signaling receptor TRPC2 being classed as a pseudogene in humans (Clapham, 2003). Analogous to the Drosophila phototransduction pathway, TRPC channels are receptor-operated channels that respond to GPCR signaling, receptor tyrosine kinases, and intracellular Ca²⁺ (Clapham, 2003). The Go₃q/PLC-dependent signaling and generation of DAG and IP₃ are proposed to be the major mechanism for activation of TRPC gating (Schaefer et al., 2000). Several TRPC members are implicated in neuropathic pain and inflammatory processes. TRPC1 and TRPC6 are “stretch-activated ion channels” that frequently share expression with TRPV4 on small and medium diameter sensory neurons (Alessandri-Haber et al., 2009). Both channels are proposed to function synergistically with TRPV4 to contribute to inflammation-induced mechanical hyperalgesia in mice. TRPC6 is also associated with wound healing and edema caused by dysfunction of endothelial cells of the lung (Davis et al., 2012; Weissmann et al., 2012). The stretch-activated ion channel TRPC5 is highly sensitive to intracellular Ca²⁺ ion concentrations and temperatures below 37°C and may contribute to cold sensitivity or allodynia in the temperature range not processed by TRPM8 or TRPA1 (Zimmermann et al., 2011). Stimulation
of Gαi/o-coupled signaling through M2 muscarinic, 5-HT1A serotonin, and μ-opioid receptors leads to direct activation of TRPC4 or TRPC5 channels (Miller et al., 2011; Jeon et al., 2012). Further studies may elucidate important functional roles for TRPC channels in itch sensation and analgesia.

III. Molecular Substates for the G Protein–Coupled Receptor–Transient Receptor Potential Channel Axis: Regulation of Transient Receptor Potential Channels by Lipid and Protein Interactions

The interaction between TRP channels, signaling proteins, and second messengers determines channel gating or cellular localization and can influence pain transmission. Hence, the intracellular N- and C-terminal regions of TRP channels are key regulatory domains that are essential for function (Phelps and Gaudet, 2007). Although some of these interactions are tissue or TRP specific, conservation of regions in the C terminus of most TRP channel family members favors interactions with PIP2 and CaM. However, new protein binding partners (e.g., AKAP) and lipid metabolites (e.g., anionic long chain acyl CoA esters) continue to be discovered (Jeske et al., 2009; Yu et al., 2014). The characterization of these lipid and protein interactions provides information about key domains that are important for TRP channel gating and may therefore be therapeutically “tuned” to avoid noxious sensory nerve excitation and inflammation in dysregulated systems. Recent advances in our understanding of TRP structure have provided information about these regulatory domains and given insight into tetrameric subunit assembly and channel gating. These processes are essential for understanding how GPCR modulation of TRP channels may occur.

A. Transient Receptor Potential Channel Structure: Improving Our Understanding of Protein Interactions

Cryoelectron microscopy has provided greater understanding of the molecular architecture of the TRP homotetramers, such as TRPV4, and has revealed that cytoplasmic regions of a TRP channel form a large basket-like domain, which is proposed to influence assembly, channel gating, and protein-protein interactions (Shigematsu et al., 2010). Advanced electron cryomicroscopy has provided the high-resolution (3.4 Å) structure of TRPV1 (Fig. 3) (Cao et al., 2013b; Liao et al., 2013). These data are consistent with X-ray crystallography studies on the isolated vanilloid TRPV1 ankyrin domain, which enabled characterization of interactions with CaM and identified ATP as a novel TRP channel ligand (Jin et al., 2006; Lishko et al., 2007; Phelps and Gaudet, 2007; Inada et al., 2012). The latest structural studies of TRPV1 provide insights into subunit assembly and reveal that a β-sheet is formed between C- and N-terminal domains (Liao et al., 2013). Specifically, the linker region between the N-terminal ankyrin domain and the first transmembrane domain associates with a single β-strand within the C terminus (Fig. 3B). The linker region is in close proximity to the ankyrin repeats, which enhances association between N and C termini and provides contacts for tetrameric subunit assembly (Liao et al., 2013). This interaction may explain biochemical studies in which PIP2 or protein interactions are observed to occur on both intracellular tails of a TRP subunit (Lau et al., 2012; Garcia-Elias et al., 2013). The TRPV1 structure is further supported by the first structure of TRPV1 bound to a peptide ligand (extracellular spider toxin) or vanilloid-based ligand (ultrapotent resiniferatoxin) to demonstrate the adaptation of TRPV1 to ligand-specific conformations and may provide clues for ion permeability through the channel pore (Cao et al., 2013b). These data also provide an enhanced 3-dimensional prototype for understanding how proteins and other ligands interact with all TRP channels. TRPV1 may be used as a structural template to build a TRP channel “interactome,” to provide new perspectives on TRP channel regulation. Figure 3 highlights distinct and overlapping binding sites and key regulatory regions of TRPV1, TRPV4, TRPA1, and TRPM8. Furthermore, structural information provides us with an understanding of phosphorylation and how proinflammatory mediators, pruritogens, and receptor signaling may influence these processes.

B. Calmodulin

CaM participates in TRP channel desensitization by binding to N-terminal ankyrin repeats and the C-terminal tail of TRPs. With respect to pain-related pathways, Ca2+-dependent CaM interacts with TRPV1-4, TRPM2, and TRPM8, predominantly to facilitate channel desensitization (Numazaki et al., 2003; Rosenbaum et al., 2004; Lishko et al., 2007; Sarria et al., 2011). The role of Ca2+ and CaM in TRP channel regulation has been reviewed (Zhu, 2005) and demonstrates how all GPCRs that signal though PLC pathways have the potential to influence TRP channel gating.

Ca2+-CaM and intracellular ATP compete for a common binding site to influence channel-gating properties. Although the mechanism remains unknown, ATP binding is proposed to prevent Ca2+-CaM desensitization (Phelps et al., 2010). The high-resolution crystal structure Ca2+-CaM bound to a C-terminal fragment of TRPV1 reveals how the two Ca2+-bound lobes of CaM clasp the helical TRPV1 peptide to form a tight interaction. In contrast, interactions between CaM and the N-terminal ankyrin repeats are predicted to occur with lower affinity yet promote greater desensitization of TRPV1 (Lau et al., 2012). The high-affinity binding with the C terminus may be important for coordinating TRP-CaM docking to facilitate interactions with the N-terminal ankyrin site for optimal TRP channel desensitization (Lau et al., 2012).
C. Phospholipids

Phosphoinositide lipids, such asPIP₂, are essential intermediates of the GPCR-TRP channel axis and are known to modulate the activity of more than 30 channels (Suh and Hille, 2005). PIP₂ is present in limiting quantities in the phospholipid bilayer and binds at distinct sites within the cytoplasmic tail of TRPV channels (Fig. 3, C and D). In TRPV1, these sites overlap with binding regions for the Ca²⁺-sensitive CaM, which can exert inhibitory effects by competing with PIP₂ (Fig. 3) (Brauchi et al., 2007; Garcia-Elias et al., 2013). For TRPV4, PIP₂ interactions within the N-terminal ankyrin region are proposed to “rearrange” cytoplasmic domains to either facilitate channel gating or expose binding sites for endogenous inflammatory lipids, such as eicosanoids (Garcia-Elias et al., 2013). Multiple signaling proteins contribute to somatosensation by modulating PIP₂-mediated TRP regulation. PLC-dependent breakdown of PIP₂ leads to increased intracellular Ca²⁺ and production of DAG, leading to PKC stimulation and TRP phosphorylation. Furthermore, the accessory protein Pirt indirectly regulates PIP₂-dependent TRPV1 activity, and phosphoinositide 3-kinase–dependent phosphorylation of PIP₂ produces PI(3,4,5)P₃ to enhance TRPV1 cell surface expression and extracellular signal-regulated kinase (ERK)–dependent thermal pain (Zhuang et al., 2004; Zhang et al., 2005; Stein et al., 2006; Zhu and Oxford, 2007; Hernandez et al., 2008; Kim et al., 2008; Ufret-Vincenty et al., 2011).

Although ion channels appear to nonselectively bind PIP₂ over other PI species, its precise regulatory role continues to be debated (Chuang et al., 2001; Prescott and Julius, 2003; Hilgemann, 2012; Cao et al., 2013a). PIP₂ interactions with TRP channels regulate their function through PLC-mediated breakdown of PIP₂, which relieves TRP channel inhibition, leading to heat/capsaicin-induced pain. Hence, TRP channel sensitization occurs as a consequence of PIP₂ turnover and generation of phosphoinositide-derived second messengers, such as AA (Huang et al., 2010; Cao et al., 2013a). A new model has been proposed to explain how PLC isofoms mediate bradykinin-dependent TRPV1 sensitization: submaximal...
PLCβ activity (and PKC phosphorylation) sensitizes TRPV1, whereas increased TRPV1 Ca²⁺ influx activates Ca²⁺-sensitive PLC8 isoforms to decrease PIP2 levels and serve as a negative feedback mechanism (Lukacs et al., 2013).

TRP channel interaction with PIP2 is one example of how receptors, phospholipases, and lipids can influence neuronal excitability. Anandamide (arachidonylethanolamide), AA, and its metabolites constitute a large group of endogenous TRP channel ligands that contribute to pain and inflammation (Zygmun and Adams, 1999). The role of AA and other fatty acid derivatives, such as eicosanoids, in TRP channel biology has been extensively studied (Hardie, 2003; Watanabe et al., 2003; Kahn-Kirby et al., 2004; Hu et al., 2006; Matta et al., 2007; Meves, 2008; Pethö and Reeh, 2012). Their roles in pathophysiologic settings are also discussed below.

D. Kinase Scaffold Proteins

1. A-Kinase Anchoring Protein and Receptor for Activated C-Kinase 1. Receptor-dependent control of TRP channel activity requires channel modification by phosphorylation and the coordinated activities of kinases and phosphatases. AKAP proteins (AKAP79 in human/ AKAP150 in rodents) have a central role in receptor-mediated TRP channel activity by facilitating associations between kinases, phosphatases, and phosphodiesterases to arrange functionally and spatially distinct pools of signaling molecules, including PKA, PKC, and cAMP (Houslay and Adams, 2003). The PGE₂-sensitive EP receptor stimulates Ga₅ pathways (Malmberg et al., 1997), and AKAPs have been confirmed as crucial scaffolding intermediates for PGE₂-stimulated TRPV1-mediated hyperalgesia (Rathee et al., 2002; Zhang et al., 2008). AKAP150 knockout mice and AKAP150 siRNA knockdown reveal how AKAP scaffolding participates in receptor-mediated nociception by orientating PKA, PKC, and the phosphatase PP2B toward TRPV1. This process mediates PKA- and PKC-dependent sensitization of TRPV1 responses to heat, capsaicin, and GPCR-dependent bradykinin and PGE₂ stimulation (Jeske et al., 2008, 2009; Schnizler et al., 2008; Zhang et al., 2008). TRPV4 phosphorylation by PKA/PKC is also enhanced by AKAP79 expression to promote channel sensitization to hypertonic cell swelling and bradykinin (Fan et al., 2009).

TRP channel interactions with these adaptor proteins can also provide negative feedback mechanisms to facilitate TRP channel desensitization. Phosphodiesterase PDE4D5 activity downregulates cAMP levels, and for the β adrenoreceptor, this regulates switching between G protein signaling pathways (Bailie et al., 2003). The PKC scaffold receptor for activated C-kinase 1 coordinates PKC and the PDE4D5 protein to regulate TRPC3 and TRPM6 activity by regulating PKC and PKA activity (Bandyopadhyay et al., 2008; Cao et al., 2008). Interactions between receptor for activated C-kinase 1 and other nociceptive TRP channels are yet to be determined.

2. β-Arrestins as Adaptors for G Protein–Coupled Receptors and Transient Receptor Potential Potential Channels. The four members of the arrestin protein family include arrestin-1 and -4 of visual sensory tissue and the nonvisual ubiquitously expressed arrestin-2 and -3, otherwise known as β-arrestin-1 and β-arrestin-2. Initially named for their capacity to prevent continued signaling of β-adrenergic receptors, β-arrestins are now known to regulate multiple GPCRs and TRP channels in the pain pathway (Rowan et al., 2014). The association of β-arrestins with GPCRs is facilitated by receptor phosphorylation, where agonist-occupied GPCRs are phosphorylated by a family of G protein–coupled receptor kinases (GRKs), in addition to PKC and PKA, which enhances association with β-arrestins. β-Arrestins uncouple GPCRs from heterotrimeric G proteins and desensitize the cell surface component of signal transduction through recruitment of GPCRs to clathrin and other endocytic adaptor proteins, to mediate receptor endocytosis. Internalized GPCRs then either recycle to the cell surface for reactivation (e.g., neurokinin 1 receptor [NK₁R] in the spinal cord) or are degraded in lysosomes (e.g., PAR₂ in sensory neurons; reviewed by Murphy et al., 2009, and Shenoy and Lefkowitz, 2011). Rapid and specific receptor phosphorylation events promote different outcomes of G protein signaling, β-arrestin recruitment, and GPCR trafficking. Highly phosphorylated receptors, such as PAR₂ and NK₁R, strongly associate with β-arrestin-1 and -2, resulting in rapid and robust internalization and sequestration into endosomal pools (Cattaruzza et al., 2013; Pal et al., 2013).

The contribution of GPCR phosphorylation and trafficking to complex pathophysiologic processes, such as inflammatory pain, are poorly understood. However, it is established that endosomes are not merely a sorting station for delivery of receptors to recycling or degradative pathways but exist as platforms for receptors to signal from intracellular locations (Murphy et al., 2009). Arrestins are crucial in this process. In the case of NK₁R trafficking, β-arrestin affords NK₁R an extended residence in the endosomes and serves as a scaffold for ERK1/2 and Src kinases, which may promote pain-transmitting signaling cascades.

Although most studies of β-arrestins have examined their interactions with GPCRs, β-arrestins can also associate with TRP channels to directly regulate channel activity. At the cell surface of sensory neurons, phosphorylation within the TRPV1 N terminus (Ser¹¹⁶ and Thr³⁷⁰) enhances channel activity and reduces pharmacological desensitization. Two additional PKA phosphorylation sites (Thr³⁷⁰ and Thr³⁸⁵) in the linker region (immediately downstream of the N-terminal ankyrin repeat domain) are proposed to increase electrostatic interactions between TRPV1 and β-arrestin-2, providing a scaffold for PDE4D5, resulting in reduced cAMP and...
IV. The G Protein-Coupled Receptor–Transient Receptor Potential Channel Axis in Visceral Pathways

GPCRs and TRP channels are essential for all gastrointestinal functions. GPCRs mediate the actions of many gastrointestinal hormones and neurotransmitters, and GPCRs and TRP channels participate in chemosensation, regulate motility, secretion, and homeostasis and contribute to visceral sensation. Dysregulation of these functions, for example, with respect to the activation properties and expression of TRP channels in visceral afferents, contribute to visceral pain and inflammation. In addition to activation by environmental stimuli and endogenous mediators, a wide variety of pungent substances commonly associated with spices are also potent TRP channel agonists, and traditional treatments for gut-related disorders often contain TRP channel activators as active ingredients (Vriens et al., 2008; reviewed by Holzer, 2011). This section provides an overview of the sensitization and function of neuronal TRP channels in the etiology of digestive disease, with a particular emphasis on pain and neurogenic inflammation.

A. Transient Receptor Potential Channels in Inflammatory Bowel Disease and Functional Bowel Disorders

Visceral pain caused by inflammation, obstruction, or functional disorders is the most common form of disease-derived pain and is often poorly managed therapeutically. The extrinsic innervation of viscera, particularly the gastrointestinal tract, has been the subject of several recent reviews (Robinson and Gebhart, 2008; Blackshaw et al., 2010; Brookes et al., 2013). The expression, activation, and sensitization of TRP channels expressed by visceral afferents are perhaps best characterized for extrinsic sensory neurons innervating the colon. Studies on knockout mice or the use of TRP-selective agents have demonstrated that TRP channels play integral roles in both the development and maintenance of colitis, as well as being major mediators of colonic afferent hypersensitivity. TRP channels are also upregulated in colonic afferents of animals with inflammatory bowel disease (IBD). Most clinical studies have focused on TRPV1, and there is an increased density of TRPV1-positive fibers innervating the colonic mucosa in both IBD and functional bowel disorders, such as irritable bowel syndrome (IBS). These changes have been positively correlated to visceral pain scores.

1. Sensitization of Transient Receptor Potential Channels: Mechanisms and Role in Colonic Hypersensitivity

The factors that lead to abdominal pain, such as occurs in IBS and IBD, are poorly understood. IBS-related pain is often associated with a past infection and inflammation, and sensory afferents may exhibit hyperexcitability long after the initial inflammation is resolved (Collins, 2001; Beyak et al., 2004; Ibeakanma et al., 2009). In visceral afferents of the colon, sensitization of TRPV1, TRPV4, and TRPA1 by GPCR agonists, inflammatory changes, and exposure of nerve terminals to acid has been reported. These pathways are illustrated in Fig. 4. The sensitization of visceral afferents, particularly after exposure to GPCR agonists, is a major mechanism through which postinflammatory visceral pain is initiated and maintained. Evidence for functional expression of TRPV1, TRPV4, TRPA1, and TRPM8 by colonic afferent neurons is provided by studies demonstrating mRNA and protein expression and by Ca2+ imaging and electrophysiologic studies that show responsiveness by these neurons to established TRP channel activators (Christianson et al., 2007, 2010; Brierley et al., 2008, 2009; De Schepper et al., 2008; Tan et al., 2008; Harrington et al., 2011).

TRP channel sensitization through activation of GPCRs has been demonstrated by a number of studies. Peripheral sensitization of colonic afferents to stretch stimulation occurs after exposure to an acidic inflammatory soup containing bradykinin, 5-HT, histamine, and PGE2 (Jones et al., 2005). Diminished afferent hypersensitivity in trpv1−/− mice demonstrates that this sensitization is partly mediated via TRPV1 and is consistent with the use of an H2 histamine receptor antagonist to reduce capsaicin-sensitive neurogenic inflammation of the colon (Okayama et al., 2004). Activation of TRPV1 by capsaicin, heat, or by protons is also augmented after pre-exposure of colonic sensory neurons to 5-HT and involves a G protein–dependent PKA/AKAP mechanism (Sugiuar et al., 2004). 5-HT2 and 5-HT4 reduce the temperature threshold for neuronal firing, an effect that is lost in neurons from trpv1−/− mice.
and requires PKC activity. Pre-exposure to the proinflammatory mediators 5-HT or histamine enhances intracellular Ca²⁺ responses of colonic afferent neurons to the TRPV4 agonist 4α-PDD, a response that is attenuated by intrathecal delivery of TRPV4 siRNA (Cenac et al., 2010). The underlying sensitization mechanism involves PLC, PLA₂, PKC, and mitogen-activated kinase, and imaging studies revealed that 5-HT and histamine treatment promoted increased cell surface expression of TRPV4 in neurons. In an analogous system, mice lacking TRPA1 show deficiencies in early responses to bradykinin-induced colonic afferent mechanosensitivity (Brierley et al., 2005, 2009; Wang et al., 2008).

Growth factors, including NGF and glial-derived neurotrophic factor, are also upregulated in IBD and in animal models of colitis (Christianson et al., 2010). Acute exposure of colonic afferent neurons to NGF potentiates TRPV1- and TRPA1-evoked Ca²⁺ signaling in colonic afferents, suggesting an important role for TrkA receptor signaling in TRP channel activity, potentially due to increased TRP channel surface expression of TRPV4 in neurons. In an analogous system, mice lacking TRPA1 show deficiencies in early responses to bradykinin-induced colonic afferent mechanosensitivity (Brierley et al., 2005, 2009; Wang et al., 2008).

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TRP channels are major mediators of inflammatory bowel disease and visceral pain. The expression of TRP channels within the colon and by colonic extrinsic primary afferent neurons is significantly increased in both IBD and IBS. The activation of TRP channels expressed by primary afferents leads to the peripheral release of proinflammatory neuropeptides (SP, CGRP), resulting in neurogenic inflammation. The release of these neuropeptides at the level of the spinal dorsal horn results in pronociceptive signaling. Agonists of cell surface receptors, including GPCRs, are released during tissue damage or inflammation. The activation of these receptors leads to the sensitization or recruitment of TRP channels (see examples shown). This effectively augments TRP channel activity in these neurons, resulting in enhanced neuropeptide release and to greater inflammation and pain. Modified from Furness et al., 2013.

Fig. 4. TRP channels are major mediators of inflammatory bowel disease and visceral pain. The expression of TRP channels within the colon and by colonic extrinsic primary afferent neurons is significantly increased in both IBD and IBS. The activation of TRP channels expressed by primary afferents leads to the peripheral release of proinflammatory neuropeptides (SP, CGRP), resulting in neurogenic inflammation. The release of these neuropeptides at the level of the spinal dorsal horn results in pronociceptive signaling. Agonists of cell surface receptors, including GPCRs, are released during tissue damage or inflammation. The activation of these receptors leads to the sensitization or recruitment of TRP channels (see examples shown). This effectively augments TRP channel activity in these neurons, resulting in enhanced neuropeptide release and to greater inflammation and pain. Modified from Furness et al., 2013.
PAR₄ activation by selective activating peptides or by cathepsin G reduces responses to colorectal distension (Annahazi et al., 2009) through presumed activation of PAR₄ expressed on nerve terminals (Annahazi et al., 2012). Prestimulation with PAR₄ activating peptide can significantly reduce allodynia and hyperalgesia in response to these agonists (Auge et al., 2009). Intracolonic PAR₁ activation similarly reduces behavioral responses, indicative of antinociceptive regulation of visceral pain (Kawao et al., 2004).

**B. Transient Receptor Potential Channels, Pancreatitis, and Pancreatic Pain**

The pancreas is innervated by both vagal and spinal afferents, with cell bodies located within nodose and dorsal root ganglia, respectively, and sensitization by inflammatory mediators likely contributes to pancreatitis pain. Several lines of evidence support the importance of neurogenic inflammation in the etiology of pancreatitis, including increased pancreatic innervation, elevated neurotransmitter content in pancreatic afferents, and reduction in pancreatitis severity by destruction of primary afferent neurons (Nathan et al., 2002; Hartel et al., 2006; Noble et al., 2006; Schwartz et al., 2013).

1. **Transient Receptor Potential Channels and Pancreatitis**

   The involvement of TRPA1, TRPV1, and TRPV4 in pancreatitis and pancreatic pain has been investigated, and observations support a role for TRPV1 and TRPA1 in neurogenic inflammation of the pancreas. TRPV4-positive nerve fibers innervate the pancreas, and pancreatic DRG afferents express functional TRPV4, although no functional changes in TRPV4 have been associated with acute pancreatic inflammation (Ceppa et al., 2010). Nerve fibers positive for both TRPV1 and CGRP are also in close association with pancreatic acinar cells and predominantly originate from spinal afferent DRG neurons (Fasanella et al., 2008). TRPV1 mRNA and protein are upregulated in animals with pancreatitis, in line with enhanced responses by pancreatic afferent neurons to capsaicin (Xu et al., 2007; Schwartz et al., 2011, 2013; Zhu et al., 2011). Consistent with these studies, capsaicin-treated animals are less susceptible to pancreatitis and treatment with the TRPV1 antagonist capsazepine or resiniferatoxin-induced denervation protects against caerulein-induced pancreatitis (Nathan et al., 2001; Noble et al., 2006). TRPA1-positive afferent nerve fibers innervate the pancreas and respond to the TRPA1 activators allyl isothiocyanate and endogenous agonists produced in pancreatic inflammation (15dPGJ2 and 4-HNE), and responses are attenuated by TRPA1 inhibitors or in trpa1⁻/⁻ mice (Ceppa et al., 2010; Schwartz et al., 2011, 2013; Li et al., 2013). The administration of TRPV1 and TRPA1 agonists into the pancreatic duct induces c-fos expression and NK₁R internalization in spinal cord and causes nocifensive behavior, which together suggest activation of pain pathways. These effects are diminished by intrathecal delivery of NK₁R and CLR antagonists, TRPV1/TRPA1 antagonists, or genetic deletion of TRPA1 (Wick et al., 2006; Ceppa et al., 2010; Schwartz et al., 2013).

2. **Sensitization and Activation of Transient Receptor Potential Channels in Pancreatitis and Pancreatic Pain**

   The inflamed pancreas is an ideal environment in which TRP channels may be directly activated or indirectly sensitized. The initial tissue injury associated with pancreatitis leads to premature activation of trypsin, an established agonist of PAR₂. PAR₂ has proinflammatory and protective roles in pancreatitis, depending on the model used (Kawabata et al., 2006; Laukkarinen et al., 2008). PAR₂ activation by intraductal delivery of PAR₂-AP or trypsin results in c-fos induction in spinal neurons that is attenuated by capsazepine (Nishimura et al., 2010). PAR₂-dependent activation of spinal ERK is also blocked by capsazepine and is thus mediated through PAR₂-TRPV1 interaction (Fukushima et al., 2010). PAR₂ sensitization of other TRP channels has not been examined with respect to pancreatic pain.

   NGF promotes upregulation of TRPV1 gene expression, sensitization of TRPV1 currents, and increased functional TRPV1 at the surface of nociceptive neurons (Bron et al., 2003; Zhuang et al., 2004; Zhang et al., 2005). Blockade of NGF signaling using NGF neutralizing antibodies reduces pancreatic hyperalgesia in an intraductal model of chronic pancreatitis, induced with 2,4,6-trinitrobenzenesulfonic acid (Zhu et al., 2011). The AA derivative leukotriene B₄ induces pancreatic inflammatory damage, which is diminished by capsazepine or inhibition of the leukotriene B₄ biosynthesis (Vigna et al., 2011). Other potential TRPV1 activators are produced in pancreatitis, including protons, anandamide, and endovanilloids, although their specific roles are not well defined.

   In conclusion, considerable evidence supports an integral role for TRP channels in the control of normal visceral function and sensation. Changes in TRP channel expression and sensitivity are associated with inflammatory disease and visceral hypersensitivity in animal models, and these changes may also occur in human disease. Although the functional interaction between GPCRs and TRP channels may underlie visceral hypersensitivity, the mechanisms of these interactions, particularly in functionally relevant cells, such as visceral afferent neurons, remain unclear. GPCR-TRP signaling pathways require greater characterization and may be supported by further studies on human tissue or innovative approaches, such as the assessment of visceral diseases in mice lacking key signaling proteins, such as the AKAP scaffold (Zhang et al., 2011).
V. Not-So-Painful: Contributions of the
G Protein–Coupled Receptor–Transient
Receptor Potential Channel Axis for Irritant
Sensation and Inflammation

The first line of defense against noxious substances and
invasive organisms is formed by epithelial layers
such as the epidermis (skin), the mucosal lining of the
airways, and the gastrointestinal tract. These barriers
are highly innervated for detection of potentially harm-
ful stimuli, including noxious temperatures, chemical
irritants, and microorganisms. Exposure to noxious
stimuli triggers pain, which leads to withdrawal from
or avoidance of damaging agents. However, noxious
stimuli also activate reflexes that remove the stimulus
(e.g., cough, scratching) and initiate protective measures
(e.g., mucus secretion, activation of an inflammatory
immune response). As is the case in pain, GPCRs and
TRP channels play a major role in these protective
mechanisms. Thus, sensory processes stimulated by
GPCRs and TRP channels are integral to the body’s
defense mechanisms, but are not always painful. More-
over, abnormalities of the GPCR-TRP axis may contribute
to dysregulation and chronic disease, such as chronic
cough, pruritus, and inflammation.

A. The G Protein–Coupled Receptor–Transient
Receptor Potential Axis in Pruritus

Itch (pruritus) is a defense mechanism that has been
defined as an “unpleasant skin sensation that elicits
the desire or reflex to scratch” (Rothman, 1941). When
peripheral nerve terminals in the skin and mucosal
surfaces detect pruritogenic stimuli they elicit a scratch-
ing response that protects by removing the associated
irritant. By contrast, chronic itch is a common clinical
problem that exacerbates cutaneous conditions and leads
to further injury (reviewed in LaMotte et al., 2014). The
most well established pruritogen, histamine, is secreted
by dermal mast cells and excites nearby sensory fibers
by acting on histamine H1 receptors (Liu et al., 2009).
However, chronic pruritus is insensitive to antihistamine
treatment. Chronic itch is commonly associated with
dermal diseases and metabolic disorders and can be
classified according to the source or cause of the pruritic
symptoms: dermal or peripheral itch (e.g., dermatitis,
psoriasis), neurogenic itch (e.g., chronic renal failure,
cholestatic liver disease), and neuropathic itch (e.g.,
multiple sclerosis, diabetes) (Binder et al., 2008).

In contrast to the classic theory that itch as a “sub-
threshold pain” evoked by weak activation of nociceptors,
the “specificity theory of itch” describes the differentia-
tion of pain and itch sensory pathways (Han et al., 2013;
Tóth and Bíró, 2013). The sensations of itch and pain are
distinct; however, they are both protective mechanisms
and involve a similar neuronal pathway of transmission.
Like pain, itch is mediated by subpopulations of un-
myelinated small diameter C-fiber sensory neurons with
cell bodies in the DRG and TG and is transmitted centrally
to second-order neurons of the spinal cord (Caterina and
Julius, 1999; Paus et al., 2006; Shim and Oh, 2008; Han
et al., 2013). Moreover, there are important functional
interactions between pain and itch pathways that are
exemplified by the phenomenon that painful scratching
can inhibit itch, and intrathecal analgesics can cause itch.
These interactions depend on regulatory mechanisms,
such as itch-inhibitory interneurons, that are stimulated
to reduce the postsynaptic transmission of itch when
nociceptive neurons are activated. For example, the
administration of opiates can cause itch by both inhibiting
synaptic transmission of the pain signal and preventing
activation of the itch-inhibitory interneurons (Xiao and
Patapoutian, 2011; Baraniuk, 2012). In addition, MrgrpA3
is a pruritogen-responsive GPCR expressed in neurons
that innervate the epidermis. Genetic ablation of
MrgrpA3-positive neurons in mice substantially reduced
scratching behavior to a range of stimuli (histamine,
chloroquine, dry skin, allergic inflammation) without
affecting pain sensitivity (Han et al., 2013). Interestingly,
in mice engineered to exclusively express TRPV1 in
MrgrpA3-positive neurons, scratching behavior in res-
ponse to capsaicin remains intact, whereas pain
responses are absent (Han et al., 2013). There is evidence
for multiple nociceptive TRP channels to contribute to
pruritus, and Han et al. (2013) hypothesize that ex-
pression of specific GPCRs within a neuronal subpopu-
lation may be a key determinant that influences the
ability of a TRP channel to contribute to either nociceptive
or pruritic processes.

The circuitry controlling itch is complex and involves
many pruritogenic substances (histamine, tryptase,
serotonin, kinins, chemokines) released from neurons
and cutaneous cells, including keratinocytes, mast,
endothelial, and immune cells (Tóth and Bíró, 2013).
TRPA1, TRPV1, TRPV3, and TRPV4 are expressed on
all of these cell types and contribute to acute and
chronic itch processes either by facilitating pruritogen
release or sensory itch responses in neurons (Ikoma
et al., 2006). A more comprehensive understanding of
the fundamental molecular basis of itch, including the
GPCR-TRP axis, is required to improve the treatment
of chronic itch conditions. GPCRs expressed in the
periphery contribute to the processing of itch and
include those receptors sensitive to histamine (H1R,
H4R), proteases (PAR2), bradykinin (B1R, B2R), bile
acids (TGR5), prostanoids (EP1R), SP (NK1R), and the
Mas-related GPCRs (MrgrpA3, MrgrpC11). Their roles
in pruritus and regulation of TRP channels have been
reviewed (Ikoma et al., 2006; Bautista et al., 2014).
GPCR-TRP sensitization pathways that have been
clearly defined to promote acute or chronic itch are
summarized in Fig. 5.

1. Transient Receptor Potential Vanilloid 1. TRPV1
has been implicated in multiple itch pathways, and
although TRPV1 can directly induce itch by capsaicin, it
primarily functions downstream of receptors that are stimulated by pruritogens (Imamachi et al., 2009; Sikand et al., 2009). Histamine is released by dermal mast cells during inflammation or in response to allergens and stimulates H1 and H4 GPCRs on unmyelinated C-fibers to cause itch (Bell et al., 2004; Dunford et al., 2007; Shim et al., 2007). Two major intracellular signaling pathways have been postulated to mediate H1 receptor responses. In CHO cells, H1 causes the release of intracellular calcium stores mediated by the PLC/IP3 pathway (Leurs et al., 1994). In rat DRGs, H1 receptor activation stimulates PLA2 and lipoxygenase activity to increase gating of TRPV1 (Kim et al., 2004). The DRG model is supported by reduced histamine-evoked scratching in mice exposed to inhibitors of PLA2, lipoxygenase, H1, and TRPV1, or in trpv1−/− mice. However, these mice still exhibit scratching behavior, which suggests that alternate histamine-dependent pruritic pathways exist (Shim et al., 2007). Histamine receptor signaling can stimulate kinase activity and the production of sensitizing agents, such as prostaglandin PGE2, suggesting the involvement of other TRP-sensitizing pathways (Belghiti et al., 2013).

Neurogenic inflammation can lead to pruritus through the local release of NGE and neuropeptides, such as tachykinins and CGRP (Holzer, 1998; Costa et al., 2008). PAR2-dependent sensitization of TRPV1 on sensory afferents is also proposed to be pruritogenic (Belghiti et al., 2013). However, itch induced by the PAR2 activating peptide SLIGRL is potentially mediated by the MrgrpC11, rather than PAR2. In contrast, a truncated peptide SLIGR activates PAR2 to cause pain but does not induce MrgrpC11-dependent scratching (Liu et al., 2011). Although the signaling mechanisms by which MrgrpC11 regulates TRPV1 are not fully understood, MrgrpC11 activates TRPA1 via Gaq/11-PLC signaling (Wilson et al., 2011). Alternatively, MrgrpC11 may cause mast cell degranulation, neurogenic inflammation, and the release of a multitude of mediators, including proteases and kinins, all of which contribute to itch and TRPV1 activation (Liu et al., 2011; Wilson et al., 2011).

Pirt regulates PIP2-dependent activation of TRPV1 and TRPM8 (Kim et al., 2008) and participates in histamine-dependent and -independent (chloroquine, 5-HT, endothelin-1) itch, as well as TRPV1-dependent and -independent responses (Patel et al., 2011). However, Pirt does not contribute directly to the activation of other TRP channels, such as TRPA1, and it is therefore hypothesized that Pirt may modulate specific pruritogen-induced GPCR signaling cascades, such as PLCβ3 activity (Patel et al., 2011).

2. Transient Receptor Potential Ankyrin 1. TRPA1 is a major target of GPCR-dependent itch processes and is a key mediator of chronic itch, either directly or via clinically relevant histamine-independent pruritogens including BAM8-22 and chloroquine, which stimulate Mrg receptors MrgrpC11 and MrgrpA3, respectively (Liu et al., 2009). MrgrpA3- and MrgrpC11-induced itch is absent in trpa1−/− mice, and both receptors can activate TRPA1 in vitro (Wilson et al., 2011). These two GPCRs activate TRPA1 via distinct signaling mechanisms; pharmacologic experiments suggest that MrgrpC11 is functionally linked to TRPA1 by stimulating PLC activity, whereas MrgrpA3

Fig. 5. Sensory and inflammatory pathways leading to sensitization or activation of TRP channels in the skin. Exposure of the skin to pruritogens results in activation of sensory neurons, resulting in the generation of itch responses. In addition, endogenous pruritogens are released from various cell subtypes, such as keratinocytes, mast cells, and endothelial cells. Several itch pathways are now known to exist, and TRP channels are integral components of these. TRPV1 is thought to largely mediate histamine-dependent itch downstream of H1 receptors via PLA2 and lipoxygenase. Nonhistaminergic itch involves the activation of TRPA1. Key intracellular mechanisms of GPCR-TRP channel interaction are outlined.
couples to TRPA1 via the Gβγ subunits, independently of PLC activation (Wilson et al., 2011). Related studies have also demonstrated that chloroquine-induced sensitization of TRPV1, TRPA1, and TRPM8 in DRG neurons occurs via similar PLC-independent pathways (Than et al., 2013). This intracellular signaling diversity highlights the complexity of itch sensation yet may help to explain the selectivity of the distinct transduction pathways.

Several animal models of chronic itch have been used to understand human processes of itch (Bautista et al., 2014). Comprehensive gene expression-profiling analyses in mouse models of chronic itch (alcohol-ether-water dry skin) reveal that TRPA1 activity is a key mediator of chronic itch phenotypes. TRPA1 activity induces expression changes in many genes contributing to scratch behavior, dermatitis, dry skin, and other sensory, inflammatory, or immune responses (Wilson et al., 2013a). This study also highlighted a potential role for TRPA1 in itch hypersensitivity incurred through increases in the expression of itch receptors (e.g., B2R) and innervation by itch-detecting primary afferent nerve fibers, as previously observed in dry skin mouse models (Tominaga et al., 2007).

Conditions of chronic pruritus, such as atopic dermatitis, psoriasis, cholestasis, and diabetes, are associated with high levels of oxidative stress. TRPA1 is an established mediator of oxidant-induced pain (Andersson et al., 2008; Bessac and Jordt, 2008), and, unsurprisingly, TRPA1 has also been implicated in itch associated with oxidative-stress induced by hydrogen peroxide and tert-butylhydroperoxide. In contrast, this itch is not influenced by blockade of histamine receptors. This evidence reinforces the concept that TRPA1 is a mediator of nonhistaminergic pruritus (Wilson et al., 2011). More recently neuronal TRPA1 has also been linked to the actions of the cytokine thymic stromal lymphopoietin (TLSP) in atopic dermatitis-associated itch (Wilson et al., 2013b). A relationship between proteases, PAR2 signaling, and TLSP has been reported for models of dermatitis (Briot et al., 2009, 2010). The study by Wilson et al. (2013b) extends these findings to show that protease-dependent stimulation of PAR2 in keratinocytes results in intracellular Ca2+ release, stimulation of endoplasmic reticulum STIM-1 protein, and the cell surface Orai1 Ca2+ channel to initiate expression and secretion of TLSP. The activation of TLSP receptors on adjacent sensory neurons subsequently stimulates TRPA1 activity. Together, this mechanism reveals pruritogenic processes that require communication between cells and between GPCRs and ion channels to generate itch responses in chronic skin conditions (Wilson et al., 2013b).

3. Transient Receptor Potential Vanilloid 3. A role for TRPV3 in itch sensation has been demonstrated using an experimentally induced dry skin pruritus model, where trpv3-/− mice show decreased scratching in response to acetone-ether-water (Yamamoto-Kasai et al., 2012). Moreover, an increase in the density of invasive nerve fibers is observed in this model. This observation is consistent with atopic dermatitis patients, where increased epidermal C-fiber innervation is thought to aggravate the disease (Paus et al., 2006).

A genetic basis for itch disorders involving TRPV3 hyperactivity has been identified, where a gain-of-function mutation in the trpv3 gene (Gly573Ser) causes spontaneous dermatitis and itch in rodents (Asakawa et al., 2006). Gain-of-function missense mutations in TRPV3 (Gly573Ser, Gly573Cys, and Trp692Gly) have been described in Olmstead syndrome, a rare congenital skin condition that causes severe itching. Mutant TRPV3 channels expressed in HEK cells or in keratinocytes of Olmstead Syndrome patients show increased inwardly rectifying currents and apoptotic cell death (Lin et al., 2012). TRPV3 can be sensitized via the PKC signaling pathway (Hu et al., 2006) and could contribute to itch downstream of GPCR activation; however, this has not been reported to date.

4. Bile Acids. Bile acids are essential for the digestion and absorption of dietary fat and can also activate the bile acid receptor TGR5 (GPBA), a GPCR that couples to Gαs, adenylyl cyclase, and cAMP production (Kawamata et al., 2003; Rajagopal et al., 2013). Bile acids are markedly increased in the circulation and tissues of patients with cholestatic liver disease and may contribute to the chronic and intractable pruritus that is a common feature of this condition (Hayashi and Majima, 1999). TGR5 is expressed in peptidergic small diameter primary sensory neurons innervating the skin, and activation by bile acids causes a TGR5-dependent hyperexcitability and stimulates the secretion of the itch-selective transmitter gastrin releasing peptide (Alemi et al., 2013). The intradermal injection of bile acids and TGR5-selective agonists also caused robust scratching in wild-type but not tgr5−/− mice, whereas gain-of-function transgenic mice exhibit a spontaneous pruritus. Evidence suggests that TGR5 agonists promote pruritus via a TRPA1-dependent process (Lieu et al., 2014). Bile acids can also evoke scratching by alternative mechanisms. The intradermal administration of deoxycholic acid to rats, for example, activates kallikreins to generate bradykinin and stimulate B2R signaling (Hayashi and Majima, 1999). The role of B1R and B2R activity in PAR2-dependent pruritus has also been investigated and suggests that kinin receptors function downstream of protease-induced pruritic responses (Costa et al., 2010). Although the bile acid-bradykinin system has not been clearly defined, TRPV1 sensitization was recently demonstrated to contribute to liver disease-induced itch and is predicted to play a major role, potentially via receptors for bradykinin, histamine, serotonin, or PAR2 (Belghiti et al., 2013). In addition, TRPV1 and TRPA1 are expressed in distinct and overlapping populations of peptidergic and IB4+ neurons (Bhattacharya et al., 2008; Kim et al., 2010), and coexpression of TRPA1 and TRPV1 on peptidergic C-fibers is hypothesized to result in Ca2+-dependent
channel cross-talk (Bautista et al., 2006). Although TRPA1 is a major downstream target of Gs-stimulated TGR5 signaling in the presence of elevated endogenous pruritogens (Lieu et al., 2014), it is tempting to speculate that TRPV1-induced Ca\(^{2+}\)-dependent upregulation of TRPA1 may contribute to scratching behavior via a regulatory pathway similar to that observed for bradykinin-induced inflammation via a TRPV1-TRPA1 and Ca\(^{2+}\)-dependent pathways (Bautista et al., 2006).

**B. The G Protein–Coupled Receptor–Transient Receptor Potential Axis in Airway Diseases**

The lungs are constantly exposed to the external environment, and the respiratory epithelium provides the first point of contact with the majority of inhaled chemicals, pathogens, allergens, and pollutants. The immune system plays a crucial role in maintaining lung health by detecting and removing potentially harmful agents. Inflammatory processes are driven by an orchestrated interplay between the respiratory epithelium, the innate immune system, and the adaptive immune system. Nonspecific protection also includes anatomic barriers, mucus, saliva, cough, and the recruitment of inflammatory cells. The cough reflex is a protective mechanism that helps to clear foreign material from the lungs and aids in immune defense. However, chronic cough can lead to additional injury, airway inflammation, and exacerbation of disease (Ford et al., 2006). The mechanisms driving cough have only recently started to be elucidated, and TRP ion channels appear to play a fundamental role in both the healthy reflex and exacerbated inflammatory cough pathologies (Grace et al., 2013).

The prevalence of asthma, chronic obstructive pulmonary disease (COPD), and other respiratory diseases is increasing on a global scale, and the etiology of asthma and COPD has been extensively reviewed (Rabe et al., 2007; Mathers et al., 2008; Barnes, 2011, 2013). Briefly, asthma pathology is characterized by chronic airway inflammation, variable airflow obstruction, airway hyperresponsiveness, and symptoms, such as cough, dyspnea, wheezing, and chest tightness. COPD encompasses a heterogeneous group of airway pathologies, including chronic bronchitis, bronchiectasis, and emphysema (Bateman et al., 2008). In contrast to asthma, COPD is characterized by airflow limitation that is not fully reversible and is usually progressive because of persistent inflammation and typical functional changes in the airways resulting from repeated tissue injury and repair (Di Stefano et al., 1998).

The potential for TRP channels to contribute to early airway responses is evident after inhaled constituents of food products that contain noxious or pungent TRPV1 and TRPA1 agonists (e.g., capsaicin, mustard, cinnamon, wasabi). Environmental particles, such as cigarette smoke, wood smoke, or air pollution, also contain TRPA1 agonists (e.g., acrolein, crotonaldehyde), which cause reflex cough or bronchoconstriction (Grace and Belvisi, 2011). In contrast, substances such as menthol (TRPM8) are reported to inhibit cough and elicit bronchodilation (Materazzi et al., 2009). Much of what we know about TRP channels in the lung has been inferred from studies investigating the pain pathway. However, in some instances, the airways show important differences to the rest of the body. For example, PGE\(_2\) is generally considered to be a proinflammatory mediator in the periphery, which causes pain and is implicated in diseases, such as rheumatoid arthritis and ultraviolet B-induced cutaneous inflammation (Materazzi et al., 2008). However, in the lungs PGE\(_2\) has both beneficial (e.g., bronchodilatory and anti-inflammatory) as well as unwanted (e.g., protussive) effects (Pavord et al., 1993; Gauvreau et al., 1999). The following discussion focuses on the importance of TRP channels in the airways and our current understanding of how GPCRs contribute to TRP channel function in this context. These airway GPCR-TRP sensitization pathways are summarized in Fig. 6.

1. **Transient Receptor Potential Vanilloid 1**

   In diseases such as asthma and COPD, activators and sensitizers of TRPV1 are present at increased concentrations in the lung. This can induce tonic activation of TRPV1 and is proposed to contribute to underlying airway disease pathology (Profita et al., 2003; Gatti et al., 2006). The biologic response to TRPV1 stimulation is consistent with respiratory disease symptoms and processes, including cough, bronchoconstriction, and inflammation. Moreover, cigarette smoke exposure, allergy, and virus cause hypersensitivity of the cough reflex to TRPV1 agonist inhalation, which can be prevented by TRPV1 antagonists (reviewed by Adcock, 2009, and Banner et al., 2011). Increased expression of TRPV1 in airway nerves and smooth muscle is also correlated with sensitization of the cough reflex in humans (Groneberg et al., 2004; Mitchell et al., 2005; Butler et al., 2010). In contrast to cough, a direct role for TRPV1 in the etiology of other airway diseases is still unclear. For example, in animals, TRPV1 agonists cause tracheal smooth muscle contraction in vitro and bronchoconstriction in vivo via neurogenic pathways (Belvisi et al., 1992; Laloo et al., 1995). Conversely, capsaicin has been observed to cause bronchodilation in humans (Ichinose et al., 1988).

2. **Transient Receptor Potential Ankyrin 1**

   TRPA1 is expressed by bronchial epithelia and TRPV1-positive vagal afferent fibers innervating the lung (Nassenstein et al., 2008; Mukhopadhyay et al., 2011). TRPA1 is highly sensitive to respiratory irritant exposure, including pungent cysteine-reactive chemicals, or irritants, such as cigarette smoke, to cause TRPA1-dependent protussive events in human and animal models and is therefore a key therapeutic target for respiratory disorders (Trevisani et al., 2007; Birrell et al., 2009; Preti et al., 2012). The pathophysiologic consequences of this include neurogenic release of the proinflammatory neuropeptide SP.
In a positive feedback cycle, SP released from sensory nerve terminals increases NK1R-dependent generation of reactive species, including the lipid peroxidation product 4-HNE, which can further increase TRPA1 activity (Rahman et al., 2002; Boldogh et al., 2005; Taylor-Clark et al., 2008a). Together, an initial TRPA1 response to respiratory irritants, followed by secondary responses to inflammatory mediators, implicates TRPA1 in sensory processing and augmented dysregulation in airway diseases due to enduring inflammation and lung remodeling (Springer et al., 2007; Li et al., 2008). TRPA1 is also likely to mediate allergen-induced airway inflammation in patients with asthma, as demonstrated using trpa1−/− mice or by treatment with the selective TRPA1 inhibitor HC-030031 [2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4-isopropylphenyl)acetamide], which reduces allergen-induced leukocyte infiltration, cytokine and mucus production, and airway hyper-responsiveness (Caceres et al., 2009; Raemdonck et al., 2012). Animal models also show that TRPA1 plays an important role in the early phase of bronchial inflammation to cigarette smoke, causing release of interleukin-8, which is a chemoattractant for neutrophils, T cells, and monocytes (Mukhopadhyay et al., 2011). Contraction of bronchial rings to cigarette smoke extract, acrolein, and crotonaldehyde are also reduced by inhibition of TRPA1 but not by TRPV1 inhibition (Andre et al., 2008). TRPA1 can also mediate the irritant effects of certain volatile anesthetics in the airways, where activation triggers the release of neuropeptides that can cause bronchospasm and neurogenic inflammation (Matta et al., 2008; Eilers et al., 2010).

3. Transient Receptor Potential Vanilloid 4. Although a loss-of-function mutation in TRPV4 does not show significant association to asthma (Canco Recasens et al., 2010), diesel exhaust particles can activate PAR2 expressed by isolated human bronchial epithelial cells, leading to activation of TRPV4 and secretion of matrix metalloproteinase-1, which plays a role in tissue remodeling and contributes to COPD and emphysema pathogenesis (Li et al., 2011). Moreover, bronchial smooth muscle cells and nerve endings may become exposed to hypotonic bronchial fluid in patients with asthma because of airway remodeling (Liedtke and Simon, 2004). A hypotonic solution such as this is proposed to elicit TRPV4-dependent smooth muscle contraction in isolated human and rodent airways (Jia et al., 2004). A genome-wide association study has also revealed multiple TRPV4 single-nucleotide polymorphisms associated with susceptibility to COPD (Zhu et al., 2009). Loss of TRPV4 Ca2+ permeability has also been linked to cystic fibrosis pathology, rendering the epithelia unable to respond to hypotonic stress (Arniges et al., 2004).

In murine ciliated tracheal cells, functional TRPV4 regulates ciliary beat frequency associated with mild temperature and ATP stimulation, implicating it in mucociliary clearance of the lungs (Lorenzo et al., 2008). Moreover, osmotic stress can cause ATP release (and activation of inflammatory purinergic receptors) from human bronchial epithelial cells via TRPV4 and the RhoA pathway (Eltom et al., 2011; Seminario-Vidal et al., 2011). It is possible that genetic mutations in TRPV4 could contribute to disease pathogenesis through inability of cilia to respond to environmental stimuli and hampered mucus clearance associated with respiratory diseases such as COPD (Zhu et al., 2009).

4. Transient Receptor Potential Melastatin 8. A lack of selective pharmacologic tools and the fact that many
TRPM8 agonists can also modulate TRPA1 have led to an ongoing debate about the functional importance of TRPM8 in airway processes. Breathing cold air can induce respiratory autonomic responses that include cough, airway constriction, plasma protein extravasation, and mucosal secretion (Yoshihara et al., 1996; Carlsen and Carlsen, 2002). Conversely, TRPM8 has been shown to inhibit the cough reflex, although data are contradictory. Although menthol efficacy is not greater than placebo in clinical trials, menthol remains a key ingredient in many over-the-counter antitussive therapies (Kenia et al., 2008).

The modestly selective TRPM8 antagonist BCTC [N-(4-tert-butylphenyl)-4-(3-chloropyridin-2-yl)piperazine-1-carboxamide; also described as a TRPV1 antagonist] and siRNA knockdown can inhibit TRPM8-induced inflammatory cytokine production and suggests that antagonism of TRPM8 could be beneficial for patients with cold-induced asthma or related respiratory disease (Sabinis et al., 2008; Banner et al., 2011). Moreover, menthol has been claimed as an adjuvant agent and is associated with relief of dyspnea in diseases such as COPD (Eccles, 2003). Indeed, menthol is regularly used in nasal decongestant therapies. However, there is no effect of menthol on nasal airflow, although menthol does increase the perception of nasal patency (Kenia et al., 2008).

5. G Protein–Coupled Receptor–Dependent Sensitization of Transient Receptor Potential Channels in Airway Diseases. The activation of TRP channels by GPCR-stimulated intracellular signaling is important in respiratory disease and congruent with pain transmission mechanisms. High concentrations of inflammatory mediators that indirectly activate or sensitize TRPV1 and TRPA1 (e.g., PGE2, bradykinin, lipoxygenase metabolites, proteases) are found in the blood and lungs of patients with inflammatory airway disease (Profita et al., 2003; Birring et al., 2004; Gatti et al., 2006; Grace et al., 2012). Several of the pathways initiated by these mediators activate Gαs and Gαq-coupled pathways, which converge on PKA, PKC, or PLC (Bessac and Jordt, 2008) (Fig. 6).

Vagal bronchopulmonary C-fibers play an important role in inducing bronchoconstriction, hypersecretion of mucus, and the cough reflex (Lee et al., 2002a). In particular, the tussive effects of PGE2 have been linked to EP3 receptor-dependent activation of TRPV1 and TRPA1 (Maher et al., 2009; Grace et al., 2012). In other pathways, PGE2 sensitizes TRPV1 responses in cultured vagal neurons by activation of adenyl cyclase and through PKA-mediated TRPV1 phosphorylation, likely via the EP2 receptor (Kwong and Lee, 2002; Gu et al., 2003).

Kinins are released in response to tissue injury and inflammation, and expression of B2R by pulmonary cells is upregulated under inflammatory conditions (Newton et al., 2002). Several pathways have been suggested to mediate the effects of bradykinin on TRPV1 and TRPA1. Activation of B3R stimulates Gαq-coupled PKA signaling and PLC-dependent PKC activity and bradykinin-induced lipooxygenase products (Premkumar and Ahern, 2000; Ferreira et al., 2004; Grace et al., 2012; Gregus et al., 2012).

A balance between proteases that digest structural proteins (e.g., elastin) and protease inhibitors that protect against digestion is important in maintaining the integrity of the lungs. Inhaled irritants, including cigarette smoke, cause the release of proteases from a variety of cells. Proteases activate PARs, digest connective tissue in the lung parenchyma leading to emphysema, and also stimulate mucus hypersecretion (Barnes, 2010). In addition, individuals with α1-antitrypsin deficiency carry a greater risk of developing COPD (Brebner and Stockley, 2013). Activation of PAR2, presumably on sensory afferents of the lung, potentiates TRPV1-induced cough responses through activation of PKC, PKA, and the release of prostanoids (Gatti et al., 2006). Therefore, endogenous release of proteases may contribute to the sensitized cough reflex to TRPV1 stimulation observed in patients with asthma and COPD. Interestingly, the PAR2-activated induction of TRPV4 in airways requires Gαq signaling and the activity of PLC and PI3K (Li et al., 2011). This may indicate cell type–specific regulatory mechanisms and a requirement of Gβγ subunits.

Increased activation of TRPV4 by mechanical and osmotic stimuli in oviductal ciliated cells may depend on PLAr activation and production of AA metabolites such as EET. Under conditions of low PLA2 activation, ATP (likely via the P2Y2 receptor) may contribute to TRPV4 gating by activating PLC, leading to IP3 generation (Fernandes et al., 2008; Lorenzo et al., 2008). Importantly, alterations in the activity of PLAr and AA levels have been reported in cystic fibrosis airway epithelia, which could explain the lack of TRV4 activation by cell swelling (Arnings et al., 2004).

VI. Targeting G Protein–Coupled Receptors, Transient Receptor Potential Channels, and the G Protein–Coupled Receptor–Transient Receptor Potential Channel Axis

A. The Need for Alternative Therapeutics

GPCRs and TRP ion channels are attractive targets for the treatment of pain and related disorders, such as chronic pruritus and inflammation. Current analgesics include those that act locally (e.g., lidocaine) or attenuate neurotransmitter release through inhibition of ion channels (voltage-sensitive Ca2+, Na+, or K+ channels) in central nerve pathways. Anticonvulsant and anesthetic sodium channel blockers have long been used to treat acute or chronic pain, such as trigeminal neuralgia, by reducing the generation of action potentials and nerve excitation (Bhattacharya et al., 2009). For peripheral pain, nonsteroidal anti-inflammatory drugs have mild
analgesic, anti-inflammatory, and antipyretic properties that inhibit production of algesic prostaglandins and are effective for inflammatory conditions such as arthritis (Andersson et al., 2011). Opioids reduce potassium channel activity and TRP channels to reduce presynaptic neurotransmitter release and central nerve excitability and remain a first-line treatment of postoperative, acute, and chronic pain (Finnegan et al., 2006). Drawbacks associated with these treatments include overdose, development of tolerance, seizures, confusion, sedation, and undesirable off-target effects (e.g., morphine causes respiratory depression and constipation). In addition, the promise of key nociceptive receptors (e.g., bradykinin and neurokinin family members) has led to the development of many selective, high-affinity antagonists that block noxious responses in animal pain models yet have failed to yield successful drugs in the market. Hence, therapeutic intervention of peripheral sensory pathways targeted toward GPCRs or TRP channels at the source of pain transmission could be a better strategy (Patapoutian et al., 2009).

Analgesics directed toward GPCRs and TRP channels within peripheral cells are currently providing promise in the clinic (Brederson et al., 2013). However, this strategy may prove challenging, given the widespread expression of TRPs and their broad physiologic significance (Ramsey et al., 2006). As with so many therapeutic targets, development of new treatments is predominantly limited by our understanding of how these receptors or ion channels function in different cells or disease states. Species differences can also lead to different outcomes in human studies compared with animal models (Steinhoff et al., 2014). To assess the potential to generate novel therapeutics to alleviate chronic conditions while keeping basal physiologic functions intact is an enormous challenge and requires deeper understanding of all processes, including the mechanisms of GPCRs and TRP channel cross-talk.

B. Lessons from Analysis of Transient Receptor Potential Channel Expression in Sensory Neurons

1. Improved Techniques for Expression Analysis. TRP channels are expressed in primary sensory neurons and are active targets of drug discovery efforts directed toward the identification of novel ligands for pain therapies. Crucial to the outcomes of such drug discovery programs is the knowledge of the sites of TRP channel expression. TRPV1, TRPV2, TRPV3, TRPV4, TRPA1, and TRPM8 are expressed by primary sensory neurons, which were once considered to be the sole site of expression. However, these pain targets are also expressed in other cell types, where they can have distinct roles. Difficulties in detecting TRP channels include lack of selective antibodies for anatomic studies and of agonists and antagonists for functional studies. However, some of these challenges have been overcome by studies of reporter mouse lines. For example, the availability of TRPV1Cre/R26R-lacZ mice and TRPV1PLAP-nlacZ mice has improved the reliability of TRPV1 detection and has revealed new and unexpected information about TRPV1 expression. Unexpected findings include the expression of TRPV1 in a smaller subpopulation of sensory neurons than previously reported and the detection of TRPV1 in arteriole smooth muscle, which may explain the cardio-protective effects induced by capsaicin or endogenous TRPV1 ligands (Peng and Li, 2009; Cavanaugh et al., 2011a,b). Clearly, a detailed understanding of the sites of TRP channel expression in normal and diseased states is necessary for the interpretation of clinical trials of TRP agonists or antagonists.

2. Upregulation of Transient Receptor Potential Channels in Pain Conditions. There are marked changes in TRP expression in pain states that have important implications for therapeutic targeting. Damage to one branch of the spinal root of a dorsal root ganglion leads to upregulation of TRPV1 in the adjacent undamaged nerve that can still transmit pain signals (Hudson et al., 2001). TRP channel expression is also altered in human tissues during chronic pain. A study of TRPV1, TRPV3, TRPV4, and TRPM8 expression revealed that TRPV1 is upregulated in the injured brachial plexus nerves and that TRPV1 and TRPV3 are upregulated in hypersensitive skin after nerve repair, whereas TRPV4 is unchanged (Facer et al., 2007). In the same study, TRPV1-immunoreactive nerves were present in injured dorsal spinal roots and dorsal horn of control spinal cord but not in ventral roots, whereas TRPV3 and TRPV4 were detected in spinal cord motor neurons. The upregulation of TRP channels in painful conditions may contribute to neuronal sensitization and resultant hyperalgesia. Indeed, TRP channel antagonists may have more selective and beneficial therapeutic actions in diseases associated with a robust upregulation of TRPs in sensory neurons compared with other tissues (Basbaum et al., 2009).

C. Current State of Transient Receptor Potential Agonists and Antagonists

TRP channels are attractive therapeutic targets: preclinical studies from multiple laboratories show that TRP channels play a major role in pain, pruritus, and neurogenic inflammation and the channels are “drugable,” because selective agonists and antagonists have been developed for many channels. However, there are formidable challenges with targeting TRP channels. First, the preclinical models of chronic pain, itch, and inflammation in experimental animals may be poor representations of the complexity of human diseases, and the translation of information from studies of laboratory animals to humans is always problematic. Second, there is marked redundancy among TRP channels. Sensory nerves coexpress multiple TRP channels that participate in sensory transduction and neurogenic inflammation, often with overlapping functions. Third, TRP channels
are not confined to sensory nerves and participate in many normal physiologic processes, such as thermoregulation (e.g., TRPV1) and osmoregulation (e.g., TRPV4). The on-target disruption of these homeostatic mechanisms represents significant challenges to the targeting of TRP channels. Despite these concerns, several TRP agonists and antagonists have advanced to clinical trials for pain. In general, two different approaches have been used to target TRPs for pain therapeutics: agonists have been screened to desensitize sensory neurons and antagonists have been generated as antihyperalgesic agents.

1. Transient Receptor Potential Vanilloid 1 Agonists. The burning sensation evoked by capsaicin eventually desensitizes or defunctionalizes primary nerve terminals and results in analgesia of the affected area. This well-known phenomenon has led to the topical use of capsaicin creams in the treatment of neuropathic and musculoskeletal pain (Mason et al., 2004). Despite its widespread use, capsaicin cream has moderate to poor clinical efficacy in treating chronic musculoskeletal and neuropathic pain, although it may be useful in some cases where other treatments are inadequate. The use of capsaicin is problematic mainly because of the slow time for the analgesic effect to become apparent (hours to weeks) and because of the risk of spreading the painful substance to sensitive areas of skin and organs, such as wounds or mucus membranes containing TRPV1-positive C-fibers. An effective alternative for some neuropathic pain conditions includes the recently available high-concentration (8% capsaicin) transdermal patch Qutenza (Acorda Therapeutics Inc., Ardsley, NY). The controlled release of capsaicin into the skin for 60 minutes in healthy patients results in loss of epidermal nerve fibers for more than 12 weeks (Kennedy et al., 2010), and preliminary studies of the high-dose patch on postherpetic neuralgia patients indicate that the patch is reasonably well tolerated and improves analgesia compared with low-dose topical creams (Martini et al., 2013).

The highly potent TRPV1 agonist resiniferatoxin has been used experimentally to treat intractable detrusor hyper-reflexia of the bladder (Evans, 2005; Apostolidis et al., 2006). Although the clinical efficacy of resiniferatoxin in treating pain is still unknown, it is currently being tested for its ability to treat severe pain associated with advanced cancer in National Institute of Dental and Craniofacial Research–sponsored phase I and phase II trials (ClinicalTrials.gov identifier: NCT00804154).  

2. Other Transient Receptor Potential Agonists. Folk remedies and over-the-counter liniments and rubs have been used to relieve muscle soreness for many years. Eucalyptus oil, ginger extract, and oil of wintergreen contain substances that are agonists of TRPV1 (gingerol) (Morera et al., 2012), TRPM8 (menthol) (Peier et al., 2002a), and TRPA1 (methyl salicylate, menthol, and gingerol) (McKemy et al., 2002; Bandell et al., 2004; Morera et al., 2012). However, when used topically, there is little objective evidence that they act at sensory nerves and are analgesic.

Dietary agonists of TRPV1 (capsaicin, gingerol), TRPV3 (thymol, eugenol, carvacrol), TRPM8 (menthol), and TRPA1 (cinnaemaldehyde, mustard oil) stimulate Ca2+ flux in the oral mucosa and gastrointestinal tract to regulate mucosal blood flow. Mediterranean diets rich in oregano (carvacrol) have been proposed to promote cardioprotective effects via arterial dilatation, yet this has not been therapeutically investigated (Xu et al., 2006; Earley et al., 2010; Okumi et al., 2012). This may be attributed to the fact that in most cases the oral bioavailability of these compounds is extremely limited. Caution should also be taken regarding modulation of TRPV3 because of observations of agonist-induced augmentation of Ca2+-dependent activity with repeated stimulation (Xiao et al., 2008). Constitutive TRPV3 activity is also attributed to chronic itch, dermatitis, hair loss, and cell proliferation (Asakawa et al., 2006; Yoshioka et al., 2009; Yamada et al., 2010).

3. Transient Receptor Potential Vanilloid 1 Antagonists. A detailed understanding of the pharmacological actions of capsaicin in the 1960s prompted the development TRPV1 antagonists before cloning of the capsaicin receptor (Jancso et al., 1967; Caterina et al., 1997). Capsazepine, the first TRPV1 antagonist, reverses persistent nociceptive and neuropathic pain behavior in rats (Walker et al., 2003), and many new antagonists have since reported effectiveness in animal models of pain (reviewed in Kort and Kym, 2012).

Although TRPV1 antagonists can provide therapeutic benefit for pain in humans, many compounds affect normal thermoregulation and temperature perception (reviewed in Brederson et al., 2013). A phase I trial of SB-705498 given orally to healthy volunteers showed effectiveness in reversing heat-evoked pain and skin sensitization induced by capsaicin or ultraviolet B irradiation (Rami et al., 2006; Chizh et al., 2007). One volunteer experienced a modest increase (1.3°C) in core body temperature, but no other adverse events were reported. A phase 1b trial of AMG517 to treat dental pain was terminated because of marked hyperthermia in some subjects who experienced an increase in core body temperature of over 4°C (Doherty et al., 2007; Gavva et al., 2008). After initial safety trials for the TRPV1 antagonist AZD-1386 on healthy volunteers and GERD patients (Krarp et al, 2011; ClinicalTrials.gov identifier: NCT00692146), a double-blind phase II trial of dental pain from third molar extraction revealed that AZD-1386 provided pain relief and was well tolerated, with an average 0.4°C increase in core body temperature (Quing et al., 2013). MK-2295 markedly blunted heat perception in healthy human subjects measured using quantitative sensory testing of pain evoked by immersion of the hand into heated water or sipping hot liquids without attenuation by repeated dosing (Eid, 2011; ClinicalTrials.gov Identifier: NCT00387140). A
multiple-dose, double-blind, placebo-controlled, randomized trial of healthy subjects showed that that ABT-102 caused a reversible change in oral and cutaneous sensitivity to heat but not cold without adverse effects (Gomtsyan et al., 2008; Rowbotham et al., 2011). Thus, although TRPV1 antagonists may blunt pain, there are variable effects on core body temperature and consistent effects on the perception of heat, where potentially noxious noxious temperatures were perceived as innocuous (Eid, 2011). The variability of effects of TRPV1 antagonists on core body temperature are compound selective and may thus depend on structure and could be “engineered out.” However, the uniform effects of TRPV1 antagonists on the threshold of oral heat-pain are unacceptable, because they introduce a risk of scalding injury from hot drinks or food (Rowbotham et al., 2011). Given that this latter effect is likely an on-target action of TRPV1 antagonists, it may represent a challenging problem for development of TRPV1 antagonists. Possibly allosteric modulators of TRPV1 activity will be more useful analgesics than full antagonists.

4. Other Transient Receptor Potential Antagonists. Research programs to identify and characterize novel antagonists selective for TRPA1, TRPV3, TRPV4, and TRPM8 to treat conditions of pain, itch, cough, and inflammation have also massively expanded in the past 10 years, and the now abundance of literature of preclinical antagonist development is heavily supported and scrutinized by the pharmaceutical industry (reviewed by Eid, 2011; Brederson et al., 2013). TRPA1, for example, is a tractable therapeutic target as demonstrated by reduced inflammatory or neuropathic responses in antagonist-treated or trpa1−/− mice. Selective TRPA1 antagonists, such as HC-030031, have been identified as effective inhibitors of TRPA1 activity in primary neurons when stimulated by irritants, bradykinin receptor signaling, and cold temperatures. Numerous TRPA1-selective compounds from Glenmark, Abbott, and Amgen followed, with varying degrees of oral bioavailability (Eid et al., 2008; Eid, 2011; Moran et al., 2011). Phase I and phase II human trials by Glenmark, Cubist, and Hydra are currently in progress, and the findings are yet to be published. One could speculate that topical application or inhalation of TRPA1 antagonists may also be beneficial for the reduction or prevention of itch or chronic cough (Bautista et al., 2013). Although TRPA1 antagonist patent applications have been filed with intention to treat these disorders, it is yet to be investigated in detail. TRPV3 antagonists also have efficacy in models of inflammatory pain, formalin-induced flinching, thermal injury, spinal sensitization, and neuropathic pain (Brederson et al., 2013). These treatments may have additional benefit in pruritus and skin conditions, such as Olmsted Syndrome (Lai-Cheong et al., 2012; Lin et al., 2012). TRPV4 antagonists have wide potential for alleviating disorders of inflammation, central nervous system water retention, metabolism, bladder distention, and circulation, and several recent TRPV4 antagonist patent applications have been filed (e.g., US20140135369 A1, 2014; WO2013/201352109 A1, 2013; WO2013/2014089013 A1, 2013). There is limited information on these compounds progressing beyond preclinical development, which may indicate complications in enhancing specificity for target organs or tissues (Willette et al., 2008; Eid, 2011).

Toxins have also been the focus of drug screening efforts to identify natural analgesics that target a range of proalgesic ion channels (Malberg and Yaksh, 1995; Diochot et al., 2012). Recently, this has led to the identification of ligands that selectively bind and regulate TRP ion channels, including two distinct Tarantula venoms that antagonize TRPA1 (Gui et al., 2014) and stimulate TRPV1 (Bohlen et al., 2010). Continued drug discovery efforts and development of structure-activity relationship studies on toxins as pharmacophore models may uncover new classes of therapeutically important analgesics (Lewis et al., 2012).

D. Therapeutic Targeting of G Protein–Coupled Receptor–Transient Receptor Potential Interactions

GPCRs play a major role in regulating TRP channels in disease by stimulating intracellular signaling pathways that can lower the activation thresholds of TRP channels. These mechanisms are likely to be cell type specific and require second messengers and auxiliary proteins that may offer alternative targets for drug discovery. For example, mice lacking Pirt, which interacts with TRPV1 and TRPM8, exhibit decreased sensitivity to heat, itch, or cold in selective neurons (Kim et al., 2008; Patel et al., 2011; Tang et al., 2013). TRPV4 is a PAR2 receptor-operated ion channel and can contribute to neurogenic inflammation through a mechanism that requires generation of endogenous TRPV4 agonists and phosphorylation of a tyrosine residue 110 in TRPV4 (Poole et al., 2013). Thus, in addition to direct targeting of GPCRs and TRP channels, therapies that target key kinases, lipids, or auxiliary proteins that mediate GPCR-TRP coupling offer an alternative therapeutic approach for sensory disorders and neurogenic inflammation. In support of this concept, the tyrosine kinase inhibitor bafetinib was recently shown to block PAR2 coupling to TRPV4 in cell lines and attenuate PAR2-dependent mechanical hyperalgesia in mice (Grace et al., 2014a). Similarly, the novel PAR2 antagonist GB88 was recently shown to selectively block PAR2-induced Goq signaling events and inflammatory pain in mice (Suen et al., 2014) and demonstrates the potential for receptor-operated ion channels to be selectively blocked via treatment with biased antagonists.

More than 400 kinases are associated with human diseases and they are a well studied therapeutic target (Zhang et al., 2009). Moreover, kinase-selective inhibition is a successful treatment of inflammatory disorders (Patterson et al., 2014). Small-molecule inhibitors of
protein-protein interactions have had limited success owing to difficulty in disrupting the large interfaces between interacting proteins (Jin et al., 2014). However, several approaches that have been explored to disrupt such interactions may allow for TRP channel-specific inhibition. Pepducins are small membrane-associated peptides that mimic intracellular loop 3 of PARs and which disrupt the interactions between PARs and heterotrimeric G proteins and thereby inhibit inflammatory signaling (Sevig et al., 2011). TRPducins are palmitoylated peptide sequences from the C-terminal "TRP domain" of TRPs (Fig. 3B). TRPV1 TRPducins selectively inhibits TRPV1 over other TRPV channels and can block heat-, acid-, and voltage-evoked TRPV1 currents. This process inhibits CGRP release from cultured neurons and lowers chemically- but not mechanically-induced pain (Valente et al., 2011). Similarly, small PKC sequence peptides fused to membrane-permeating TAT sequences can selectively inhibit specific PKC isoforms and have been shown to reduce ischemic heart damage (Chen et al., 2001). Although these kinase inhibitors may selectively prevent inflammatory pathways (e.g., PKCε is critical for PAR2 sensitization of TRPV1), penetrating peptides directed toward the β-strands, linker region, or ankyrins may similarly affect interaction sites in TRP channels (Amadesi et al., 2009).

Finally, local anesthetics exert their analgesic effect by inhibiting voltage-gated sodium channels by binding to sites in the inner face of the plasma membrane. It has been observed that some TRP ion channels undergo activation-dependent pore dilation, and consequently, large cations can be transported into sensory nerves. This pore dilation might be a means of producing TRP channel subtype-dependent inhibition. Specifically, coadministration of capsaicin with a local anesthetic enhances TRPV1 pore dilation to facilitate the delivery of membrane impermeant local anesthetics to voltage-dependent sodium channels, which inhibits action potentials in TRPV1-positive sensory neurons (Binshtok et al., 2007). This represents a novel means of exploiting ion channels for drug delivery that may be used to increase the specificity and efficacy of therapeutics (Roberson et al., 2011). This approach has also been used to investigate distinct neuronal itch pathways and indicates the potential to selectively inhibit pain-transmitting nerves where inflammatory states have increased TRP channel expression (Roberson et al., 2013).

VII. Concluding Remarks

GPCRs and TRPs are frontline partners for the detection of noxious, irritant, and inflammatory stimuli. These integral membrane proteins are coexpressed at the surface of sensory neurons and many other cell types in most tissues. Although GPCRs and TRPs can independently detect diverse chemical, mechanical, and thermal stimuli, the GPCR-TRP functional axis is of vital importance. Through this axis, signals that emanate from multiple GPCRs converge on a smaller number of TRP channels, leading to channel activation and sensitization or altered channel expression and plasma membrane insertion. This convergence often amplifies the effects of GPCRs, which results in exacerbated responses to noxious, irritant, and inflammatory stimuli. Accordingly, the GPCR-TRP axis is vitally important for the normal protective processes of pain, itch, cough, and neurogenic inflammation and also participates in chronic conditions that underlie disease.

The realization that GPCRs and TRPs are key mediators of disease processes has provided the impetus for efforts to develop antagonists of GPCRs and TRPs to treat chronic pain, itch, cough, and inflammation. Antagonists and agonists of GPCRs and TRP channels are effective treatments for many preclinical models of sensory and inflammatory disorders in experimental animals and in some cases are useful treatments for human diseases. However, the widespread distribution of GPCRs and TRPs and their involvement in diverse physiologic processes can lead to off-target side effects that can limit the effectiveness of receptor- and channel-directed drugs.

Whether targeting the GPCR-TRP axis is a more selective, effective, and viable approach to treat chronic disorders remains to be determined. A prerequisite for the success of this approach is a detailed understanding of the molecular mechanisms that underlie the GPCR-TRP axis in functionally relevant cell types and disease settings. Our current understanding of this axis mostly derives from studies of GPCRs and TRPs that are overexpressed in model cell lines. Such studies have identified multiple mechanisms, which are often overlapping, by which GPCRs can regulate the activity of TRP channels. In many cases it is not known whether the same mechanisms also underlie the GPCR-TRP axis in functionally relevant cells, such as primary nociceptive neurons. Furthermore, whether these same mechanisms also operate in disease settings or whether defects in the GPCR-TRP axis are involved in the etiology of chronic disorders of sensation and inflammation remains to be determined. However, the recent explosion of high-resolution information of GPCR and TRP channel structures has provided invaluable insights into the mechanisms of GPCR and TRP activation and signaling. Whether such structural information can illuminate the mechanisms of the GPCR-TRP axis remains to be determined, but if it does it is likely to provide valuable knowledge of potential therapeutic targets for conditions that are a major cause of suffering.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Veldhuis, Poole, Grace, McIntyre, Bunnett.
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