Mas and Its Related G Protein–Coupled Receptors, Mrgprs

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Abstract—The Mas-related G protein-coupled receptors (Mrgprs or Mas-related genes) comprise a subfamily of receptors named after the first discovered member, Mas. For most Mrgprs, pruriception seems to be the major function based on the following observations: 1) they are relatively promiscuous in their ligand specificity with best affinities for itch-inducing substances; 2) they are expressed in sensory neurons and mast cells in the skin, the main cellular components of pruriception; and 3) they appear in evolution first in tetrapods, which have arms and legs necessary for scratching to remove parasites or other noxious substances from the skin before they create harm. Because parasites coevolved with hosts, each species faced different parasitic challenges, which may explain another striking observation, the multiple independent duplication and expansion events of Mrgpr genes in different species as a consequence of parallel adaptive evolution. Their predominant expression in dorsal root ganglia anticipates additional functions of Mrgprs in nociception. Some Mrgprs have endogenous ligands, such as β-alanine, alamandine, adenosine, RF-amide peptides, or salusin-β. However, because the functions of these agonists are still elusive, the physiologic role of the respective Mrgprs needs to be clarified. The best studied Mrgpr is Mas itself. It was shown to be a receptor for angiotensin-1–7 and to exert mainly protective actions in cardiovascular and metabolic diseases. This review summarizes the current knowledge about Mrgprs, their evolution, their ligands, their possible physiologic functions, and their therapeutic potential.

I. Introduction and History

Mas was described more than 25 years ago (Young et al., 1986) and later became eponymous for the whole family of Mas-related G protein–coupled receptors (Mrgprs), formerly called Mas-related genes (Mrgs). Intriguingly, the first three main discoveries about this gene turned out to be erroneous, mostly due to the fact that the quantity of collected data and the general knowledge at that time obscured the interpretation of the obtained results and led to inappropriate conclusions.

The human MAS gene was originally isolated from DNA of a human epidermoid carcinoma cell line because of its ability to transform NIH3T3 cells upon transfection (Young et al., 1986) and therefore was thought to be a proto-oncogene. Because computer analysis of the MAS amino acid sequence suggested that the protein belongs to the class of G protein–coupled receptors (GPCRs) with seven transmembrane domains (Young et al., 1986), this provided the first direct evidence for an oncogenic activity of a GPCR. Although such tumorigenicity was confirmed in independent experiments (Janssen et al., 1988; van’t Veer et al., 1993), the oncogenic potential of Mas was challenged. In these experiments, the transfected cells or the tertiary tumor in nude mice contained amplified MAS sequences characterized by rearrangements in 5′- and 3′-noncoding regions, such as an insertion of human centromeric a-satellite repeat DNA (van’t Veer et al., 1993). However, the original tumor DNA used in the first round of transfection was neither rearranged nor amplified or mutated in the MAS coding sequence and therefore cannot be considered as the driving cause for tumor development. Presumably, the MAS 5′-region represents a hot spot of recombination, and the rearrangement of the 5′-noncoding sequence, which occurred during transfection, was responsible for the activation of the MAS gene in the tumorigenicity assay. Thus, Mas is not an oncogene and has never been found amplified in a primary tumor but can transform cells when artificially overexpressed.

The first attempts to clarify the function of the Mas protein were done by Jackson et al. (1988) by its transient expression in Xenopus frog oocytes and stable transfection of a mammalian cell line. Under voltage-clamp conditions, oocytes injected with Mas RNA exhibited a dose-dependent induction of an inward current in response to the angiotensins (Ang) I, II, and III, whereas in the Mas-transfected cells, stimulation with Ang II and III led to the mobilization of intracellular Ca2+ and to the initiation of DNA synthesis. On the basis of these results Mas was proposed to be a functional angiotensin receptor—a molecule whose identification had been pending for years. Although a number of further studies were in agreement with these assumptions (Jackson and Hanley, 1989; McGillis et al., 1989; Poyner et al., 1990; Andrawis et al., 1992), the activation of inward currents by Ang in Mas-mRNA injected oocytes was not inhibited by Ang antagonists (Jackson et al., 1988).

Moreover, Ambroz et al. (1991) could show that the intracellular Ca2+ increase in Mas-transfected cells after Ang II treatment was only observed in cells already expressing endogenously Ang II receptors. Therefore, doubts arose as to whether the Mas gene product, per se, is an Ang II receptor. Moreover, cloning of the Ang II receptor type 1 (AT1) in 1991 (Murphy et al., 1991; Sasaki et al., 1991) did not favor the original hypothesis of Ang II being a ligand for Mas. The later identification of Ang-(1–7) as a Mas agonist in 2003 and of the direct interaction between Mas and AT1 receptors in 2005 (Kostenis et al., 2005; Santos et al., 2007) partly explained the original observations of Jackson et al. (1988) in

ABBREVIATIONS: AKT, protein kinase B; Ang, angiotensin; AT1, Ang II receptor type 1; BAM, bovine adrenal medulla; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; eNOS, endothelial nitric oxide synthase; GPCR, G protein–coupled receptor; KO, knockout; Mrg, Mas-related gene; Mrgpr, Mas-related G protein–coupled receptor; MSH, melanocyte-stimulating hormone; PACAP, pituitary adenylate cyclase–activating peptide; POMC, proopiomelanocortin; RAS, renin-angiotensin system; RTA, rat thoracic aorta; siRNA, small interfering RNA; SNSR, sensory neuron-specific receptor; TM, transmembrane; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1; VSMC, vascular smooth muscle cells.
conserved part of the M1 muscarinic receptor or for Mas screening of cDNA libraries with probes either for the Mas1L called Mas, homology to et al., 1990) and of the with cloning of the rat thoracic aorta (RTA) gene (Ross et al., 1991; Schweifer et al., 1997). Imprinting of the maternally expressed Igf2r gene is controlled by an intronic imprint control element that contains the promoter of the long noncoding RNA, Airn (antisense Igf2r RNA noncoding), which overlaps the silenced paternal Igf2r promoter and partially the Mas gene in an antisense orientation (Wutz et al., 1997; Lyle et al., 2000). However, our work with Mas-deficient mice clearly demonstrated that Mas is biallelically expressed (Alenina et al., 2002a). Thus, because of the lack of strand selectivity in the reverse-transcription polymerase chain reaction assays used by Villar and Pedersen (1994) and Miller et al. (1997), the maternally imprinted RNA detected by them was most probably not the coding mRNA but the antisense RNA of the Mas gene as part of Airn. Altogether, these data demonstrate that Mas antisense RNA but not the mRNA underlies monoallelic expression in mouse and human.

The expansion of the Mrgr family started in 1990 with cloning of the rat thoracic aorta (RTA) gene (Ross et al., 1990) and of the mas-related gene (mrg, now called Mas1L) (Monnot et al., 1991) with 34 and 35% homology to Mas, respectively, both identified by screening of cDNA libraries with probes either for the conserved part of the M1 muscarinic receptor or for Mas itself. Ten years later, a comparative analysis of the transcriptome of dorsal root ganglia (DRG) of Ngn1-deficient mice, which lack a subclass of nociceptive neurons, enlarged the family by several genes expressed in such neurons, which were called Mas-related genes A, MrgA (Dong et al., 2001). Subsequent screening of murine DRG cDNA libraries and bioinformatic analysis of databases led to the identification of approximately 50 Mrgs (also called SNSR or sensory neuron-specific receptors by some authors) in mouse, rat, human, and macaque (Dong et al., 2001; Wittenberger et al., 2001; Lembo et al., 2002; Takeda et al., 2002; Zhang et al., 2005), which are now divided into several subfamilies and have been renamed according to a new nomenclature (http://www.guidetopharmacology.org): MrgrpA, B, C, D, E, F, G, H, and a primate-specific MRGPRX subfamily (Table 1). Interestingly, different subfamilies consist of multiple duplicated genes in different species. Primates have several MRGPRX genes, whereas rodents have multiple MrgrpA, B, and C genes, and even different rodent species differ in the amount of genes per subfamily (Dong et al., 2001; Zylka et al., 2003).

Although being structurally homologous to Mas, which is widely recognized as a component of the renin-angiotensin system (RAS), the function of most other Mrgrs turned out to be unrelated to the RAS with a broad range of peptidic and nonpeptidic ligands and implications in the perception of itch and pain. Nevertheless, our recent discovery of the putative new RAS peptide, alamandine [Ala1-Ang-(1–7)], which binds and activates mMrgprD (Lautner et al., 2013), suggests that the regulation of the RAS and of the cardiovascular system is not restricted to Mas among the Mrgrs.

In the following, we will summarize the current knowledge about Mrgrs, in particular about their evolution, their ligands, their possible physiologic functions, and their therapeutic potential. Despite being the most extensive, the part about Mas is far from being comprehensive, because the amount of literature about the

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**TABLE 1**
Mrgrp genes in different subfamilies and species

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Genes</th>
<th>Rat</th>
<th>Human</th>
</tr>
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<tbody>
<tr>
<td>MrgrpA</td>
<td>mMrgrpA1, mMrgrpA2, mMrgrpA3, mMrgrpA4, mMrgrpA5, mMrgrpA6, mMrgrpA7, mMrgrpA8, mMrgrpA9, mMrgrpA10</td>
<td>rMrgrpA</td>
<td>—</td>
</tr>
<tr>
<td>MrgrpB</td>
<td>mMrgrpB1, mMrgrpB2, mMrgrpB3, mMrgrpB4, mMrgrpB5, mMrgrpB6, mMrgrpX2</td>
<td>rMrgrpB2, rMrgrpB3, rMrgrpB4, rMrgrpB5, rMrgrpX2-like, rMrgrpX2-like</td>
<td>—</td>
</tr>
<tr>
<td>MrgrpC</td>
<td>mMrgrpX1 (mMrpC11)</td>
<td>rMrgrpX1 (rMrpC)</td>
<td>—</td>
</tr>
<tr>
<td>MrgrpX</td>
<td>—</td>
<td>—</td>
<td>hMRGPRX1, hMRGPRX2, hMRGPRX3, hMRGPRX4</td>
</tr>
<tr>
<td>MrgrpD</td>
<td>mMrgrpD</td>
<td>rMrgrpD</td>
<td>hMRGPDRD</td>
</tr>
<tr>
<td>MrgrpE</td>
<td>mMrgrpE</td>
<td>rMrgrpE</td>
<td>hMRGPRE</td>
</tr>
<tr>
<td>MrgrpF</td>
<td>mMrgrpF</td>
<td>rMrgrpF (RTA)</td>
<td>hMRGPRF</td>
</tr>
<tr>
<td>MrgrpG</td>
<td>mMrgrpG</td>
<td>rMrgrpG</td>
<td>hMRGPRG</td>
</tr>
<tr>
<td>Mas1L</td>
<td>—</td>
<td>—</td>
<td>hMAS1L (mrg)</td>
</tr>
<tr>
<td>MrgrpH</td>
<td>mMrgrpH (GPR90)</td>
<td>rMrgrpH</td>
<td>—</td>
</tr>
<tr>
<td>Mas</td>
<td>mMas</td>
<td>rMas</td>
<td>hMAS</td>
</tr>
</tbody>
</table>

Original names are given in parentheses. The subfamilies are grouped by evolutionary relationship (see Fig. 1).
functions of this protein exceeds the scope of a review about all Mrgrps. For more information about the eponymous gene of the Mrgrp family, the reader is referred to more comprehensive recent reviews (Xia and Lazartigues, 2010; Rabelo et al., 2011; Xu et al., 2011; Ferreira et al., 2012; Bader, 2013; Passos-Silva et al., 2013; Santos et al., 2013a; Simoes e Silva et al., 2013; Regenhardt et al., 2014a).}

II. Evolution of Mas-Related G Protein–Coupled Receptors

Mrgrps belong to the class A family of the superfAMILY containing all GPCRs with more than 800 genes [in the human genome (Fredriksson et al., 2003)]. We did not find members of the Mrgrp family outside Tetrapoda, neither in the complete genomes of bony fish (e.g., zebrafish, Danio rerio) nor in sequences from incompletely sequenced species, such as coelacanth or lungfishes, which are closer relatives to Tetrapoda than bony fish. Thus, we conclude that this family emerged after the divergence of bony fish from the line leading to Tetrapoda.

To pinpoint the different groups of sequences within the Mrgrps, we constructed a phylogenetic tree with the sequences of 10 human members and all homologs from a selection of completely sequenced Tetrapoda species (see Supplemental Table 1 for details). Following Dong et al. (2001), we used the Frp1 orthologs from mouse and the frog Xenopus, which, as members of a related subfamily, work as outliers and help to find the root of the branch containing the Mas related sequences (Fig. 1).

Here we summarize the most outstanding features derived from the tree, which are mainly confirming earlier publications (Dong et al., 2001; Lembo et al., 2002; Choi and Lahn, 2003; Zylka et al., 2003; Yang et al., 2005; Zhang et al., 2005). The branches of the tree have been swapped to present these facts roughly from top to bottom.

The top branch contains the two outliers (Frp1) followed by a frog-specific branch (5 members). The next branch includes Mas and rodent (but no primate) MrgrpH genes, and seems to be the oldest one because it includes a frog and two chicken members. Thus, the name Mas-related G protein–coupled receptors, Mrgrps, for the whole family, which historically arose since Mas was the first gene discovered, is also correct in evolutionary terms. The next branch contains three duplicated chicken members, indicating that it evolved in the lineage leading to aves. Then there are two branches that emerged later (no frog or chicken members): one that includes MrgrpD, MAS1L, MrgrpF, MrgrpE, and MrgrpG, and another with the human MRGPRX and the rodent-specific MrgrpA, B, and C genes. The MrgrpC subfamily, however, consists only of pseudogenes in mouse, except mMrgrC11 (Zylka et al., 2003). The two functional genes rMrgrC and mMrgrC11 have been renamed rMrgrpX1 and mMrgrpX1, respectively, despite the fact that they are not particularly close homologs of the human hMRGPRX1 gene (Fig. 1). Regardless, the Mrgrp gene family contains more pseudogenes than functional genes.

The most striking feature, however, is the expansion of Mrgrp subfamilies by repeated duplication events, which seem to have happened mainly independently in different branches of vertebrate evolution (Dong et al., 2001; Lembo et al., 2002). Whereas the A and B subfamilies were expanded in both the mouse and rat lineages, the C family got larger only in the mouse lineage; however, most of the duplicated genes of this subfamily were inactivated at some point (Zylka et al., 2003). In primates, the MRGPRX family was expanded (Choi and Lahn, 2003; Yang et al., 2005; Burstein et al., 2006). But such massive expansion can also be found in other Tetrapod species. Marsupials, such as the opossum and the Tasmanian devil, each have more than 20 Mrgrp-like genes, and reptiles, such as the Chinese soft turtle and the Anole lizard, have more than 15 orthologs, which are more homologous to each other in each species than to any other Tetrapod Mrgrp gene (ENSEMBL GeneTree ENSGT0055000074531). This massive duplication of Mrgrp genes may have been facilitated by insertion of retrotransposons close to the ancestral gene, because it was observed in the rat and mouse MrgrpA–C clusters (Dong et al., 2001; Zylka et al., 2003). However, such a mechanism will most probably not explain the effect for all vertebrates. Instead, the species-specific expansion of certain Mrgrp subfamilies strongly supports the notion that there was a powerful evolutionary drive to gain more Mrgrps, which was independently acting in several Tetrapod lineages. Accordingly, it has been suggested that the evolution of Mrgrps was adaptive by showing that the amino acid alterations mainly affected the extracellular domains of the receptors and thereby, most likely, the ligand binding sites (Choi and Lahn, 2003; Yang et al., 2005; Fatakia et al., 2011). Thus, the receptors seem to have been optimized for the different sets of ligands each species was independently facing in its environment. Later in this review, we will present a hypothesis that may explain this phenomenon as well as the appearance of the whole Mrgrp family only in Tetrapods.

III. Gene Structure and Expression of Mas-Related G Protein–Coupled Receptors

A. Chromosomal Localization and Gene Structure

All genes for Mrgrps other than Mas, Mas1L, and MrgrpH are located on a single chromosome in human (chromosome 11), rat (chromosome 1), and mouse (chromosome 7), forming in each case two large clusters in syntenic regions as a result of the evolutionary recent repeated duplications (Fig. 2). In rat, the genes for Mas and MrgrpH are also on chromosome 1, but in mouse these two genes are located on chromosome 17, and the human MAS and MAS1L genes are situated on chromosome 6, with
Fig. 1. Phylogenetic tree of the 10 human MRGPR proteins, together with Mrgr proteins from the selected Tetrapoda species *Pan troglodytes* (gorilla, 10 sequences), *Macaca mulatta* (rhesus monkey, 10 sequences), *Mus musculus* (mouse, 25 sequences), *Rattus norvegicus* (rat, 18 sequences), *Gallus gallus* (chicken, 4 sequences), *Xenopus tropicalis* (frog, 6 sequences), and two sequences from the related Frp1 subfamily (from frog and mouse) that were used as homologous outliers to fix the root of the Mrgr subfamily. Genes without associated protein products in the database (e.g., pseudogenes) were not included in the analysis. The 10 human MRGPR protein sequences were obtained from the NCBI Protein database (NCBI Resource Coordinators, 2013). All known genes of the same family were obtained for selected Tetrapoda species, via Homologene groups of the human proteins (NCBI Resource Coordinators, 2013) and through sequence searches (list of database identifiers in Supplemental Table 1). The homology to the Mrgpr subfamily was tested using reciprocal sequence searches to the human proteome to verify that the closest hit was not a protein from another close GPCR subfamily. Ultimately, an iterative process of producing increasingly large multiple sequence alignments and the corresponding phylogenetic tree allowed to discriminate genes that did not belong to the Mrgr subfamily. The final selection of genes (Supplemental Table 1) included 73 additional Tetrapoda sequences and 2 Frp1 members from a related GPCR subfamily that were used as outliers. The multiple sequence alignments were produced using the MUSCLE server at European Bioinformatics Institute (Edgar, 2004) and the phylogenetic tree was constructed using ClustalX (Larkin et al., 2007).
MAS1L in a region associated with diabetes type 1 (Aly et al., 2008).

Nearly all coding sequences of Mas and Mrgprs are present on a single exon, only hMRGPRF, rMrgprG, mMrgprx1, mMrgprB1, and all mMrgprAs, except mMrgprA4, have two coding exons, and mMrgprF and rMrgprF have 3. Nevertheless, in all genes there are several 5′-noncoding exons, with Mas itself carrying more than 10 of them (N. Alenina, unpublished data).

B. Expression of Mas-Related G Protein–Coupled Receptors in Dorsal Root Ganglion Neurons

The Mrgpr family was originally discovered by its expression in DRGs (Dong et al., 2001; Lembo et al., 2002). All members are expressed there and in the trigeminal ganglia in small diameter nociceptive neurons in mice; however, Mas, as well as MrgprF, G, and H are expressed at low levels (Cox et al., 2008; Costa et al., 2012; Avula et al., 2013; Cao et al., 2013a). mMrgpr-expressing neurons are nonpeptidergic, characterized by the presence of immunoreactivity for the Griffonia simplicifolia lectin IB4 and the glial-cell line–derived neurotrophic factor coreceptor c-Ret and the absence of transient receptor potential vanilloid 1 (TRPV1), substance P, and, in most but not all cases, of calcitonin gene-related peptide (CGRP) (Dong et al., 2001; Han et al., 2013) (Fig. 3). These neurons are unmyelinated and project to the superficial layer of the epidermis and the stratum granulosum, and in the dorsal horn of the spinal cord, the axons project to the inner lamina II (Fig. 3) (Zylka et al., 2005). They are developed from neural crest precursors in the last trimester of mouse embryogenesis and the first weeks of life (Lallemend and Ernfors, 2012). The transcription factors Runx1 and tlx3 are essential for the development of this class of neurons and directly regulate the expression of mMrgprD and mMrgprX1 starting around embryonic day 16 (Chen et al., 2006; Luo et al., 2007; Liu et al., 2008; Lopes et al., 2012; Xu et al., 2013). Runx1 also induces c-Ret in some neurons, which, in turn, switch on the expression of mMrgprA1, mMrgprA3, and mMrgprB4 in this subset of cells (Luo et al., 2007; Franck et al., 2011). After birth, Runx1 becomes a repressor of mMrgprAs, mMrgprB4, and mMrgprX1 and is turned off in this cell lineage, although it remains expressed in the mMrgprD subclass of neurons where it consequently leads to the exclusive expression of mMrgprD (Liu et al., 2008). Consequently, two subclasses of small-diameter nonpeptidergic neurons are defined in the adult mouse, the D compartment (30% of DRG neurons) expressing MrgprD and the A/B/C compartment (3–5% of DRG neurons) expressing the other Mrgprs (Fig. 3) (Liu et al., 2008; Abdel Samad

![Fig. 2. Chromosomal (Chr.) localizations of Mrgpr genes in human, rat, and mouse. The genes are shown as arrows in their direction of transcription. The deleted fragment in the cluster-knockout mice (Liu et al., 2009) is marked.](image-url)
et al., 2010). The Mrgprs, however, are not essential for the development and projection of these neurons, because mice deficient for mMrgprD (Zylka et al., 2005) (Table 2) and cluster-knockout mice lacking 12 Mrgpr genes (Liu et al., 2009) (Fig. 2; Table 2) show a normal amount and wiring of the corresponding cells.

The MrgprA/B/C compartment can be further subdivided into subsets of cells by the differential expression and coexpression of mMrgprAs, Bs, and X1 (Dong et al., 2001). A recent transgenic mouse study defined an mMrgprA3-expressing subset, which was peptidergic and expressed CGRP (Han et al., 2013). In addition, first evidence showed that Smad4 and Bone morphogenetic peptide signaling is essential for the expression of mMrgprB4 in another subset of neurons (Liu et al., 2008). Mouse MrgprX1 is always coexpressed with an MrgprA and never with MrgprBs in DRG neurons (Liu et al., 2008). In the rat, the situation is different (Lembo et al., 2002; Zylka et al., 2003): rMrgprX1 is never coexpressed with rMrgprA and rMrgprD is always coexpressed with rMrgprA and, in some cells, with rMrgprB4. Furthermore, rMrgprX1-positive DRG neurons in the rat are also peptidergic (Lembo et al., 2002; Hager et al., 2008). In humans and macaques also, all the MRGPRX orthologs and MRGPRD are found exclusively expressed in DRG neurons (Dong et al., 2001; Lembo et al., 2002; Robas et al., 2003; Zhang et al., 2005), but not much is known about the characteristics of these cells, despite the fact that in these species a significant proportion of them also seem to be peptidergic and express TRPV1, CGRP, and substance P (Zhang et al., 2005).

C. Expression of Mas-Related G Protein–Coupled Receptors in Mast Cells

A second major cell type expressing several Mrgprs in considerable amounts in addition to DRG neurons is the mast cell. In humans, mainly hMRGPRX2 is expressed in human umbilical cord mast cells as well as small amounts of hMRGPRX1, but not hMRGPRX3 and hMRGPRX4 (Tatemoto et al., 2006; Subramanian et al., 2011b). In rats, expression of rMrgprB3 and B8 was detectable in peritoneal mast cells but only low levels of rMrgprB1, B2, B6, and B9 were detected, and neither rMrgprA, rMrgprX1, nor any other rMrgprB...
was expressed in this cell type (Tatemoto et al., 2006). In contrast, rMrgprX1 was detected by immunohistochemistry in skin mast cells (Hager et al., 2008). mMrgprB10 was found in mucosal mast cells in mouse intestine in inflammatory conditions (Avula et al., 2013). The presence of other Mrgprs (A and E–H) was not tested in mouse mast cells other than in one study showing the absence of mMrgprA3 transcripts (Liu et al., 2009).

### D. Expression of Mas-Related G Protein–Coupled Receptors in Other Tissues

Although most mouse MrgprAs, MrgprBs, and MrgprX1 seem to be exclusively expressed either in DRG neurons (Dong et al., 2001) or in mast cells, some Mrgprs have been shown to be expressed also in other tissues. Only low expression of mMrgprB3 and mMrgprB8 has been described for bladder and testis, respectively (Huang et al., 2013). Human and mouse MrgprE are widely expressed in neurons in the brain, and hMRGPRE is expressed also in placenta but not in any other human tissue (Zhang et al., 2005; Cox et al., 2008). rMrgprE is mainly expressed in DRG but also in the brain, spinal cord, and testis (Milasta et al., 2006). hMRGPRF, mMrgprF (originally named RTA), and mMrgprH were shown to be mainly expressed in cerebellum and smooth muscle–containing tissues, such as large vessels (Ross et al., 1990) (Patent WO2003004528A1, 2001). More recently, hMRGPRF was found to be drastically upregulated in monocyte-to-macrophage differentiation (Hohenhaus et al., 2013). mMrgprH was originally discovered as GPR90 in 2001, and its mRNA was detected by Northern blot mainly in heart but less so in kidney (Wittenberger et al., 2001). hMRGPRX2 expression was highest in DRGs and could also be found in testis and gut, but less so in several other tissues (Robas et al., 2003; Table 2).

### Animal models with alterations in an Mrgpr

<table>
<thead>
<tr>
<th>Name/Affected Mrgpr</th>
<th>Genetic Alteration</th>
<th>References</th>
<th>Phenotype Described</th>
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<tr>
<td>mMrgprA1-tetO-TG</td>
<td>Knockout mouse</td>
<td>Xu et al., 2000</td>
<td>+</td>
</tr>
<tr>
<td>mMrgprA2-KO</td>
<td>Knockout mouse</td>
<td>Han et al., 2013</td>
<td>+</td>
</tr>
<tr>
<td>mMrgprA3-Cre</td>
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<td>Han et al., 2013</td>
<td>+</td>
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<tr>
<td>mMrgprB4-PLAP</td>
<td>Knockin of placental alkaline phosphatase, knockout</td>
<td>Liu et al., 2007</td>
<td>+</td>
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<tr>
<td>mMrgprB4- eGFPf -2A-Flp</td>
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<td>Vrontou et al., 2013</td>
<td>+</td>
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<tr>
<td>mMrgprD-KO</td>
<td>Knockin of tdTomato-2A-Cre-recombinase, knockout</td>
<td>Vrontou et al., 2013</td>
<td>+</td>
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<tr>
<td>mMrgprD-eGFPf-Cre</td>
<td>Knockin of Farnesyl-enhanced GFP knockin mouse, knockout</td>
<td>Zylka et al., 2005</td>
<td>+</td>
</tr>
<tr>
<td>mMrgprD-eGFPf</td>
<td>IRES-Farnesyl-enhanced GFP knockin mouse, no knockout</td>
<td>Zylka et al., 2005</td>
<td>+</td>
</tr>
<tr>
<td>mMrgprD-eGFPf-PLAP</td>
<td>Knockin of placental alkaline phosphatase, knockout</td>
<td>Zylka et al., 2005</td>
<td>+</td>
</tr>
<tr>
<td>mMrgprD-ChR2-Venus</td>
<td>Knockin of channel rhodopsin-Venus, knockout</td>
<td>Wang and Zylka, 2009</td>
<td>+</td>
</tr>
<tr>
<td>mMrgprF-KO</td>
<td>Knockout mouse</td>
<td>Cox et al., 2008</td>
<td>+</td>
</tr>
<tr>
<td>mMrgprG-KO</td>
<td>Knockout mouse</td>
<td>id=TF0924</td>
<td>—</td>
</tr>
<tr>
<td>mMrgprH-KO</td>
<td>Knockout mouse</td>
<td>id=TF0274</td>
<td>—</td>
</tr>
<tr>
<td>mMrgprX1-KO</td>
<td>Knockout mouse</td>
<td>id=TF2901</td>
<td>—</td>
</tr>
<tr>
<td>hMRGPRX3-actin-TG</td>
<td>Transgenic rat with hMRGPRX3 gene under chicken actin promoter control</td>
<td>Kaisho et al., 2005</td>
<td>+</td>
</tr>
</tbody>
</table>

DTA, diphtheria-toxin A; DTR, diphtheria-toxin receptor; GFP, green fluorescent protein; IRES, internal ribosomal entry site; PLA, phospholipase A.
Allia et al., 2005; van Hagen et al., 2008). This was in contrast to the original description of the hMRGPRX family showing no expression in any other tissue than in DRGs (Lembo et al., 2002). rMrgprD expression was also found outside the main expressing tissue, DRG, in tissues such as testis, urinary bladder, uterus, and arteries, but less so in other tissues, including cerebellum, gut, and fat (Shinohara et al., 2004; Milasta et al., 2006). Rat MrgprX1 and rMrgprA were also most highly expressed in DRG and trigeminal ganglia but also broadly in nearly every tested tissue (Bender et al., 2002; Gustafsson et al., 2005). In these studies, quantitative reverse-transcription polymerase chain reaction was used with total RNA from the tissues; thus, the expressing cell type was not distinguished and mast cells cannot be excluded. However, the localization of rMrgprA expression in the vasculature and in the collecting duct of the kidney was recently reported in detail, and an inhibition of vasopressin signaling by rMrgprA was postulated (Kishore et al., 2013).

One group has systematically analyzed the expression of all Mrgprs in mouse intestine (Avula et al., 2011, 2013). With a few exceptions, they are all expressed on different types of enteric neurons in varying levels; however, the function of Mrgprs in these cells is elusive. Under normal conditions, mMrgprA4 and mMrgprA7 are most highly expressed, and in inflammation, mMrgprA2, mMrgprA5, mMrgprB2, mMrgprB10, mMrgprX1, and mMrgprD get upregulated, which is partly due to the invasion of mast cells (mMrgprB10) (Avula et al., 2013).

E. Expression of Mas

The founder gene of the Mrgpr family, Mas, is more broadly expressed than most other Mrgprs. Highest expression is found in brain and testis but low levels are also detected in other organs. In the rat and mouse brain, Mas transcripts are localized in the hippocampus and cerebral cortex (in particular in the dentate gyrus), the CA3 and CA4 areas of the hippocampus, the olfactory tubercle, the piriform cortex, and the olfactory bulb, but also at lower levels all over the neocortex and especially in the frontal lobe (Young et al., 1988; Bunnemann et al., 1990). More recently, Mas expression was also discovered in regions of the rat brain important for cardiovascular regulation (Becker et al., 2007) and in the monkey retina (Kitaoka et al., 1994). Mas expression in the brain starts in the rat at postnatal day 1, with a pattern similar to that seen in the adult (Martin et al., 1992), and stays plastic because it is transiently upregulated after seizure episodes in the hippocampus (Martin and Hockfield, 1993).

In the rodent testis, Mas expression is not detectable in newborn animals, but rather, starts a few weeks after birth and continuously increases during puberty (Metzger et al., 1995; Alenina et al., 2002b). On the cellular level, Mas is confined to Leydig and Sertoli cells, with a clear preference for Leydig cells (Alenina et al., 2002b).

However, Mas expression was also discovered in other tissues of mice and rats, such as heart, kidney, lung, liver, spleen, tongue, and skeletal muscle (Villar and Pedersen, 1994; Metzger et al., 1995; Ferrario et al., 2005; Alenina et al., 2008). This ubiquitous low-level presence of Mas mRNA may partly be due to its expression in the endothelial layer of vessels in different organs, as has been shown for brain (Kumar et al., 1996), heart (Alenina et al., 2008), and corpus cavernosum (Goncalves et al., 2007), supporting an important role of this protein in the function of the endothelium.

IV. Structure, Ligands, and Signaling of Mas-Related G Protein-Coupled Receptors

Several receptors of the Mrgpr family, when being analyzed for ligand-induced activation, showed a high constitutive activity (Chen and Ikeda, 2004; Burstein et al., 2006; Uno et al., 2012). The structural reason for this ligand-independent signaling is not yet clear because there is no experimentally generated three-dimensional structure for any of the Mrgpr family members. Three-dimensional structures of Mas, rMrgprA, and mMrgprX1 have been predicted from the comparison with the rhodopsin structure (Heo et al., 2007a,b; Prokop et al., 2013). However, later trials to validate the deduced predictions for the agonist binding sites of rMrgprA by specifically mutating them failed, raising doubts on the validity of the structure predictions based on remotely related homologs (Knope et al., 2013). Nevertheless, it is obvious from the primary sequence that all Mrgprs lack otherwise highly conserved features among class A GPCRs, which may be involved in blocking agonist-independent activity: the Cys-Cys bridge from transmembrane domain (TM) 3 to extracellular domain 2 (Knope et al., 2013; Prokop et al., 2013) and the so-called “ionic-lock” from the amino acids E/DRY in TM3 to a glutamate in TM6 (Rosenbaum et al., 2009). This ionic lock has been shown to inhibit activation and constitutive activity in rhodopsin and some class A GPCRs (Scheer et al., 1997; Palczewski et al., 2000), but not in others (Schneider et al., 2010). However, in the absence of a crystal structure of any Mrgpr, the molecular basis of the intrinsic activity of these receptors cannot be resolved.

Despite their high constitutive activity, Mrgprs can be further stimulated by agonists, but for most of them, only a single agonist could be identified. Moreover, even closely related members of the Mrgpr family showed quite divergent ligand specificities. However, all available data about agonists are based on gain-of-function experiments in transfected cells. There currently is no proof that any of the described agonists is the natural ligand of the respective Mrgpr. Furthermore, in most cases, the effective concentrations of the agonists are hardly reached in vivo. Thus, nearly all of the Mrgprs still have to be deorphanized. The only exceptions may be Mas itself and MrgprD, for which
angiotensin (Ang)-(1–7) and β-alanine, respectively, have been described as agonists, the physiologic effects of which are absent in corresponding knockout mice (Santos et al., 2003; Rau et al., 2009) (Tables 2 and 3). For mMrgrpRX1, the absence of effect of its possible endogenous ligand bovine adrenal medulla (BAM) peptide 8–22 has been shown in the cluster knockout mice (Fig. 2; Table 2) lacking 12 Mrgrp genes, including the one for mMrgrpRX1; thus, the data are not totally conclusive (Guan et al., 2010). For no other Mrgrp has the absence of effect of a naturally existing ligand in a deficient animal model been shown. For hMRGPRX2, the knockdown by short hairpin RNA in a mast cell line also leads to an attenuation of the effects of known agonists, but again, these experiments are done in cell culture with relatively high agonist concentrations and do not reflect the in vivo situation (Subramanian et al., 2013). Therefore, and based on the high constitutive activity of Mrgrps, the hypothesis has been coined that their basic state is the active one and that their yet unknown natural ligands should be inverse agonists, which inhibit their activity (Burstein et al., 2006). Nevertheless, no screen of naturally occurring inverse agonists has yet been performed, and thus, we may still miss the physiologic ligands of Mrgrps.

Another possibility is that Mrgrps were not evolved for the recognition of endogenous ligands but for eliciting itch or pain for warning an organism of noxious substances released to its skin by toxic plants or animals or by parasitic infections. Evidence for this hypothesis are 1) their relatively high promiscuity with best affinities for itch-inducing substances; 2) their nearly exclusive expression in sensory neurons and mast cells in the skin, the main cellular components of the itch pathway; and 3) their evolutionary appearance in tetrapods together with arms and legs that allow scratching. Fish cannot fight skin invaders by scratching, thus, itch-inducing sensory receptors would be useless. However, on land, it is a great advantage to be able to remove parasites or other noxious substances before they create more harm. Because the parasites coevolved with the Tetrapods, and as a consequence each species faced different parasitic challenges, the parallel adaptive evolution of the Mrgrps by multiple independent duplication and expansion events in different species could also be explained. Nevertheless, some of the Mrgrps have endogenous ligands and elicit effects beyond itch and pain. Thus, the evolution of the Mrgrps may not have only been driven by the invention of itch.

In the following, the agonists described for the different Mrgrps are listed (Table 3).

A. Mas

After the initial erroneous description of Ang II as Mas-agonist (Jackson et al., 1988) (see section 1), we characterized Ang-(1–7) as functional ligand for this GPCR (Santos et al., 2003). In this study, specific binding of [125I]Ang-(1–7) to kidney sections was annulled by genetic deletion of Mas. Specific binding of [125I]Ang-(1–7) was also reported in cells transfected with fluorescently labeled Mas (Gironacci et al., 2011). In these two studies, the Ang-(1–7) antagonist, A-779 (Santos et al., 1994), competed for the binding of [125I]Ang-(1–7) to the Mas-transfected cells with an IC50 close to the EC50 of [125I]Ang-(1–7). Nevertheless, standard pharmacologic binding experiments with [125I]Ang-(1–7) are limited by the high nonspecific binding of the radiolabeled peptide, possibly due to the introduction of the bulky iodine isotope in the peptide sequence, which may favor low affinity binding to Mas-unrelated sites. In contrast, the nonspecific binding observed in experiments using fluorescent Ang-(1–7) is very low. By using this method, a significant, specific binding of Fam-Ang-(1–7) to Mas-transfected CHO cells has been reported (Pinheiro et al., 2004; Savergnini et al., 2010; Jankowski et al., 2011).

Accordingly, specifically binding of Fam-Ang-(1-7) to platelets (Fraga-Silva et al., 2008) and Leydig cells in testes (Leal et al., 2009) is absent in Mas-knockout (KO) mice. The blockade of the effects of the Ang-(1–7) mimic, AVE0991, by A-779 (Wiemer et al., 2002) and the functional effects produced by Ang-(1–7) in Mas-transfected cells (Santos et al., 2003; Pinheiro et al., 2004; Sampaio et al., 2007b) are in keeping with the significant body of evidence that Ang-(1–7) is an endogenous ligand for Mas. A recent in silico docking experiment explains the different affinities of Mas for Ang II and Ang-(1–7) (Prokop et al., 2013): the additional Phe8 of Ang II compared with Ang-(1–7) interacts with amino acid residues of the AT1 and AT2 receptors, which lack in Mas.

As further evidence for the specific interaction of Ang-(1–7) with Mas, we showed that Mas-KO mice lost responsiveness to Ang-(1–7) with respect to vasodilation, antidiuresis, and antithrombosis (Santos et al., 2003; Fraga da Silva et al., 2008, 2011). Likewise, the antihypertrophic effect of Ang-(1–7) in cardiomyocytes was blunted by downregulation of Mas by antisense RNA (Tallant et al., 2005). Nevertheless, there are reports about other receptors for Ang-(1–7), such as the Ang II AT2 receptor (De Souza et al., 2004; Walters et al., 2005) and other natural ligands for Mas (Dong et al., 2001; Gembardt et al., 2008; Jankowski et al., 2011). Thus, it is not clear whether the interaction between Ang-(1–7) and Mas is mutually exclusive.

It should be pointed out that all the described effects of Ang-(1–7) or AVE0991 in Mas-transfected cells are related to a Ca2+-independent activation of nitric oxide synthase (via the phosphatidylinositol 3-kinase/protein kinase B [AKT] pathway) or to activation of phospholipase A2 (Santos et al., 2003; Pinheiro et al., 2004; Sampaio et al., 2007b; Savergnini et al., 2013; Than et al., 2013) (Fig. 4). Our recent phosphoproteomic study identified several further possible signaling pathways of cells induced by Ang-(1–7) stimulation, such as the
forkhead box protein O1 (Verano-Braga et al., 2012). It is not yet clear how these early signaling events induced by Ang-(1–7) are linked to the NO and phospholipase A2 pathway. In the phosphoproteomic study, we also saw an Ang-(1–7)–induced dephosphorylation of mitogen activated protein kinases in contrast to some cell types of the kidney, in which an activation was observed (Zimpelmann and Burns, 2009; Liu et al., 2012b). Some kidney cells also showed an increase in cAMP and activation of protein kinase A after Ang-(1–7) challenge, suggesting Gs activation (Magaldi et al., 2003; Liu et al., 2012b). Nevertheless, in these studies there is only indirect evidence that Mas is involved in the Ang-(1–7) effects based on the fact that they could be inhibited with A-779.

### TABLE 3

Ligands of Mrgprs

Some structures are shown on the bottom of the table. The EC50 values are different depending on the assay system used in each publication and can only be taken as estimation concerning the specificity of the receptors.

<table>
<thead>
<tr>
<th>Mrgpr</th>
<th>Ligands</th>
<th>Structure or Peptide Sequence</th>
<th>EC50</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mas</td>
<td>Angiotensin-(1–7)</td>
<td>DRVYIHP</td>
<td>N.D.</td>
<td>Dong et al., 2001; Santos</td>
</tr>
<tr>
<td></td>
<td>NPFF</td>
<td>FLFQPQRF-amide</td>
<td>0.4 nM</td>
<td>et al., 2003; Jankowski</td>
</tr>
<tr>
<td></td>
<td>Angioprotecin</td>
<td>PEYYIHP</td>
<td>N.D.</td>
<td>et al., 2011</td>
</tr>
<tr>
<td>mMrgprA1</td>
<td>FLRF</td>
<td>FLRF-amide</td>
<td>0.02–0.4</td>
<td>Dong et al., 2001; Han</td>
</tr>
<tr>
<td></td>
<td>NPFF</td>
<td>FLFQPQRF-amide</td>
<td>0.2–2.1</td>
<td>et al., 2002; Wang et al., 2006</td>
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<td>Salusin-β</td>
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<td>mMrgprA3</td>
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<td>see below</td>
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<td>Liu et al., 2009</td>
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<td>mMrgprA4</td>
<td>NPAF</td>
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<td>ACTH</td>
<td>39 amino acids</td>
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<td></td>
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<td>von Kügelgen et al., 2008</td>
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<td></td>
<td></td>
<td></td>
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<td>Bender et al., 2002; Gorzalka et al., 2005</td>
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<td>mMrgprX1</td>
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<td>0.017</td>
<td>Han et al., 2002; Liu et al., 2011; He et al., 2014</td>
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<tr>
<td></td>
<td>γ2-MSH</td>
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<td>FLRF</td>
<td>FLRF-amide</td>
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<tr>
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<td>NPFF</td>
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<td>SLGRL</td>
<td>SLGRL</td>
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<td>YGGMFRVGRPEWMDYQKYRG</td>
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<td>rMrgprX1</td>
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<td>MrgrD (rat, mouse, and human)</td>
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<td>Alamanidine</td>
<td>ARVYIHP</td>
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<td>Lembo et al., 2002</td>
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<td>YVMGHFRWDRF-amide</td>
<td>0.013</td>
<td>Lembo et al., 2002; Tatemoto et al., 2006; Hager et al., 2008; Liu et al., 2009; Kashem et al., 2011</td>
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<tr>
<td>SNSR3</td>
<td>BAMB22</td>
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<tr>
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<td>0.028</td>
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<td>YVMGHFRWDRF-amide</td>
<td>0.6</td>
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<td>Chloroquine</td>
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<td>hMRGPRX2</td>
<td>MCD</td>
<td>MCIICKNGKPLPGFICRKICMMEETHamide</td>
<td>1.25</td>
<td>Robas et al., 2003; Kamohara et al., 2005; Nothacker et al., 2005; Tatemoto et al., 2006; Subramanian et al., 2013</td>
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<td>PACAP</td>
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<td>Somatostatin</td>
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<td></td>
<td>Cathelicidines</td>
<td>35–40 amino acids</td>
<td>65</td>
<td></td>
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</table>

N.D., not determined; NPAF, neuropeptide AF; NPFF, neuropeptide FF.
On the other hand, there is no evidence for an effect of Ang-(1–7) and AVE0991 on the stimulation of other Mas-mediated signaling pathways such as Ca\(^{2+}\) influx/release or inositol 1,4,5-trisphosphate accumulation (Dias-Peixoto et al., 2008; Shemesh et al., 2008; Gomes et al., 2012; Zhang et al., 2012) (Fig. 4), which are particularly pronounced in strongly Mas-overexpressing cells. These Ang-(1–7) independent signaling pathways are most likely the ones induced by the constitutive activity of the receptor and are responsible for its transforming activity. Some studies have found ligands of Mas that modulate these pathways, especially at high concentrations (> 1 \(\mu\)M), such as the agonists MBP7 (Bikkavilli et al., 2006), AR234960 (Zhang et al., 2012), and CGEN-856S (P61 in Shemesh et al., 2008) with serine instead of cysteine (Savergnini et al., 2010), and the inverse agonist, AR244555 (Zhang et al., 2012). Interestingly, CGEN-856S has been shown to activate both pathways, Ca\(^{2+}\) and AKT—but Ca\(^{2+}\) only in Go16 transfected cells with a diverted signaling (Shemesh et al., 2008; Savergnini et al., 2013). Thus, the still limited evidence about Mas signaling suggests that Mas behaves like other GPCRs, for which it has also been shown that different ligands activate different signaling pathways, a phenomenon called functional selectivity or biased signaling (Urban et al., 2007; Kenakin and Christopoulos, 2013) (Fig. 4).

**B. hMRGPRX1 (hSNSR4)**

hMRGPRX1 stably expressed on mast cells was shown to be activated by BAM22, a product of proenkephalin A metabolism, and by compound 48/80, a classic mast cell activator (Kashem et al., 2011) (Table 3). This was consistent with the findings of Lembo et al. (2002), who had cloned hMRGPRX1 as SNSR4 and had discovered that it can be activated by peptides derived from proenkephalin A at an EC\(_{50}\) of 16 nM. The signaling of this receptor was not abolished by pertussis toxin and, thus, was transmitted by Gq proteins. When they analyzed the binding domain on BAM22, they found that the first 7 amino acids are not relevant for hMRGPRX1 binding, in contrast to its binding to opioid receptors. The peptide lacking this Met-enkephalin motif, BAM8–22, had the same affinity to hMRGPRX1 as BAM22 itself. BAM8–22 also activated rat neurons stably expressing hMRGPRX1 (Chen and Ikeda, 2004). In these cells, hMRGPRX1 inhibited the activity of high-voltage-activated Ca\(^{2+}\) and M-type K\(^+\) channels. Whether this is also true in naturally hMRGPRX1 expressing cells remains elusive. First small-molecule antagonists for hMRGPRX1 have also already been developed (Kunapuli et al., 2006; Bayrakdarian et al., 2011).

**C. rMrgprX1 (rSNSR1, rMrgC), mMrgprX1 (mMrgC11)**

Peptides derived from proenkephalin A and proopiomelanocortin (POMC) have been shown to bind cells expressing rMrgprX1 (Grazzini et al., 2004) and mMrgprX1 (Han et al., 2002) with EC\(_{50}\) values of 11 to 150 nM (Table 3). In particular, the POMC derivatives, \(\gamma\)-1- and \(\gamma\)-2-melanoocyte-stimulating hormone (MSH), show highest affinity and selectivity for these receptors.

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**Fig. 4.** Signaling of Mas. The most important known signaling pathways of Mas involve phospholipase A (PLA) to generate arachidonic acid (AA) and phosphoinositide 3 kinase (PI3K) and AKT to activate eNOS by phosphorylation at serine 1177 and dephosphorylation at threonine 495. Ang-(1–7), AVE0991, CGEN-856S, and angiotensin have been shown to activate these pathways and A-779 antagonizes these actions. Ang-(1–7) also activates forkhead box protein O1 by dephosphorylation at serine 236. The ligands for the PLA and PI3K pathways (other than CGEN-856S), however, do not activate a phospholipase C (PLC)/Ca\(^{2+}\) signaling pathway observed in Mas-overexpressing cells, which in some studies were additionally transfected with promiscuous G proteins. This pathway is activated by the ligands neuropeptide FF (NPFF), AR234960, MPF7, and CGEN-856S and blocked by AR244555. Thus, different Mas ligands activate distinct signaling pathways, a phenomenon called biased agonism.
γ2-MSH elicits Ca²⁺ release but no change in cAMP metabolism in mMrgrpX1 expressing cells, supporting Gq coupling of this receptor (Han et al., 2002). Because rMrgrpX1 and mMrgrpX1 also bind the proenkephalin A metabolite BAM22, they may be functional orthologs to the human MRGPRX1 receptor (see above). However, one study unraveled a difference between the human and the rodent MrgrpX1s: Solinski et al. (2010) showed that hMRGPRX1 is not internalized after agonist binding, in contrast to mMrgrpX1 and rMrgrpX1.

Another functionally important ligand of mMrgrpX1 is the peptide SLIGRL, which is released from the protease activated receptor 2 upon trypsin digestion (Liu et al., 2011), and which is responsible for the itch response induced by intradermal trypsin injections. He et al. (2014) recently showed that the hMRGPRX1 antagonist, 2,3-disubstituted azabicyclo-octane (Kunapuli et al., 2006), also blocks rMrgrpX1 and mMrgrpX1. They also describe a first synthetic MrgrpX1 agonist, JHU58 (He et al., 2014).

D. mMrgrpA1, mMrgrpA4, mMrgrpX1 (mRgC11)

Already the first description of Mrgrps had discovered that peptides terminating with the amino acids RF/Y-amide or RF/YG are ligands for some members of this family, including Mas itself (Dong et al., 2001). Such peptides include the already mentioned BAM and MSH derivatives. In addition, the tetrapeptide FLRF and neuropeptide AP (NPAF) (Table 3) were shown to bind to mMrgrpA1 and mMrgrpA4, respectively, with EC₅₀ values of lower than 100 nM (Dong et al., 2001). The authors could also demonstrate that these receptors desensitize after activation with the preferred ligand. Moreover, mMrgrpX1, in addition to its affinity for proenkephalin A and POMC metabolites, is also activated by RF-amide peptides with comparable EC₅₀ as the MrgrpA family members (Han et al., 2002). These peptides elicit Ca²⁺ influx in cells expressing mMrgrpA1 or mMrgrpX1 only depending on Gq proteins.

A high-throughput approach testing 16,000 compounds identified two other agonists for MrgrpA1, which, however, are not further chemically defined (Zhang et al., 2007). Furthermore, salusin-β, a peptide of yet not well defined functions, has been characterized as agonist for mMrgrpA1, but no MRGPR ortholog in humans seems to react on this substance, questioning the physiologic importance of this finding (Wang et al., 2006).

The specific activation of mMrgrpA1 by FLRF and its normally very restricted expression in DRGs was exploited for the generation of transgenic mice in which Ca²⁺ signaling in all astrocytes can be activated by FLRF (Table 3). These animals were created by breeding mice expressing the tetracycline repressor under the control of the astrocyte-specific glial-fibrillary acidic protein promoter, with animals carrying the mMrgrpA1 coding region under the control of the tet operator (Fiacco et al., 2007) (Table 2). When they are given doxycycline, the mice ectopically express mMrgrpA1 only on astrocytes and have been used to study the role of this cell type in synaptic transmission by applying the agonist, FLRF (Fiacco et al., 2007; Agulhon et al., 2010; Lacar et al., 2012; Xie et al., 2012; Cao et al., 2013b; Devaraju et al., 2013; Wang et al., 2013b).

E. mMrgrpA3, rMrgrpA, hMRGPRX1

There is yet no endogenous ligand known for MrgrpA3. However, it has been shown to be activated by the antimalaria compound, chloroquine (Table 3), and to be responsible for its pruritogenic actions (Liu et al., 2009, 2011; Wilson et al., 2011). When activated, mMrgrpA3 opens transient receptor potential (TRP) A1 channels via Gβγ release (Wilson et al., 2011). In the rat and in humans, rMrgrpA and hMRGPRX1, respectively, seem to be the responsible receptors for chloroquine sensing (Liu et al., 2009). EC₅₀ values are in the high micromolar range, which is sufficient to explain the itch racking chloroquine-treated patients, because the drug accumulates in the skin (Liu et al., 2009).

F. hMRGPRX2, rMrgrpB3

hMRGPRX2 on mast cells was shown to be activated by compound 48/80 (Kashem et al., 2011) (Table 3). Moreover, an artificial C3a receptor agonist, E7, also stimulates hMRGPRX2 (Kashem et al., 2011). The same group showed that antimicrobial peptides of two classes, β-defensins (e.g., hBD2 and hBD3) and cathelicidines (e.g., LL-37), are ligands at the hMRGPRX2 receptor on human mast cells and activate Gq- and Gi-dependent signaling pathways (Subramanian et al., 2011a, 2013). On rat mast cells, the rMrgrpB3 receptor probably confers this function, but it is not linked to Gq proteins, and there are no receptors for these peptides detectable in functional assays using the mouse defensin, mCRAMP, on mouse mast cells (Subramanian et al., 2013). Another group has confirmed in human and rat mast cells that hMRGPRX2 and rMrgrpB3 (but not rMrgrpA, B2, B6, B8, and B9), respectively, are transmitting the degranulating action of numerous basic substances including mast cell degranulating peptide, substance P, pituitary adenylate cyclase–activating peptide (PACAP), and platelet activating factor 4, with EC₅₀ values in the low micromolar range (Tatemoto et al., 2006).

Furthermore, cortistatin-14 (EC₅₀: 25 nM) and -17 (EC₅₀: 99 nM) have been shown to be agonistic ligands of hMRGPRX2 (Robas et al., 2003) (Table 3), but their tissue distribution does not overlap much and there is no hMRGPRX2 homolog in rodents, which challenges the physiologic relevance of this finding (Kamohara et al., 2005; Siehler et al., 2008; van Hagen et al., 2008). Cortistatin increases Ca²⁺, but not cAMP, in hMRGPRX2-expressing cells, arguing in favor of Gq coupling of hMRGPRX2 (Robas et al., 2003). However, the same publication lists several other peptides with only slightly higher EC₅₀ (>100 nM). Furthermore, the
poadrenomedullin C-terminal peptides, PAMP-12 and -20, showed comparable affinities to hMRGPRX2, and activated Gi and Gq proteins in hMRGPRX2-expressing cells (Kamohara et al., 2005; Nothacker et al., 2005). These data again show that Mrgprs are quite promiscuous and that ligands bind these receptors with considerably lower affinity than the ligands of most other GPCRs. Nevertheless, the expression of short hairpin RNA against hMRGPRX2 leads to an attenuation of the effects of cortistatins and b-defensins in a mast cell line (Subramanian et al., 2013), which suggests a physiologic role of this receptor in mast cell degranulation by these agonists.

**G. hMRGPRX3 (hSNSR1), hMRGPRX4 (hSNSR6)**

For hMRGPRX3 (hSNSR1) and hMRGPRX4 (hSNSR6), no ligand has been described yet.

**H. rMrgrpA (rMrgrpX3), mMrgrpA9, mMrgrpA10**

The only rat MrgrpA receptor (also called rMrgrpX3) has been shown to bind adenine (Table 3) with a dissociation constant of 24 nM and to inhibit cAMP generation by Gi protein coupling (Bender et al., 2002). In mouse, two members of the MrgrpA subfamily, mMrgrpA9 and A10, have also been described to bind adenine, albeit with somewhat lower affinities (Kd: 113 and 286 nM, respectively), and to inhibit cAMP generation (von Kügelgen et al., 2008; Knospe et al., 2013; Thimm et al., 2013). The same group has discovered an adenine binding homolog in Chinese hamster, but there has not yet been any adenine receptor of the Mrgrp family found in humans. The binding of adenine to its receptors is very specific, because even small molecular alterations markedly reduce affinity of the ligand for MrgrpA and MrgrpA10 (Gorzalka et al., 2005; von Kügelgen et al., 2008; Borrmann et al., 2009). A first small-molecule antagonist for rMrgrpA has also been developed (Kishore et al., 2013).

**I. MrgrpD, MrgrpE**

The first ligand described for MrgrpD of human, rat, mouse, and monkey was b-alanine (Shinohara et al., 2004) (Table 3). This amino acid analog is produced in the liver from uracil or from dietary carnosine by the enzyme carnosinase (Stellingwerff et al., 2012). MrgrpD activation is transmitted by both Gq and Gi proteins onto changes of intracellular Ca2+ and cAMP, respectively (Shinohara et al., 2004). The EC50 of b-alanine for a rise in intracellular Ca2+ in MrgrpD-expressing cells was found to be between 14 and 44 μM, depending on the species (Shinohara et al., 2004). b-Alanine binding leads to rapid internalization of MrgrpD (Shinohara et al., 2004), which is inhibited when MrgrpE is coexpressed in transfected cells, because these two receptors most likely form heterodimers (Milasta et al., 2006). When KCNQ2/3 potassium channels are coexpressed with MrgrpD, as in most cells expressing the receptor naturally, a potent inhibition of the channels is initiated by b-alanine (Crozier et al., 2007). Also, a coupling to calcium-activated chloride channels via Gq proteins, phospholipase C, and inositol-3 phosphate–induced Ca2+ release has been described, albeit in Xenopus oocytes (Zhuo et al., 2014).

A recent study, which systematically searched for agonists in the Library of 640 Pharmacologically Active Compounds (LOPAC) using a fluorescence image plate reader assay confirmed the G protein coupling and found even lower EC50 values for b-alanine [4 μM (Ajit et al., 2010)]. In addition, this study showed that GABA as well as 5,7-dihydroxytryptamine and HA-966 are potent agonists of MrgrpD (Ajit et al., 2010). Another high-throughput study using a fluorescence image plate reader and 8000 compounds identified two further agonists for MrgrpD, which, however are not further chemically defined (Zhang et al., 2007). Recently, b-aminobutyric acid and diethylstilbestrol were added to the list of agonists with EC50 values for Ca2+ release of 53 and 10 μM, respectively (Uno et al., 2012). This study also demonstrated the very high constitutive activity of MrgrpD: as Ajit et al. (2010) had observed, cells overexpressing MrgrpD at too-high levels could no longer be stimulated by any agonist (Uno et al., 2012). Uno et al. (2012) also describe an antagonist for MrgrpD: the chemical compound Mu-6840 inhibits constitutive and b-alanine-induced activity of MrgrpD in transfected cells and can therefore be described as the first inverse agonist of an Mrgrp, albeit not a natural one.

However, small molecules are not the only agonists of MrgrpD. Gembardt et al. (2008) discovered weak effects of angiotensin peptides [Ang III and Ang-(1–7)] on MrgrpD-expressing cells. More recently, we described a novel component of this peptide family, alamandine, Ala1-Ang-(1–7), as potent agonist of MrgrpD (Lautner et al., 2013). Peptides seem to interact differently with the receptor as b-alanine, because b-alanine does not inhibit the vasodilatory actions of alamandine (Lautner et al., 2013). However, D-Pro7-Ang-(1–7) was shown to be an inhibitor of alamandine binding to MrgrpD in addition to its blocking effect on Ang-(1–7) binding to Mas (Lautner et al., 2013).

**J. MrgrpF (RTA), MASIL (mrg)**

The first two Mrgrps discovered after Mas were MrgrpF (originally named RTA and expressed in all mammals) and MASIL (originally named mrg and only found in primates) (Ross et al., 1990; Monnot et al., 1991). In both papers describing the discoveries, no ligands could be identified, but Ang II could be excluded (Ross et al., 1990; Monnot et al., 1991). Nevertheless, angiotensins stimulated MASIL-expressing Xenopus oocytes, indicating stimulatory cross-talk between MASIL and angiotensin receptors opposite to what was shown for Mas itself (Kostenis et al., 2005). For MrgrpF, this information is not available. More recently, Gembardt et al. (2008) showed that Ang III and Ang-(1–7) and also, slightly, Ang II can stimulate MASIL-expressing cells. They did not, however, exclude cross-talk to potentially coexpressed angiotensin receptors.
K. MrgrpG, MrgrpH

For MrgrpG and MrgrpH receptors, no ligand has been described yet.

L. Receptor Hetero-Oligomerization

GPCRs are known to form hetero-oligomers with functions distinct from monomers or homo-oligomers (Fuxe et al., 2008). Also, Mrgps seem to influence the signaling of other GPCRs by such interactions. Accordingly, it has been shown that hMRGPRX1 can form dimers with the δ-opioid receptor, and binding of hMRGPRX1 ligands to this heterodimer inhibits δ-opioid signaling (Breit et al., 2006). rMrgrpX1 activation modulates μ-opioid receptor coupling in rat DRG neurons, in which they are coexpressed (Wang et al., 2013a), and activation of rMrgrpA inhibits the signaling of coexpressed vasopressin V2 receptors in the collecting duct of the kidney (Kishore et al., 2013), but in both cases a direct interaction of the receptors was not analyzed. Moreover, Mas was found to bind the Ang II AT1 receptor (Kostenis et al., 2005; Santos et al., 2007). This may explain the early findings that Ang II could activate Mas-expressing cells more than control cells (Jackson et al., 1988) and that permanent transfection of kidney cells with Mas can change the hypertrophic actions of Ang II into a proliferative response (Wolf and Neilson, 1992) despite Mas not being a receptor for Ang II. In these cells, however, as well as in the study of Kostenis et al. (2005), an antagonizing interaction between the two receptors was shown (Wolf et al., 1995).

Also different Mrgps can form hetero-oligomers with each other when they are coexpressed in one cell: as already mentioned above, rMrgrpE binds to rMrgrpD and inhibits its internalization and increases its signaling (Milasta et al., 2006). Thus, hetero-oligomerization with other GPCRs may be a common way by which Mrgps exert their actions.

V. Physiologic and Pathophysiologic Functions of Mas-Related G Protein-Coupled Receptors

A. Mas-Related G Protein-Coupled Receptors in Sensory Neurons

Most Mrgps (all MrgrpA, MrgrpB, and MrgrpC subfamily members, as well as MrgrpD) are nearly exclusively expressed in specific dorsal root and trigeminal ganglia neurons. Therefore, it is not surprising that for these Mrgps functions, mainly nociception, itch, or pruritus, and thermosensation have been described.

Mouse MrgrpA3 and MrgrpX1 are mostly coexpressed in the same DRG neurons (Fig. 3) (Zylka et al., 2003). When both genes were ablated (together with 10 other ones in the same cluster; Fig. 2) the itch response to chloroquine, SLIGRL, and BAM8–22 was blunted (Liu et al., 2009, 2011). These primary sensory neurons have endings in the epidermis of the skin and seem to be responsible for itch induced by pruritogens in the skin (Fig. 3). When these neurons were specifically ablated by injecting diptheria toxin into transgenic mice, in which the diptheria toxin receptor gene was activated by Cre recombinase expressed under the control of the mMrgrpA3 promoter (Table 2), itch was much less inducible by most pruritogens. This included substances known to activate Mrgprs, such as chloroquine and BAM8–22 (see section IV), and also histamine and serotonin, at least in juvenile mice (Han et al., 2013). In older animals, the cells become more selective most likely by changes in receptor expression (Akiyama et al., 2012). The itch specificity of these neurons was further validated by an elegant gain-of-function experiment. When the expression of TRPV1, which is involved in itch and thermosensation (Mishra and Hoon, 2013), was only rescued in mMrgrpA3-expressing neurons of a TRPV1 knockout mouse, these animals reacted only with itch response on intradermal injections of capsaicin, which in normal animals would also induce pain (Han et al., 2013). Concordantly, the overexpression of mMrgrpA3 in TRPV1-positive nociceptors induced by a BRAF-expressing transgene led to a marked increase in their response to pruritogens (Zhao et al., 2013). Nevertheless, the main channel activated by mMrgrpA3 during pruriception is not TRPV1 but transient receptor potential ankyrin 1 (TRPA1), because TRPA1 knockout mice hardly respond to the mMrgrpA3 agonist chloroquine (Wilson et al., 2011), whereas in TRPV1 knockout mice only histamine-induced itch is blunted (Imamachi et al., 2009). Furthermore, TRPA1 knockout mice are not responsive anymore to the mMrgrpX1 agonist BAM8–22 despite that mMrgrpX1 cannot directly open TRPA1 channels. In this case, mMrgrpX1 activation seems to open first TRPV1 channels by phospholipase C activation, which, in turn, activates TRPA1 channels for effective signaling (Wilson et al., 2011). These results were confirmed in a recent experiment using chemical silencing or pruriceptors, which also showed that TRPA1 mediates the itch response to the mMrgrpA3 agonist chloroquine and to mMrgrpX1 agonists chloroquine and SLIGRL, respectively (Table 3), and that TRPV1+ cells are responsive to histamine (Roberson et al., 2013). mMrgrpX1 is also responsible for an itch-inducing cross-talk between mast cells and DRG neurons: mast cells release neuropeptide FF upon IgE stimulation, which induces itch by binding to mMrgrpX1 (Table 3) on neighboring nerve endings (Lee et al., 2008).

Interestingly, itch could still be induced by β-alanine in mice depleted of the mMrgrpA-expressing neurons (Han et al., 2013) (Table 2). The reason may be that the receptor for β-alanine, mMrgrpD, is expressed in other neurons, and thereby defines as second subclass of pruriceptors besides the one characterized by mMrgrpA3 expression. When these mMrgrpD-expressing neurons, which do not express TRPV1 (Pogorzala et al., 2013), were ablated by the diptheria toxin receptor approach (Table 2), it was revealed that they are, indeed, not involved in the itch response to most pruritogens (however,
β-alanine was not tested) (Imamachi et al., 2009). Instead the cells are responsible for sensing of pain induced by noxious mechanical stimulation (Cavanaugh et al., 2009) and involved in the response to noxious hot and cold temperatures (Pogorzala et al., 2013) but not for the nocifensive responses to formalin (Shields et al., 2010).

The role of mMrgrpD in the nociceptive function of these neurons is, however, not completely clear. Although, as expected, β-alanine induced itch is virtually abolished in mMrgrpD knockout mice (Liu et al., 2012c) (Table 2), these animals show no impairment in mechanical or thermal nociception (Rau et al., 2009). Nevertheless, isolated nociceptive neurons were clearly less responsive to mechanical and thermal stimuli in the absence of functional mMrgrpD (Rau et al., 2009). Thus, mMrgrpD seems to sensitize nociceptive neurons, either by the known presence of β-alanine in the skin (Crush, 1970) or, more likely, by the notorious constitutive activity of MrgrpD (Uno et al., 2012). However, the lack of sensitization may be compensated in the sustained absence of mMrgrpD, possibly by other nociceptive fibers in knockout mice.

For other Mrgrps, their influence on pain perception is not completely resolved. Activators of mMrgrpX1, rMrgrpX1, and hMRGPRX1, such as BAM8–22, have been shown to induce both hyperalgesia and hypoalgesia (Grazzini et al., 2004; Ndong et al., 2009; Guan et al., 2010; Jiang et al., 2013). Recent experiments with the cluster knockout mice lacking 12 Mrgrps, including mMrgrpX1 (Fig. 2; Table 2), shed some light on this controversial issue (Guan et al., 2010). These animals exhibit unaltered nociceptive thresholds at baseline, showing that normal pain processing is not dependent on the Mrgrps deleted in the cluster. The same was true in rats with downregulation of the rMrgrpX1 gene by local siRNA injection in the spinal cord (Ndong et al., 2009). However, the cluster-knockout mice and the rMrgrpX1-siRNA– injected rats exhibited prolonged pain sensitivity after hind paw inflammation (Ndong et al., 2009; Guan et al., 2010). The so-called “windup effect,” the activity-dependent sensitization of wide dynamic range neurons in the spinal cord, is involved in this hyperalgesia observed in inflammation. Intrathecal administration of BAM8–22 inhibited this effect in wild-type but failed to do so in the cluster-knockout mice (Guan et al., 2010). Interestingly, BAM8–22 even had the opposite effect in these mice by inducing hyperalgesia, which may explain some of the inconsistency in the published data about this molecule. Accordingly, BAM8–22 and the MrgrpX1 agonist JHU58 inhibited neuropathic pain induced by nerve ligation (He et al., 2014). This effect was abolished by the MrgrpX1 inhibitor 2,3-disubstituted azabicyclo-octane (Kunapuli et al., 2006) and absent in Mrgrp-cluster knockout mice and rMrgrpX1-siRNA–treated rats (He et al., 2014). Furthermore, BAM8–22 inhibits the upregulation of inflammatory mediators, such as CGRP, in the spinal cord (Jiang et al., 2013), and it changes the signaling of μ-opioid receptors, enhancing and maintaining their antinociceptive activity even in the presence of morphines, which normally would induce tolerance (Cai et al., 2007b; Chen et al., 2010; Wang et al., 2013a). Thus, Mrgrps binding BAM8–22, such as mMrgrpX1, rMrgrpX1, and hMRGPRX1, exert different effects in the skin, where they elicit itch and pain (Liu et al., 2009; Sikand et al., 2011), and in the spinal cord, where they exert mainly hypoalgesic but also, albeit less potently, hyperalgesic actions (Guan et al., 2010). These actions are more pronounced in inflammatory conditions, probably because the expression of BAM22 (Cai et al., 2007a) and of rMrgrpX1 (Jiang et al., 2013) are upregulated in DRGs in inflammation, e.g., induced by intrapaw injection of complete Freund’s adjuvant; however, there are also conflicting data in the same model (Ndong et al., 2009), and a downregulation of rMrgrpX1 has been observed after spinal nerve ligation (Gustafson et al., 2005).

The role of mMrgrpE in pain perception was studied using a knockout mouse for the protein (Table 2). However, despite the high expression of this Mrgrp in DRGs, spinal cord, and brain, there were only slight alterations in nociception after its ablation, namely a delayed development of allodynia after sciatic nerve injury (Cox et al., 2008). This mere absence of effect may be due to the observed, probably compensatory, upregulation of mMrgrpF in mMrgrpE-deficient mice. mMrgrpB4 marks a subset of sensory neurons that have recently been shown to confer the sensation of gentle touch and induce anxiolytic effects when activated in mice (Liu et al., 2007; Vrontou et al., 2013). However, the function of mMrgrpB4 itself in these effects, if any, was not reported, and because there is no agonist known yet, it cannot easily be tested. Some indirect evidence for a role of mMrgrpB4 in behavior comes from knockout mice for the acid-sensing ion channel 3, which show changes in maternal behavior and a drastic dysregulation of the mMrgrpB4 gene in the dorsal root and trigeminal ganglia (Huang et al., 2013).

Taken together, one major function of Mrgrps is the induction of itch by a specific subset of primary sensory neurons with endings in the epidermis and cell bodies in a DRG (Fig. 3). These neurons have recently been shown to use brain natriuretic peptide as transmitter activating natriuretic peptide A receptors on secondary pruriceptor neurons (Mishra and Hoon, 2013). Furthermore, these neurons also seem to be involved in nociception, but the respective functions of the Mrgrps in these cells remain to be clarified.

B. Mas-Related G Protein–Coupled Receptors in Mast Cells

Some Mrgrps are probably the long-sought low-affinity, low-selectivity receptors on mast cells that mediate IgE-independent degranulating actions of numerous, mainly
basic compounds. As already mentioned above (section IV.F), hMRGPRX2 and rMrgprB3, which are highly expressed in mast cells, are activated by mast-cell degranulating peptide, substance P, PACAP, compound 48/80, β-defensins, cathelicidines, and platelet activating factor 4 (Table 3) and trigger the release of histamine (Tatemoto et al., 2006; Kashem et al., 2011; Subramanian et al., 2011a, 2013). Histamine, in turn, activates pruriceptive neurons in the skin different from the ones mentioned above that express Mrgprs. Thus, the final effect of Mrgprs on mast cells is again the induction of itch, corroborating the concept that they have evolved as pruritics. Pruritogens in the skin can either activate Mrgpr family members directly on somatosensory neurons (e.g., hMRGPRX1 in humans) or other members on mast cells (e.g., hMRGPRX2 in humans), which via the release of histamine activate another class of neurons altogether, eliciting the sensation of itch. Thereby the selectivity of the Mrgprs is not very high, enabling the response to a large variety of substances that may be evolutionarily advantageous and allows the reaction on diverse skin-penetrating noxious stimuli.

C. Mas-Related G Protein–Coupled Receptors in Tumors

As mentioned in section I, Mas was originally but erroneously described as an oncogene (Young et al., 1986, 1988). Other Mrgprs are overexpressed in tumors, such as hMRGPRD, and the expression of hMRGPRD, hMRGPRX1, and hMRGPRX4 as well as of rhesus monkey MRGPRX genes elicit hyperproliferation in transfected cells, most likely by their strong constitutive activity (Andrawis et al., 1992; Burstein et al., 2006; Nishimura et al., 2012). Similar effects are described from Mrgpr-overexpressing transgenic animals (Table 2). Transgenic rats overexpressing hMRGPRX3 controlled from the opsin promoter (Table 2), degeneration of photoreceptors is the consequence, which is probably induced by proliferative signaling pathways activated in these cells because of the constitutively active Mas protein (Xu et al., 2000).

This transforming mechanism of Mrgprs has best been studied for Mas itself in stably transfected cells without addition of a ligand. Mas was shown to simultaneously couple to Gi and Gq proteins, which, in turn, activate Rhos proteins, such as ract (Zohn et al., 1998; Booden et al., 2002; Chen and Ikeda, 2004; Singh et al., 2010a). Rac1 stimulates cell cycle progression, and thereby, proliferation of cells. It is conceivable that this mechanism is also engaged by other Mrgprs, which induce hyperproliferation, but there are no studies available.

However, none of the Mrgprs has yet been shown to be a real oncogene causing cancer, even not Mas (see section I). In the opposite, Ang-(1–7), the specific agonist of Mas, has been described to have antitumor activities, which could even be therapeutically exploited (Gallagher et al., 2011). The tumorigenic and proproliferative actions of Mas and maybe also the other Mrgprs may only be observed above a certain threshold of overexpression in cells which is normally not reached in vivo.

D. Mas-Related G Protein–Coupled Receptors and Mas in Cardiovascular Organs

rMrgprA, mGprD, rMrgprF, and mMrgrH have been shown to be expressed in cardiovascular organs, but very little is known about their functions there (Monnot et al., 1991; Wittenberger et al., 2001; Shinohara et al., 2004; Milasta et al., 2006) other than the possible regulation of vasopressin signaling in the kidney by rMrgprA (Kishore et al., 2013).

In contrast, there is a fair amount of data concerning cardiovascular actions of Mas. Mas is expressed in many cardiovascular-related organs and tissues, including brain (hypothalamus, brain stem) (Becker et al., 2007; Freund et al., 2012), heart (Santos et al., 2006; Dias-Peixoto et al., 2008), kidney (Santos et al., 2003; Pinheiro et al., 2004), and blood vessels (Sampaio et al., 2007a). Most of the data related to cardiovascular actions of Mas were obtained taking advantage of the availability of Mas-deficient (Mas-KO) mice and with the use of Mas agonists [Ang-(1–7), AVE 0991 (Wiemer et al., 2002), and CGEN-856S] and the Mas antagonists, A-779 (Santos et al., 1994) and d-Pro7 Ang-(1–7) (Santos et al., 2003). Ang-(1–7) was identified as a Mas agonist in 2003 (Santos et al., 2003), whereas AVE0991 and CGEN-856S were reported later (Pinheiro et al., 2004; Shemesh et al., 2008; Savergnini et al., 2010). On the basis of evidence obtained in the last 10 years, Mas is now considered to be part of the novel axis of the RAS, ACE2/Ang-(1–7)/Mas (Xu et al., 2011; Bader, 2013; Passos-Silva et al., 2013; Santos et al., 2013a). In the following, we will summarize the numerous cardiovascular and metabolic actions of this axis, but for more comprehensive descriptions the reader is referred to other recent reviews (Xia and Lazartigues, 2010; Rabelo et al., 2011; Xu et al., 2011; Ferreira et al., 2012; Passos-Silva et al., 2013; Santos et al., 2013a; Simoes e Silva et al., 2013).

1. Mas in Heart. Mas expression was detected in cardiomyocytes (Tallant et al., 2005) and cardiac fibroblasts (Iwata et al., 2011) and, more recently, also in the sinoatrial node, providing the morphologic basis for the antiarrhythmic effect of Ang-(1–7) (Ferreira et al., 2011).

Recently, using picomolar concentrations of Ang-(1–7), Souza et al. (2013) were able to unveil a significant Mas-mediated vasodilator effect of Ang-(1–7) in isolated rat hearts. This effect was offset in hearts taken from aorta-coarcted rats. Intriguingly, the blunted vasodilation in hypertensive animals was restored by acute or chronic AT1 blockade with losartan. These observations are in keeping with the previously described interaction of AT1
receptor with Mas (Castro et al., 2005; Kostenis et al., 2005; Canals et al., 2006), which still needs to be addressed in more detail.

In cardiomyocytes, acute Mas stimulation with Ang-(1–7) has no demonstrable effect on Ca\(^{2+}\) transients but promotes NO release by activating endothelial nitric oxide synthase (eNOS) and neuronal NOS (Dias-Peixoto et al., 2008; Costa et al., 2010; Gomes et al., 2010). On the other hand, chronic Mas stimulation or genetic deletion of Mas (Mas-KO; Table 2) produces significant effects on Ca\(^{2+}\) handling proteins (Santos et al., 2006; Gomes et al., 2012). Transgenic rats expressing an Ang-(1–7) producing fusion protein in the heart present an increased Ca\(^{2+}\) transient amplitude, faster Ca\(^{2+}\) uptake, and increased expression of SERCA2 (Gomes et al., 2012). Accordingly, cardiomyocytes from Mas-KO mice presented a smaller peak Ca\(^{2+}\) transient and slower Ca\(^{2+}\) uptakes due to a decreased expression of SERCA2 (Gomes et al., 2012). These changes were turned into a decreased heart function in Mas-KO mice (Santos et al., 2006; Botelho-Santos et al., 2012; Gava et al., 2012). The changes in the calcium handling proteins were paralleled by changes in the NO production machinery (Dias-Peixoto et al., 2008). Cardiomyocytes from Mas-KO mice presented normal eNOS protein levels. However, Mas deficiency resulted in a 70% increase in caveolin 3 expression and a decrease in heat shock protein 90 (Dias-Peixoto et al., 2008). These two alterations may lead to a decrease in eNOS activity, because caveolin 3 prevents calmodulin interaction with NOS and heat shock protein 90 act as a scaffold protein for the recruitment of AKT to the eNOS complex (Wu, 2002; Takahashi and Mendelsohn, 2003).

Most of the data related to Mas and its ligand Ang-(1–7) in the heart have shown cardioprotective effects of Ang-(1–7) (Ferreira et al., 2001, 2007; Santos et al., 2004; Iwata et al., 2005, 2011; Tallant et al., 2005; Grobe et al., 2006; Iusuf et al., 2008; Mercure et al., 2008; Li et al., 2009; Giani et al., 2010; Gomes et al., 2010; Pei et al., 2010; Santiago et al., 2010; Varagie et al., 2010; Marques et al., 2011, 2012; Qi et al., 2011; Durik et al., 2012; McCollum et al., 2012; Patel et al., 2012; Zeng et al., 2012; Cunha et al., 2013; de Almeida et al., 2013) with only one exception (Zhang et al., 2012).

In transgenic rats with Ang-(1–7) elevation in the circulation produced by a fusion protein [TGR(A1-7)3292], a marked attenuation of isoproterenol-induced cardiac fibrosis was observed (Santos et al., 2004). Accordingly, He et al. (2004), and later on many other investigators (Iwata et al., 2005; Tallant et al., 2005; Grobe et al., 2006; Iusuf et al., 2008; Mercure et al., 2008; Li et al., 2009; Giani et al., 2010; Gomes et al., 2010; Pei et al., 2010; Varagie et al., 2010; Iwata et al., 2011; Durik et al., 2012; McCollum et al., 2012; Patel et al., 2012; Zeng et al., 2012; de Almeida et al., 2013), described antiremodeling effects of Ang-(1–7) and of other Mas agonists (AVE 0991/CGEN-856S) (Ferreira et al., 2007; Savergnini et al., 2010). These observations are in line with the deleterious cardiac effects of genetic ablation of Mas in mice (Santos et al., 2006; Gava et al., 2012). Interestingly, even acute blockade of Mas with A-779 produced a deterioration of heart function in isolated mouse hearts (Castro et al., 2005).

An antihypertrophic effect of Ang-(1–7) was also observed in culture cardiomyocytes treated with Ang II (Gomes et al., 2010; Flores-Munoz et al., 2012), vasopressin (Flores-Munoz et al., 2012), and endothelin (Gallagher et al., 2008).

Contrasting with several reports showing a cardioprotective effect of the Mas/Ang-(1–7) axis, overexpression of Mas in neonatal rat cardiomyocytes by means of infection with adenovirus encoding human Mas elicited a significant increase in inositol 1,4,5-trisphosphate accumulation and cellular hypertrophy. These responses were due to enhanced Gq-mediated signaling via Mas (Zhang et al., 2012). These data and others with Ang-(1–7) (Neves et al., 1995; De Mello et al., 2007) support the concept mentioned above (section V.C) that differences in signaling may arise in conditions where Mas or Ang-(1–7) are increased far above physiologic concentrations.

2. Mas in Blood Vessels. In humans and other species, Ang-(1–7) is formed in the endothelial layer of blood vessels (Santos et al., 1992) that also express Mas (Santos et al., 2000; Sampaio et al., 2007a; Xu et al., 2008). In addition, Mas is expressed in the vascular smooth muscle cells (VSMC) of different species (Goncalves et al., 2007).

Studies performed in experimental models of permanent gain- or loss-of-function of Ang-(1–7) and Mas indicate that Mas is capable of chronically influencing systemic and local hemodynamics. It has been reported that Mas-deficient mice present an important increase in the vascular resistance in many territories, such as kidney, lung, adrenal gland, mesentery, spleen, and brown fat tissue (Botelho-Santos et al., 2012). A parallel increase in total peripheral resistance and decreased cardiac index was also observed. On the contrary, in the transgenic rats, TGR(A1-7)3292, with a lifetime increase in circulating Ang-(1–7), an opposite change was reported (Botelho-Santos et al., 2007). These data suggest the existence of an Ang-(1–7)/Mas tonus in blood vessels with a previously unsuspected physiologic relevance. However, because these regional blood flow measurements were made under anesthesia, caution should be exercised in terms of transposing these finding to nonanesthetized animals.

One of the most prominent consequences of Mas deletion is endothelial dysfunction (Rabelo et al., 2008; Xu et al., 2008). In the FVB/N mouse background, the endothelial dysfunction is associated with an increase in blood pressure (Xu et al., 2008), whereas in C57Bl/6 mice, no alteration of blood pressure was observed (Rabelo et al., 2008). The endothelial dysfunction associated with Mas deficiency in two genetic backgrounds is in
keeping with the improvement of endothelial function produced by short-term Ang-(1–7) infusion in normotensive rats (Faria-Silva et al., 2005) and with the improvement of Mas-mediated vascular function in many species and conditions (Langeveld et al., 2008; Durand et al., 2010; Stegbauer et al., 2011; Beyer et al., 2013; Jarajapu et al., 2013; Tassone et al., 2013).

In addition to its effects on vascular tone, Ang-(1–7) has antiproliferative effects in VSMCs (Freeman et al., 1996; Tallant and Clark, 2003). A similar action was described for AVE0991 (Sheng-Long et al., 2012). This feature appears not to be restricted to VSMC, considering that Ang-(1–7) has well documented antiproliferative effects in other cell types, including cardiac fibroblasts (McCollum et al., 2012) and tumoral cells (Gallaher and Tallant, 2004; Ni et al., 2012). The involvement of Mas in these Ang-(1–7) effects was supported by the use of the Mas antagonist A-779.

3. Mas in Cardiovascular Centers of the Brain. As described above, Mas transcripts and protein are expressed in many brain regions (Young et al., 1986; Bunnemann et al., 1990; Martin and Hockfield, 1993; Walther et al., 1998; Becker et al., 2007; Freund et al., 2012), including those related to cardiovascular control (Becker et al., 2007; Freund et al., 2012; Jiang et al., 2013). The presence of Mas in areas, such as the hypothalamus, nucleus tractus solitarii, rostral and caudal ventrolateral medulla, provides the basis for several effects produced by its agonist, Ang-(1–7), in the brain. Modulation of sympathetic activity (Silva et al., 1993; Fontes et al., 1994; da Silva et al., 2011; Kar et al., 2011; Li et al., 2013), increase of vagal tonus (Guimaraes et al., 2012), and improvement of baroreflex sensitivity (Chaves et al., 2000) are some of the effects of Ang-(1–7) that can be blocked by the Mas antagonist A-779. In agreement with these findings, genetic ablation of Mas produces a decrease in baroreflex sensitivity and changes in sympathetic activity (Walther et al., 2000b). In addition to its role in neuronal activity, in the past few years the protective effect of Ang-(1–7) and Mas in stroke has received much attention (Mecca et al., 2011; Regenhardt et al., 2014a,b).

4. Mas in Kidney. Mas is present in the rodent kidney (Santos et al., 2003; Pinheiro et al., 2004; da Silveira et al., 2010), but it could not be detected in the human kidney in one study (Shalamanova et al., 2010). Its ablation in C57Bl/6 mice leads to reduction in urine volume and fractional sodium excretion without any significant change in free-water clearance. A significantly higher inulin clearance and microalbuminuria concomitant with a reduced renal blood flow indicates glomerular hyperfiltration in the Mas-KO mice (Pinheiro et al., 2009). Genetic ablation of Mas abolished the specific binding of $^{125}\text{I}}$Ang-(1–7) to kidney slices and the antidiuretic effect of Ang-(1–7) in water-loaded animals, whereas the binding of Ang II was unaltered (Santos et al., 2003). The absence of the antidiuretic action of Ang-(1–7) in Mas-KO mice is in agreement with the antidiuretic effect of Ang-(1–7) in water-loaded rats (Santos and Baracho, 1992). Diverging from many reports describing Mas-mediated anti-inflammatory effects in different tissues (Al-Maghrebi et al., 2009; da Silveira et al., 2010; El-Hashim et al., 2012; Giani et al., 2012; Jiang et al., 2012; Santos et al., 2012; Souza and Costa-Neto, 2012; Sukumaran et al., 2012; Chen et al., 2013; Feltenberger et al., 2013; Moore et al., 2013; Regenhardt et al., 2013; Wagenaar et al., 2013; Wang et al., 2013c; Acuna et al., 2014; Meng et al., 2014), in the kidney, the proinflammatory role for Ang-(1–7) and Mas was reported by using a model of unilateral ureteral obstruction in mice (Esteban et al., 2009). In contrast, anti-inflammatory and beneficial effects of Ang-(1–7) were observed in other models of kidney injury (Singh et al., 2010b; Moon et al., 2011; Bernardi et al., 2012; Giani et al., 2012; Harris, 2012; Chou et al., 2013; Santos et al., 2013b; Zhang et al., 2014). Interestingly, the outcome of adriamycin-induced nephropathy was similar in Mas-KO and wild-type mice. However, the beneficial effects of losartan in this model were suppressed in Mas-KO animals (Silveira et al., 2013). Further studies are obviously necessary to clarify whether influences of genetic background, the severity of the kidney disease model, an increased AT1 expression in renal vessels (Pinheiro et al., 2009), or a still unknown factor contribute to these contrasting findings (Zimmerman and Burns, 2012).

E. Mas in Metabolism

Mas is present in several tissues involved in glucose and lipid metabolism, including pancreas (Bindom and Lazartigues, 2009; Frantz et al., 2013), liver (Herath et al., 2007), adipose tissue (Santos et al., 2008; Liu et al., 2012a), vascular wall (Karpe and Tikoo, 2014), and skeletal muscle (Prasannarong et al., 2012; Acuna et al., 2014). In these organs, Mas stimulation with Ang-(1–7) promotes improvement of insulin sensitivity (Munoz et al., 2012; Marcus et al., 2013; Echeverria-Rodriguez et al., 2014), facilitates insulin signaling (Munoz et al., 2012; Chhabra et al., 2013; Santos et al., 2014), increases glucose uptake and decreases production of reactive oxygen species in adipocytes (Liu et al., 2012a), and increases glucose uptake in skeletal muscle (Echeverria-Rodriguez et al., 2014). In addition, our phosphoproteomic study in endothelial cells suggests that Mas stimulation triggers changes in the phosphorylation status of several known downstream effectors of insulin signaling, indicating an important role of Mas and Ang-(1–7) in glucose homeostasis in human endothelial cells (Verano-Braga et al., 2012). Accordingly, genetic ablation of Mas in FVB/N mice leads to a metabolic syndrome–like status comprising hypertension, increase in blood glucose, elevated plasma triglycerides and cholesterol levels, and an increase in abdominal fat (Santos et al., 2008). However, as observed for blood pressure (Rabelo et al., 2008), ablation of Mas in
C57BL/6 mice produced only minor changes in glucose and lipid metabolism. Only cholesterol levels were elevated, and this occurred in only old mice (Santos et al., 2008). In this genetic background, however, Mas deficiency produced worsening of the lipid profile and severe hepatic steatosis in apolipoprotein E knockout mice (Silva et al., 2013).

F. Mas in Behavior

There is also evidence for Mas being important for the manifestation of certain behaviors. Mas-KO mice exhibit sex-specific alterations in anxiety-like behavior, with only males showing a proanxious phenotype in the elevated plus maze (Walther et al., 1998, 2000a).

Electrophysiological experiments revealed a more pronounced long-term potentiation in the hippocampus of Mas-KO mice, which is a prerequisite of memory formation (Walther et al., 1998; Hellner et al., 2005). Surprisingly however, only insignificant improvements in the hippocampus-dependent spatial learning were observed in Mas-KO mice (Walther et al., 1998, 2000a). In contrast, object recognition memory, another hippocampus-dependent behavior, was impaired in these animals (Lazaroni et al., 2012), confirming an important but yet elusive role of Mas in hippocampal functions that is not linked to its electrophysiologic actions. However, it may be explained by the role of Mas in neuronal NO generation, which is also involved in memory formation (Yang et al., 2011).

G. Mas in Reproductive Functions

Despite the fact that Mas exhibits a localized and well ontogenically controlled expression in testis during puberty (Metzger et al., 1995; Alenina et al., 2002b), no drastic alterations in fertility are observed in Mas-KO mice, which have normal pregnancy rates and an equal number of male and female offspring (Walther et al., 1998). However, they exhibit a reduction in testis weight, an increased number of apoptotic cells during meiosis, the presence of giant cells and vacuoles in seminiferous epithelium, and a decreased amount of daily produced sperm (Leal et al., 2009), pointing to an important role of Mas in the modulation of spermatogenesis. Accordingly, a strong correlation between Mas expression in seminiferous tubules and male infertility was observed in humans (Reis et al., 2010). Moreover, Ang-(1–7) is released in the corpus cavernosum and can attenuate oxidative stress and DNA damage, processes leading to the degeneration of this tissue, and facilitate electrical-stimulated release of NO (Goncalves et al., 2007; Kilarkaje et al., 2013). Consequently, the genetic ablation of Mas results in an increase of the fibrous tissue content, leading to a compromised erectile function (Goncalves et al., 2007).

The Ang-(1–7)/Mas axis also seems to play a modulatory role in the female reproductive system, being involved in folliculogenesis, ovulation, and pregnancy. Mas expression was detected in human ovaries (Reis et al., 2011) and is regulated by gonadotropin-releasing hormone in rat ovary and in granulosa and theca cells in cattle (Pereira et al., 2009; Tonellotto dos Santos et al., 2012). It is also involved in ovulation in rabbits (Viana et al., 2011). Moreover, the Mas antagonist A-779 inhibited the germinal vesicle breakdown induced by Ang-(1–7) and reduced oocyte maturation stimulated by luteinizing hormone (Honorato-Sampaio et al., 2012), further corroborating the role of Mas in female reproduction. Mas is also present in rat uterus (Vaz-Silva et al., 2012) and in human placenta, where it is decreased in pre-eclamptic women (Velloso et al., 2007). Thus, Mas seems to be a relevant but not essential modulator of male and female fertility.

VI. Conclusions and Outlook

More than 25 years after the discovery of Mas and more than 10 years after the first description of the Mrgrp family, there is still not much known about their physiologic functions. Only Mas has been intensively studied, and it is believed to be part of a beneficial axis of the renin-angiotensin system. For most other Mrgrps, numerous low-affinity agonists have been described, but the natural ligands are still uncertain and none has been approved for therapy. However, as mentioned above, there may be no agonist, but rather, the receptors are constitutively active and we have to search for endogenous inverse agonists that modulate their functions. Alternatively, the receptors may have been evolved for promiscuous binding to several low-affinity agonists in response to challenges of noxious plants and parasites affecting the outer layer of the skin. Thus, the Mrgrps may have allowed the evolutionary invention of itch in Tetrapods, which, by initiating scratching upon their activation, avoids harmful invasion of noxious agents. Nevertheless, some members of the Mrgrp family definitively have additional functions in nociception and other physiologic processes, and therefore, several therapeutic applications of Mrgrp agonists or antagonists are conceivable.

The most advanced Mrgrp in respect to pharmacologic application is Mas itself, exploiting its protective actions in cardiovascular and metabolic diseases. Several different strategies have been developed to directly activate this receptor or to increase the concentration of its main agonist, Ang-(1–7), and some have already entered clinical trials (Steckelings et al., 2011; Bader et al., 2012; Ferreira et al., 2012). However, none is already approved for therapy.

Nevertheless, the other Mrgrps also hold potential for pharmacological applications. In particular, they may be interesting targets for antinociceptive therapies. Furthermore, inhibition of Mrgrps may be a novel working mode for anti-itch drugs. For example, antagonists of hMRGPRX1 may help block the itch occurring...
under malaria therapy with chloroquine in some patients (Liu et al., 2009). On the other hand, agonists for the same receptor, such as JH5U8, might be therapeutically exploited to inhibit inflammatory hyperalgesia and neuropathic pain (Guan et al., 2010; Guan, 2013; He et al., 2014). These obviously contradictory actions of drugs interacting with hMRGPRX1, however, may create a problem in their clinical application.

In conclusion, we are on the eve of the pharmacologic exploitation of Mrgrps in the clinic, with MAS and hMRGPRX1 as first targets. We need further basic investigations on Mrgrps to reveal their physiologic and pathophysiologic functions, and thereby, their therapeutic potential. Such research is worthwhile in view of the unsolved medical needs that Mrgrp-specific drugs may alleviate, such as chronic itch and pain as well as cardiovascular diseases and metabolic syndrome.

**Authorship Contributions**

Wrote or contributed to the writing of the manuscript: Bader, Alenina, Andrade-Vivaró, Santos.

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Mas-Related Genes


