



### An Emerging Role of MrgC in Inhibiting Neuropathic Pain

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The treatment of nonmalignant neuropathic pain continues to challenge clinicians owing to the limited efficacy of available treatments, dose-limiting adverse effects of drugs that are available, and lack of pain-specific treatment targets. G protein-coupled receptors (GPCRs) have been used frequently as drug targets for a variety of pharmacotherapies, including those for pathological pain. One novel family of GPCRs, the so-called Mas-related G protein-coupled receptors (MrGs) may play a role in the function of nociceptive neurons as well as in sensation and modulation of pain [1-3]. The Mrg family in mice can be grouped into several subfamilies (MrgA1-22, MrgB1-13, MrgC1-14, and MrgD-G). The rat genome possesses one representative each of MrgA, C, and D and 10 MrgB genes [4], and humans possess just four MrgX genes (1-4) [1,5]. MrGs are exclusively expressed in small-diameter afferent sensory neurons (presumably nociceptive) that can be visualized by lectin IB4 labeling or by expression of the glial cell line-derived neurotrophic factor co-receptor c-Ret [1,2]. Recent preclinical studies indicated that certain MrGs, especially MrgC (mouse MrgC11 and the rat homolog rMrgC), may be involved in pain modulation and represent a compelling new target for pain-specific therapy.

MrgC can function as a receptor for peptides terminating in RF/Y-G or RF/Y-amide, such as the molluscan peptide FMRamide,  $\gamma$ 2-melanocyte-stimulating hormone (MSH), and bovine adrenal medulla peptide (BAM). Intriguingly, some MrgC ligands belong to the family of endogenous opioid peptides known to be involved in pain transmission, such as proenkephalin A gene products BAM 8-22 and BAM 22 [1-3]. However, the evidence that MrgC has a role in sensory processing of pain is mixed and controversial. For example, systemic and intrathecal injection of putative MrgC ligands, such as BAM 8-22 and  $\gamma$ 2-MSH, produced a pro-nociceptive effect in acute pain models [6]. In contrast, intrathecal injection of BAM 8-22 inhibited persistent inflammatory pain, chemical pain, and spinal c-fos expression in an opioid-independent manner [7-10]. Intrathecal BAM 8-22 also dose-dependently diminished NMDA-evoked pain behaviors in rats, suggesting that it may induce spinal analgesia partially by suppressing NMDA receptor-mediated neuronal excitation [11]. The reasons for these contradictory findings remain unclear and need to be clarified in future studies. One limitation in previous peptide injection experiments is that the selectivity and mechanism of drug action were not directly addressed. Thus, the peptides studied may activate receptors that are distinct from MrGs, a potential explanation for the conflicting results [3,6-10]. Other reasons for the discrepancies may

relate to differences in animal conditions (physiological condition vs. tissue or nerve injury), etiologies (inflammatory vs. neuropathic pain) [12,13], behavioral measures (spontaneous vs. reflex), and drug dose.

Examining the roles of MrgC in pain is challenging, as deletion of a single Mrg gene may not produce a detectable phenotype change owing to the potential for functional redundancy in the Mrg family. As yet, no one has identified an MrgC antagonist that can be used to directly examine the receptor mechanisms of BAM 8-22's action in rodents, though 2,3-disubstituted azabicyclo-octane was suggested to be a selective antagonist for both MrgC11 and human MrgX1 [14]. Recently, a unique mouse line was generated in which all nociceptive neuron-expressing MrGs are deleted (Mrg-cluster $\Delta^{-/-}$ ) [15,16]. The 12 intact Mrg coding sequences that were deleted, including MrgC11, represent ~50% of the potentially functional Mrg repertoire in mice. Importantly, the Mrg-cluster $\Delta^{-/-}$  mice are viable and fertile. Deleting Mrg clusters also did not affect dorsal root ganglion (DRG) neuronal survival, nor did it alter the fate determination or differentiation of small-diameter DRG neurons. However, Mrg-cluster $\Delta^{-/-}$  mice consistently display a higher level of spontaneous pain in the second phase of the formalin test and prolonged inflammatory pain, compared to wild-type littermates [16]. The increase in c-fos+ neurons is also more evident in Mrg-cluster $\Delta^{-/-}$  mice than in wild-type mice after an intraplantar formalin injection. These findings suggest that activation of MrGs by intense noxious input inhibits the exaggeration and prolongation of pain. Importantly, deletion of the Mrg gene cluster eliminates the analgesic effect of intrathecally applied BAM 8-22 on neuropathic mechanical allodynia [16]. Furthermore, BAM 8-22 attenuates windup of wide dynamic range neuronal response to repetitive noxious inputs in wild-type mice, an effect also eliminated in Mrg-cluster $\Delta^{-/-}$  mice. Because MrgC11 is the only Mrg activated by BAM 8-22 that is absent from the Mrg-cluster $\Delta^{-/-}$  mouse, these findings suggest that the pain inhibitory effect of BAM 8-22 is mediated by Mrg signaling, most likely MrgC11, in mice. Although MrgC may be the major receptor for BAM 8-22 [2,15,17], and BAM 8-22 at the spinal site might inhibit neuropathic pain, it is critical to determine the specificity of these compounds for MrgC in pain inhibition. So far, information regarding the roles of MrgC in persistent pathological pain states is sparse and conflicting, and the therapeutic utility of MrgC ligands in neuropathic pain remains to be established. Effects of MrgC activation on afferent sensory neuronal responsiveness and spinal pain transmission are also largely unknown, leaving open the question of the underlying cellular and neurobiological mechanisms.

Altered Ca<sup>2+</sup> activity in afferent sensory neurons is essential for peripheral neuronal sensitization [18,19]. High-voltage-activated (HVA) Ca<sup>2+</sup> channels play an important role in the detection and transmission of nociceptive stimuli in DRG neurons and in neuropathic pain [19,20]. Opioids are known to inhibit HVA Ca<sup>2+</sup> currents in DRG neurons; this inhibition leads to an attenuation of neuronal excitability and reduced excitatory neurotransmitter release. Intriguingly, BAM 8-22 inhibits HVA Ca<sup>2+</sup> current in cells that express human MrgX1 [21]. Yet, whether BAM 8-22 inhibits HVA Ca<sup>2+</sup> current specifically through activation of MrGs has not been directly tested owing to the lack of Mrg-deficient neurons. Furthermore, whether BAM 8-22 can activate endogenously expressed MrgC on native rodent DRG neurons to inhibit HVA Ca<sup>2+</sup> currents

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has not been tested. Although recent studies demonstrated that MrgC ligands may function as anti-hyperalgesic agents at the spinal level, direct physiological proof of a role for MrgC in neuropathic pain is lacking. Functional and mechanistic studies are needed to elucidate details of an MrgC-mediated pain-inhibitory mechanism. Such studies could open doors that could lead to identification of a new pain-specific treatment target for neuropathic pain and development of mechanism-based pain treatment strategies.

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