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ACTIVATION OF MAS ONCOGENE-RELATED GENE (MRG) C RECEPTORS ENHANCES MORPHINE-INDUCED ANALGESIA THROUGH MODULATION OF COUPLING OF μ-OPIOID RECEPTOR TO GI-PROTEIN IN RAT SPINAL DORSAL HORN

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Abstract—Mas oncogene-related gene (Mrg) G protein-coupled receptors are exclusively expressed in small-sized neurons in trigeminal and dorsal root ganglia (DRG) in mammals. The present study investigated the effect of MrgC receptor activation on morphine analgesic potency and addressed its possible mechanisms. Intrathecal (i.t.) administration of the specific MrgC receptor agonist bovine adrenal medulla 8-22 (BAM8-22, 3 nmol) increased morphine-induced analgesia and shifted the morphine dose–response curve to the left in rats. Acute morphine (5 μg) reduced the coupling of μ-opioid receptors (MORs) to Gi-, but not Gs-, protein in the spinal dorsal horn. The i.t. BAM8-22 (3 nmol) prevented this change of G-protein repertoire while the inactive MrgC receptor agonist BAM8-18 (3 nmol, i.t.) failed to do so. A double labeling study showed the co-localization of MrgC and MORs in DRG neurons. The i.t. BAM8-22 also increased the coupling of MORs to Gi-protein and recruited Gi-protein from cytoplasm to the cell membrane in the spinal dorsal horn. Application of BAM8-22 (10 nM) in the cultured ganglion explants for 30 min increased Gi-protein mRNA, but not Gs-protein mRNA. The present study demonstrated that acute administration of morphine inhibited the repertoire of MOR/Gi-protein coupling in the spinal dorsal horn *in vivo*. The findings highlight a novel mechanism by which the activation of MrgC receptors can modulate the coupling of MORs with Gi-protein to enhance morphine-induced analgesia. Hence, adjunct treatment of MrgC agonist BAM8-22 may be of therapeutic value to relieve pain.

Key words: dorsal root ganglia, G proteins, Mas oncogene-related gene (Mrg), μ-opioid receptors, spinal dorsal horn, morphine analgesia.

INTRODUCTION

A large family of orphan guanine nucleotide-binding protein (G protein)-coupled receptors (GPCR) has been found to be related to the human Mas oncogene (Young et al., 1986) and is referred as Mas oncogene-related gene (Mrg) receptors (Dong et al., 2001) or Mas-related GPCR (Zylka et al., 2005). These receptors are also known as sensory neuron-specific receptors or SNSR (Lembo et al., 2002). Mrg family is comprised of approximately 50 members in mice and can be divided into four groups: MrgA, B, C and D (Dong et al., 2001; Zylka et al., 2003). Mrg receptors in rats also have these four groups (Zylka et al., 2003) while in humans, seven Mrg receptors have been identified and characterized as MrgX1-7 (Dong et al., 2001; Choi and Lahn, 2003; Zhang et al., 2005). Intriguingly, mice MrgA/MrgB4/MrgB5/MrgC11/MrgD (Dong et al., 2001; Zylka et al., 2003), rat MrgA/MrgC (Lembo et al., 2002; Zylka et al., 2003) and human MrgX (Lembo et al., 2002) are exclusively expressed in subsets of small-diameter neurons in trigeminal and dorsal root ganglia (DRG) where the cell bodies of nociceptors are located (Woolf and Ma, 2007). Therefore, the study of the role of Mrg receptors in nociceptive transmission may not only help to depict the pain mechanism, but also lead to the development of drugs with limited central nervous system side effects. However, the functional significance of Mrg expression is essentially not clear.

Rat MrgC receptors correspond to SNSR (Zylka et al., 2003). We have recently demonstrated that intrathecal (i.t.) administration of the specific SNSR (Lembo et al., 2002) or MrgC receptor agonist bovine adrenal medulla 8-22 (BAM8-22) (Guan et al., 2010) or (Tyr^6)-γ2-MSH-6-12 (MSH, melanocyte stimulating hormone) (Grazzini et al., 2004) prevents or reverses morphine tolerance (Cai et al., 2007b; Chen et al., 2010). As the development of tolerance greatly limits the use of opiates, which are still the most efficacious analgesics used in the clinical management of moderate to severe pain, this finding suggests that MrgC receptor agonist could be used as an adjunct for sustained use of

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Abbreviations: ANOVA, analysis of variance; DRG, dorsal root ganglia; EDTA, ethylenediaminetetraacetic acid; GPCR, (G protein)-coupled receptor; MOR, μ-opioid receptor; MPE, maximum possible effect; Mrg, Mas oncogene-related gene; PB, phosphate buffer; PBS, phosphate-buffered saline; RT-PCR, real-time-polymerase chain reaction; TFL, tail-flick latency.

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opiates to reduce tolerance. The inhibition and reversal of MrgC receptors on the decline of morphine analgesia can be attributed to their modulation on morphine tolerance since the activity of MrgC receptors suppresses chronic morphine-induced activation of protein kinase C (PKC) signaling pathway and upregulation of the pronociceptive mediators calcitonin gene-related peptide (CGRP) and neuronal nitric oxide synthase (nNOS) (Chen et al., 2010). However, it may also be possible that the activation of MrgC receptors could enhance the potency of morphine analgesia. The present study aimed to investigate whether MrgC receptors modulated morphine-induced analgesia and its possible mechanisms.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (250–320 g; Fuzhou Animal Center, Fujian, China) were housed at 22 °C with 50% humidity under a 12-h light/dark cycle and given free access to food and water. The experimental procedures described in the present study were approved by the animal care committee of the Fujian Normal University, and were in accordance with the guidelines of the treatment of animals of the International Association for the Study of Pain (Zimmermann, 1983). All efforts were made to minimize animal suffering and the number of animals used in our experiments.

Intrathecal catheter implantation

Animals were implanted with chronic indwelling catheters with modification of the previously described technique (Pogatzki et al., 2000). Briefly, a catheter was implanted i.t. under sodium pentobarbital anesthesia (50 mg/kg, i.p.). The skin was shaved along the occiput and neck. The atlanto-occipital membrane was exposed by blunt dissection. An incision was made in the dura at the atlanto-occipital junction, and a polyethylene catheter (PE-10, Stoelting, Wood Dale, IL, USA) was threaded caudally to position its tip at the L4–L5 segments of the spinal cord. The external end of the catheter was sutured to the neck muscle. Rats were allowed to recover for 7 days. Only the animals with no evidence of neurological deficits after catheter placement were used for behavioral testing. An incision was made overlying the atlanto-occipital junction, and the dura mater was exposed by blunt dissection.

Assessment of nociceptive behavior

Tail-flick latency (TFL) was determined by the radiant heat tail-flick assay using a Tail Flick Meter (IITC Life Science Inc., Woodland Hills, CA, USA). Radiant heat was focused on the underside of the tail 3 cm from its distal end and TFL was automated by equipment. Radiant heat intensity was adjusted to produce on average a baseline of 2–3 s in naïve rats. The cut-off latency was established at 10 s to avoid tissue damage. TFL at any test time point was measured three times at 1.5 min intervals and the mean value of these measurements was taken. The investigator was blind to the test drug conditions.

For assessment of the potency of acute morphine, a cumulative morphine dose–response curve was constructed. Animals were given ascending doses of i.t. morphine chloride (Northeast Pharmaceutical Group, Shenyang, Liaoning, China) every 30 min until a maximal level of antinociception was reached. TFL was determined 20 min after morphine administration, the time at which the peak effect of morphine is achieved (Jiang et al., 2006; Cai et al., 2007a). The ED_{50} values are defined as the dose that produces 50% of the maximal effect (Emax) and were determined from the morphine dose–response curve. Inhibition of the tail-flick response (i.e. analgesia) was expressed as the percentage of the maximum possible effect (% MPE) using the following formula:

$$\%\ \text{MPE} = ([\text{post-drug latency} - \text{baseline latency}]/[\text{cut-off time} - \text{baseline latency}]) \times 100\%.$$

Ganglion explant culture

Animals were sacrificed by decapitation. Trigeminal ganglia and DRG (C2–L6) were dissected under sterile technique and collected in Hank's solution. Following a wash in Hank's solution, ganglia were transferred to Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and penicillin/streptomycin. Ganglia were maintained at 5% CO_2 and 37 °C. To stabilize the culture conditions, ganglion explants were cultured for 24 h and then exposed to BAM8-22 (Huadatianyuan Biological Co., Shanghai, China). The explants were harvested and stored at −80 °C until further processing.

Trigeminal ganglia are sensory ganglia that are analogous to DRG and receive masseteric primary afferents (Wang et al., 2006). In addition, MrgC receptors have been shown to be expressed in both trigeminal ganglia and DRG, but not in sympathetic superior cervical ganglion and nodose ganglion, indicating that MrgC receptors are uniquely associated with somatosensory afferents in the rat (Lembo et al., 2002).

Spinal cord membrane and cytosolic fraction preparations

Animals were sacrificed by decapitation 30 min following i.t. administration of morphine. The dorsal half of the lumbar spinal cord was quickly harvested. Tissue samples were homogenized in lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 50 mM NaF, 1 mM NaVO3, 1% Igepal CA-630, and 0.1% SDS) supplemented with protease inhibitors (5 mM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin, all chemicals were purchased from Sigma (Shanghai, China). The spinal dorsal horn samples were fractionated into cytosolic and membrane fractions using a membrane and cytosol protein extraction kit (Beyotime Institute of