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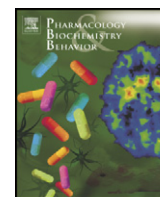

Genetic inactivation of pleiotrophin but not midkine potentiates clonidine-induced alpha-2 adrenergic-mediated analgesia

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ABSTRACT

Genetic deletion of the heparin-binding cytokines pleiotrophin (PTN) or midkine (MK) potentiates morphine-induced antinociceptive effects in animal models. Despite the known interactions between the opioid and noradrenergic systems in the control of pain, the possible roles of PTN and/or MK in analgesia induced by agonists of α_2-adrenergic receptors remained to be studied. We have now tested the antinociceptive effects of the α_2-adrenergic receptor agonist clonidine in female PTN genetically deficient (PTN −/−), MK genetically deficient (MK −/−) and wild type (WT +/+) mice. We did not find differences among genotypes in the hot-plate test, an assay in which supraspinal and spinal mechanisms contribute to nociceptive responses, suggesting that endogenous expression of PTN and MK is not key in the analgesia induced by clonidine in this test. In contrast, we found that clonidine-induced analgesia was significantly enhanced in PTN −/− mice compared to MK −/− and WT +/+ mice in the tail-immersion test. Interestingly, the α_2-adrenergic antagonist yohimbine prevented clonidine-induced analgesia in the tail immersion test in all the 3 genotypes. The data suggest that the spinal antinociceptive effects caused by stimulation of α_2-adrenoceptors are differentially regulated by endogenous expression of PTN.

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1. Introduction

Midkine (MK) and Pleiotrophin (PTN) are heparin binding growth factors (Kadomatsu et al., 1988; Milner et al., 1989) that share over 50% identity in amino acid sequence (Deuel et al., 2002). Both PTN and MK have been found to play important roles in the development of the nervous system through their actions on neuronal differentiation (Herradon and Ezquerra, 2009; Muramatsu, 2011; Gramage and Herradon, 2011). In addition, it has been hypothesized that both PTN and MK may play important roles on survival of different cell types and wound repair since both cytokines are upregulated at sites of injury and repair in inflammatory macrophages, microglia, dermal fibroblasts, endothelial cells and other cells (Blondet et al., 2005; Ezquerra et al., 2008; Gramage et al., 2010; Kikuchi-Horie et al., 2004; Martín et al., 2011; Mi et al., 2007; Sakakima et al., 2004a, 2004b; Yeh et al., 1998).

The role of MK in wound repair in the periphery has been linked to its potential actions in the guidance of neural axon regeneration in peripheral nervous system (Sakakima et al., 2009). This is supported by the delay in axonal regeneration uncovered in MK genetically deficient (MK −/−) mice with peripheral nerve injury, leading to the hypothesis that MK acts as a reparative neurotrophic factor in damaged peripheral nerves (Sakakima et al., 2009). That report significantly increased the relevance of previous findings demonstrating the consistent upregulation of MK expression levels after traumatic injury of the spinal cord and following sciatic nerve injury (Sakakima et al., 2004a, 2004b). Very interestingly, upregulation of the levels of the highly homologous cytokine PTN in the injured dorsal root ganglia (DRG) of rats with Chronic Constriction Injury (CCI) of the sciatic nerve correlates with faster recovery of neuropathic pain states (Ezquerra et al., 2008). Taking together, evidence suggests that these cytokines play a role in degeneration and regeneration after peripheral nerve injury and the recovery of subsequent chronic neuropathic pain state (Martín et al., 2011). Furthermore, we hypothesized that PTN and MK could play important roles not only in neurotrophic processes but also in nociceptive transmission as well (Gramage and Herradon, 2010; Gramage et al., 2012). Accordingly, whereas nociceptive transmission seems to be unaffected in PTN genetically deficient (PTN −/−) and MK −/− mice in the hot plate test (Gramage and Herradon, 2010; Gramage et al., 2012), PTN −/− mice showed a delayed response to nociceptive stimulus in the tail-flick test compared to MK −/− and wild type (WT +/+) mice, suggesting that endogenous PTN modulates nociceptive transmission at the spinal level (Gramage and Herradon, 2010).

In the central nervous system, PTN and MK expression levels have been found to be significantly upregulated in different brain areas in humans and rodents after administration of different drugs including morphine (Ezquerra et al., 2007; Herradon et al., 2009). Interestingly,

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the antinociceptive effects of morphine are significantly enhanced in PTN −/− and MK −/− mice (Gramage and Herradon, 2010; Gramage et al., 2012) suggesting that upregulation of these neurotrophic factors after morphine administration plays a role in the functional regulation of the pharmacological effects of morphine. It is interesting to note that descending noradrenergic inhibitory pathways in the control of pain contribute to opioid-induced analgesia (Herradón et al., 2003; Herradon et al., 2008; Millan, 2002). However, the selective α_2-adrenergic antagonist yohimbine failed to prevent morphine-induced analgesia in acute pain models in MK −/−, PTN −/− and WT +/+ mice (Gramage et al., 2012). Although opioid/α_2-adrenergic interactions do not seem to be prominent in morphine-induced analgesia in PTN −/− and MK −/− mice, the possibility of a differential analgesic effect of α_2-adrenergic drugs depending on the presence of endogenous levels of PTN or MK should not be discarded especially when both PTN and MK have been shown to regulate the cathecolamine biosynthetic pathway in different contexts (Ezquerra et al., 2004; Ezquerra et al., 2006; Hida et al., 2007; Jung et al., 2004). To test this hypothesis, we aimed to study the analgesia induced by the α_2-adrenergic agonist clonidine in PTN −/− and MK −/− compared to control, wild type mice.

2. Materials and methods

Midkine knockout (MK −/−) and PTN knockout (PTN −/−) mice were generated on a 129/Ola × C57BL/6J background by methods essentially identical to those previously described (Amet et al., 2001; Nakamura et al., 1998). The animals used in this study were 8-week old female MK −/−, PTN −/− mice and WT +/+ mice used as control. All the animals used in this study were maintained according to European Union Laboratory Animal Care Rules (86/609/ECC directive).

2.1. Hot-plate test

To assess nociceptive responses in PTN −/−, MK −/− and WT +/+ mice, we used the hot-plate test following the procedure described previously (Gramage and Herradon, 2010). A metal hot-plate was maintained at 53 ± 0.5 °C. The latency time to when the mouse first exhibited nocifensive behavior (licked its hind paw or jumping) was determined. The cut-off time for the first sign of nocifensive behavior was 75 s.

To study the effect of clonidine on hot-plate response, saline (10 ml/kg) (n = 10–13/genotype), 0.25 mg/kg of clonidine hydrochloride (Sigma, Madrid, Spain) (n = 7–9/genotype) or 0.50 mg/kg of clonidine (n = 10–14/genotype) was administered i.p. after testing the baseline response for the hot-plate maintained at 53 ± 0.5 °C. To study the time course of the effect of clonidine, the hot-plate latency was recorded 25, 75 and 125 min after clonidine (or saline, as a control) injection. The doses of clonidine were selected because they were previously shown to be effective in similar experiments in mice (Blednov et al., 2003). Four independent experiments in different days were performed using balanced cohorts of animals per experimental group (genotype and treatment) every day.

2.2. Tail-immersion test

2.2.1. Clonidine-induced antinociceptive effects in PTN −/−, MK −/− and WT +/+ mice

To assess spinal nociceptive responses in PTN −/−, MK −/− and WT +/+ mice, we used the tail-immersion test. To study the effect of clonidine on tail-immersion responses we used a bath maintained at a temperature of 55 ± 0.5 °C. We used the same doses of clonidine tested in the hot plate assay (0.25 and 0.50 mg/kg). Saline (10 ml/kg) or clonidine was administered i.p. after testing the baseline responses to the tail-immersion test using a cut-off latency of 15 s to prevent tissue damage. It is interesting to note that a small percentage of PTN −/− mice (~20%) were excluded of the pharmacological test because they reached the cut-off latency in baseline conditions which is in agreement with previous results from our group (Gramage et al., 2012). According to basal values, mice from every genotype were homogeneously distributed in saline-treated (n = 8–11/genotype), clonidine (0.25 mg/kg)-treated (n = 12–14/genotype) and clonidine (0.50 mg/kg)-treateds (n = 12–14/genotype) groups. To study the time course of the antinociceptive effect of clonidine, the tail-flick latency was recorded 25, 50, 75 and 125 min after clonidine (or saline, as a control) injection. Three independent experiments in different days were performed using balanced cohorts of animals per experimental group (genotype and treatment) every day.

2.2.2. Effects of yohimbine on clonidine-induced antinociceptive effects in PTN −/−, MK −/− and WT +/+ mice

To test the possibility that clonidine-induced analgesia was mediated by α_2-adrenergic mechanisms in PTN −/−, MK −/− and WT +/+ mice, we tested the effects of clonidine (0.25 mg/kg) in mice pretreated with the α_2-adrenergic antagonist yohimbine (Sigma, Madrid, Spain). For this purpose, after determination of basal responses, mice were injected (i.p.) with yohimbine (1.40 mg/kg) or saline (10 ml/kg; control) 10 min before clonidine (0.25 mg/kg) or saline (10 ml/kg; control) administration. As a result, we obtained four experimental groups: saline + saline (n = 7–10/genotype), saline + clonidine (n = 8–10/genotype), yohimbine + saline (n = 6–9/genotype) and yohimbine + clonidine (n = 7–8/genotype). The tail-flick latency was recorded 25, 50, 75 and 125 min after clonidine (or saline) injection. Three independent experiments in different days were performed using balanced cohorts of animals per experimental group (genotype and treatment) every day.

2.3. Statistical analysis

The statistical significance of changes within the same treatment was determined by 2-way repeated-measures ANOVA considering as factors the genotype and the 4 or 5 time points after injection depending on the experimental model (time-curse curves). Bonferroni's post hoc tests were used to detect the sources of group differences revealed by the ANOVAs.

For subsequent analysis, latencies were converted to percentages of maximal possible effect (%MPE), a way of expressing the absolute latencies relative to baseline (0% MPE) and to cut-off time (100% MPE), according to the formula:

$$\%MPE = (test - baseline)/(cutoff - baseline) \times 100.$$

In this manner, %MPE data were analyzed within each time point by 2-way ANOVA considering as factors genotype and treatment. The factor time was also analyzed as a within-subjects factor. Bonferroni's post hoc tests were used to detect the sources of group differences revealed by the ANOVAs.

Area under the curve (AUC) values obtained from PTN −/−, MK −/− and WT +/+ mice were analyzed using student's *t* test when two treatments were compared within the same genotype. In all analyses, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Hot-plate test

In the studies directed to assess clonidine-induced antinociceptive effects in the hot-plate maintained at 53 °C, we first analyzed together basal values of mice from the three genotypes used in all experiments performed (n = 24–32/genotype). The latency to the first sign of nocifensive behavior in mice of all genotypes was found to be similar in the hot-plate test (WT +/+ = 31.9 ± 2.0 s; PTN −/− = 25.2 ± 2.2 s; MK −/− = 29.1 ± 1.6 s).

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