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Short communication

Spinal nociceptin inhibits septide but not *N*-methyl-D-aspartate-induced nociceptive behavior in rats

Noemí Villanueva, Ana Lastra, Agustín Hidalgo, Ana Baamonde, Luis Menéndez *

Departamento de Medicina, Facultad de Medicina, Laboratorio de Farmacología, Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Universidad de Oviedo, C/ Julián Clavería 6, 33006 Oviedo, Asturias, Spain

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Abstract

Nociceptin can induce spinal analgesia in rats. Here, we tested the ability of nociceptin to inhibit the nociceptive behavior (biting, scratching, licking) induced by intrathecal administration of *N*-methyl-D-aspartate (4 μg) or the tachykinin NK_1 receptor agonist, septide (0.5 μg), in rats. Intrathecal nociceptin (3–30 nmol) did not modify the NMDA-induced behavior. However, coadministration of nociceptin (1–10 nmol) inhibited the septide-induced excitatory response. This inhibition was unaffected by systemic (10 mg/kg) or intrathecal (30 nmol) administration of naloxone, but intrathecal coadministration of the ORL1 (opioid receptor-like type 1) receptor antagonist [Nphe^1]nociceptin-(1-13)-NH_2 (30–90 nmol) prevented it, suggesting the involvement of ORL1 receptors.

Keywords: Spinal analgesia; Biting, scratching, licking (BSL); Septide; NMDA; [Nphe^1]nociceptin-(1-13)-NH_2

1. Introduction

Nociceptin (also termed Orphanin FQ), the most recently discovered opioid peptide (Meunier et al., 1995), binds with high affinity to the ORL1 (opioid receptor-like type 1 receptor), albeit showing a lower affinity for the classical μ-, δ- and κ-opioid receptors. The ORL1 receptor presents about 60% homology with the other opioid receptors and the opioid receptor antagonist naloxone binds it with very low affinity.

Although hyperalgesic responses have frequently been demonstrated when nociceptin is peripherally or intracerebroventricularly administered in mice or rats (see, for review, Yamamoto et al., 1999), analgesic effects have also been reported, specially after its spinal administration. In mice, intrathecal nociceptin induces biphasic responses in such a way that low doses (femtomole order) provoke hyperalgesic reactions and higher doses (nanomole order) induce analgesia (Inoue et al., 1999). Interestingly, in rats, the intrathecal administration of nociceptin yields analgesic responses without any behavioral sign of hyperalgesia.

These analgesic effects induced by intrathecal nociceptin in rats have been demonstrated in several models of tonic experimental pain (carrageenan hyperalgesia, formalin test or the ligature of the sciatic nerve) (Yamamoto et al., 1997a,b; Corradini et al., 2001), usually considered to be closer to clinical pain than models based on phasic noxious stimuli. Since both NMDA and tachykinin NK_1 receptors are the main ones involved in the spinal processing of tonic nociceptive signals, it seemed interesting to explore the effects exerted by nociceptin on the excitatory responses triggered through the activation of these receptors. The administration of NMDA and the tachykinin NK1 receptor agonist, septide, directly into the spinal cord provokes in rats a nociceptive behavior (biting, scratching, licking) (Menéndez et al., 1997) that has been used to study the analgesic effects of different drugs, such as morphine or local anesthetics (Álvarez-Vega et al., 1998). Whilst NMDA is the prototypical agonist of NMDA receptors, septide binds to the tachykinin NK_1 receptor at a different site from that of the endogenous agonist, substance P (Glowinski, 1995). In spite of that, septide behaves as a functional tachykinin NK_1 receptor agonist when injected directly into the spinal cord (Sakurada et al., 1994) and, in fact, it has been shown that in tachykinin NK_1 receptor knockout mice, septide becomes inactive (Cao et al., 1999).

In this work, we have studied the effects of spinal nociceptin on the behavioral responses induced by intrathecal injection of NMDA and septide. Furthermore, since

* Corresponding author. Tel.: +34-985-102755; fax: +34-985-103551.
E-mail address: luismen@correo.uniovi.es (L. Menéndez).

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one feature of current interest related to the analgesia induced by nociceptin in rats is the involvement of either the classical opioid or the ORL1 receptors, we have also addressed this topic by using their corresponding receptor antagonists, naloxone and [Nphe^1]nociceptin-(1-13)-NH_2 (Calo et al., 2000).

2. Material and methods

2.1. Animals

Male Wistar rats, weighing 250–350 g, from the Animalario of the Universidad de Oviedo (Reg. 33044 13A) were exposed to a light–dark cycle of 12 h and supplied with water and food "ad libitum." For intrathecal (i.t.) injections (Menéndez et al., 1997), rats were lightly anesthetized with ether and then a small cut was made in the skin and the needle tip of a hypodermic syringe (0.33×13 mm) was introduced between the L_4 and L_5 vertebrae. All experiments were conducted according to ethical guidelines (Zimmermann, 1983) and approved by the Comisión de Ensayos Clínicos y Bioética del Principado de Asturias (Spain).

2.2. Drugs

N-methyl-D-aspartate (NMDA) (Tocris) and septide (Sigma) were administered through an intrathecal injection dissolved in 25 μl of distilled water either alone or coinjected with nociceptin (Tocris). Naloxone hydrochloride (Sigma) was intraperitoneally (i.p.) injected 15 min before intrathecal administration dissolved in saline in a final volume of 1 ml/kg. When injected intrathecal, both naloxone and the ORL1 receptor antagonist [Nphe^1]nociceptin-(1-13)-NH_2 (Neosystem) were dissolved in 25 μl of distilled water, alone or coinjected with septide and/or nociceptin. In all cases, control groups received the corresponding intraperitoneal or intrathecal injections of their respective solvents.

2.3. Biting, scratching and licking behavior assays

As described in previous reports (Menéndez et al., 1997), immediately after the intrathecal administration of NMDA or septide, rats were placed in a transparent plastic cage and the time spent in biting, scratching or licking the hindquarters during 15 min was measured. The intrathecal administration of solvent, nociceptin, naloxone or [Nphe^1]nociceptin-(1-13)-NH_2 did not produce any behavioral effect by itself.

2.4. Statistical analysis

The mean values of the time spent in nociceptive behavior and their corresponding S.E.M. were calculated and intergroup comparisons were made using an initial one-way analysis of variance (ANOVA) followed by the Dunnett's *t*-test or the Newman–Keuls tests, when appropriate. The level of significance was set at $P<0.05$.

3. Results

NMDA (4 μg/rat i.t.) induced a nociceptive reaction measured 15 min immediately after the drug administration (Fig. 1A). The coadministration of nociceptin (3–30 nmol/rat) did not inhibit this behavior at any of the doses assayed. Since in this experiment, some rats treated with 30 nmol of intrathecal nociceptin exhibited a slight hypotonus of the hindpaws, higher doses of nociceptin were not further assayed.

Septide (0.5 μg/rat) also induced a robust nociceptive behavior during the 15 min following its administration (Fig. 1B). The coinjection of nociceptin (1–10 nmol/rat) with septide produced a dose-dependent reduction of this biting, scratching and licking response that became statistically significant with the doses of 3 and 10 nmol of nociceptin.

We assayed the effects of naloxone and [Nphe^1]nociceptin-(1-13)-NH_2, the corresponding antagonists of classic and ORL1 receptors, respectively. The prior administration of naloxone (10 mg/kg i.p.) did not modify the inhibition of the septide-evoked biting, scratching and licking induced by 3 nmol of nociceptin (Fig. 2A). Accordingly, when 30 nmol of naloxone were coadministered intrathecal with septide and nociceptin, the inhibitory effect persisted (Fig. 2B). In contrast, as shown in Fig. 2C, the coadministration of [Nphe^1]nociceptin-(1-13)-NH_2 (30–90 nmol) together with 3 nmol of nociceptin and septide abolished in a dose-dependent way the effect induced by nociceptin. Thus, the time spent in nociceptive behavior obtained when 90 nmol of the antagonist were coinjected with nociceptin and septide showed no statistical differences with that induced by septide alone. When these doses of [Nphe^1]nociceptin-

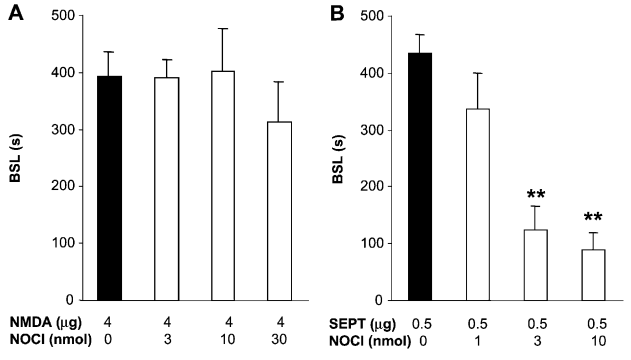

Fig. 1. (A) Lack of effect of the coadministration of nociceptin (NOCI, 3–30 nmol/rat) on the nociceptive behavior (BSL) induced by 4 μg intrathecal NMDA. (B) Inhibitory effect of the coadministration of nociceptin (1–10 nmol/rat) on the biting, scratching, licking (BSL) induced by intrathecal septide (0.5 μg). The means and corresponding standard errors are represented. $^{**}P<0.01$ related to septide-treated group, Dunnett's *t*-test.

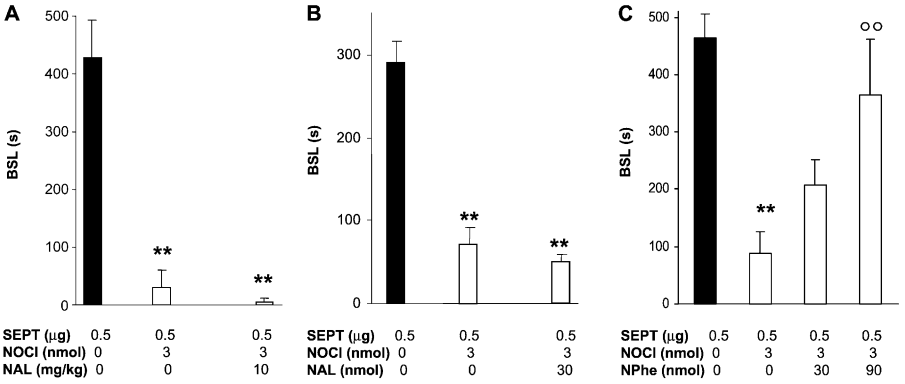

Fig. 2. Lack of effect of naloxone (NAL) either administered intraperitoneally (A; 10 mg/kg;) or intrathecally (B; 30 nmol) on the inhibition induced by nociceptin (3 nmol/rat) on septide-evoked biting, scratching, licking (BSL). (C) Antagonism by [Nphe^1]nociceptin-(1-13)-NH_2 (Nphe, 30–90 nmol) on the inhibition induced by nociceptin (3 nmol/rat) on septide-evoked BSL. The means and corresponding standard errors are represented. $^{**}P<0.01$ related to septide-treated group, $^{\circ\circ}P<0.01$ related to septide- and nociceptin-treated group, Newman–Keuls test.

(1-13)-NH_2 were coinjected with septide but without nociceptin, no increase in the time spent in biting, scratching and licking behavior was observed (data not shown).

4. Discussion

The analgesia that spinal nociceptin produces in several models of tonic experimental pain in rats prompted us to study if this peptide could inhibit the excitatory effects induced through the activation of NMDA and tachykinin NK_1 receptors at the spinal level. We found that nociceptin does not modify the excitatory response evoked by intrathecal NMDA but is able to inhibit, acting through ORL1 receptors, the nociceptive behavior induced by the tachykinin NK_1-receptor agonist, septide.

Related to NMDA, it has been reported that in medullary neurons, nociceptin can inhibit some NMDA receptor-mediated excitatory responses (Wang et al., 1996), but this inhibition does not seem to operate in the spinal cord when tested using a behavioral approach. In any case, we cannot rule out that a nociceptin-mediated inhibition of glutamate release (Nicol et al., 1996) could prevent NMDA receptor-mediated events triggered by peripheral noxious stimuli, since in the type of experimental testing used by us only postsynaptic effects induced by drugs can be detected. This could explain why, for example, such a potent analgesic drug as morphine shows only a limited ability for inhibiting NMDA-induced nociceptive behavior (Álvarez-Vega et al., 1998). In any case, despite the fact that morphine is not very effective in this assay, a difference seems to exist between the effect induced by this standard μ-opioid receptor agonist and the complete lack of effect of nociceptin to inhibit NMDA-induced effects.

In contrast, spinally administered nociceptin (1–10 nmol) inhibits the excitatory behavior induced by septide. This effect of nociceptin on septide-induced behavior is unaltered by the administration of either a high dose of systemic naloxone (10 mg/kg) or a intrathecal dose of naloxone (30 nmol) previously described as selective for the blockade of opioid receptors without affecting ORL1 receptors (Yamamoto et al., 1997a). Interestingly, the coadministration of the selective ORL1 receptor antagonist, [Nphe^1]nociceptin-(1-13)-NH_2 (30–90 nmol) was able to prevent the inhibitory effect of nociceptin (3 nmol). This strongly supports the fact that nociceptin inhibits septide-induced nociceptive behavior through the activation of ORL1 receptors.

It must be noted that the standard μ-opioid receptor agonist, morphine, could also inhibit the biting, scratching and licking induced by septide (Álvarez-Vega et al., 1998). Thus, although both drugs, nociceptin and morphine, produced the same effect qualitatively, the potency of nociceptin is higher than that obtained with morphine (ED_{50} value ~130 nmol)(Álvarez-Vega et al., 1998). Finally, bearing in mind that the spinal pharmacology of nociceptin is rather different between mice and rats, this result obtained in rats is compatible with that obtained by Inoue et al. (1999), showing that intrathecal nociceptin in the analgesic dose range in mice can inhibit the nociceptive reaction induced by the spinal injection of the endogenous tachykinin NK1 receptors substance P. All these data support the idea that the modulation of tachykinin NK_1 receptors-mediated responses, through the activation of ORL1 receptors, could be a pharmacological substrate involved in the spinal analgesia induced by nociceptin.

Globally, our results demonstrate that nociceptin can specifically modulate particular pharmacological signals involved in nociceptive processing at the spinal level. In fact, this peptide, through the activation of ORL1 receptors, can inhibit the nociceptive responses mediated through the stimulation of spinal tachykinin NK_1 receptors without modifying those triggered by intrathecal NMDA. We hope that these results will help towards a better understanding of the action of this intriguing peptide on nociception and, thus, contribute to envisaging the ORL1 receptor as a

pharmacologically distinct target for the modulation of spinal nociception.

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