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Role of spinal opioid receptor on the antiallodynic effect of intrathecal nociceptin in neuropathic rat

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HIGHLIGHTS

- Intrathecal nociceptin attenuated mechanical allodynia by spinal nerve ligation.
- The effect of nociceptin was reversed by opioid receptor antagonist.
- Spinal μ, δ, and κ opioid receptor types were involved in the activity of nociceptin.
- Intrathecal nociceptin increased δ opioid receptor protein level in neuropathic pain.

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ABSTRACT

The purpose of this study was to examine the effects of intrathecal nociceptin for neuropathic pain and determine the role of spinal opioid receptor types. Neuropathic pain was induced by ligation of L5 and L6 spinal nerves in male Sprague-Dawley rats. Several antagonists were intrathecally administered to evaluate the action mechanisms of nociceptin: nonselective opioid receptor antagonist (naloxone), μ opioid receptor antagonist (CTOP), δ opioid receptor antagonist (naltrindole) and κ opioid receptor antagonist (GNTI). The levels of opioid receptor proteins were examined by Western blotting. Intrathecal nociceptin produced dose-dependent antiallodynia. Intrathecal naloxone reversed the antinociception of nociceptin. Intrathecal CTOP, naltrindole and GNTI reversed the antinociceptive effect of nociceptin. Western blots showed that the levels of spinal opioid receptor proteins did not differ between rats with neuropathic pain and naïve rats. Intrathecal nociceptin increased the level of δ opioid receptor protein compared with that of nerve ligated rats, while the levels of μ, and κ opioid receptor proteins were unchanged. These results suggest that intrathecal nociceptin produced antiallodynic effect in spinal nerve ligation-induced neuropathic pain. All three types of spinal μ, δ, and κ opioid receptors were involved in the antiallodynic mechanism of nociceptin.

1. Introduction

Neuropathic pain develops after peripheral or central nerve injury [4,33]. A variety of clinical symptoms have been noted, primarily increased responsiveness to painful stimuli (hyperalgesia) and pain responses to normally innocuous stimuli (allodynia). Non-steroidal anti-inflammatory drugs are lack of effectiveness in neuropathic pain management. Moreover, opioids are not first line drugs for neuropathic pain because of lower efficacy. Thus, continuous efforts are indispensable to solve such unmet needs.

Remarkable progress has been made toward elucidating the processes of nociceptive transmission, especially at the spinal level, where many receptors and transmitters modulate nociception [5]. Of particular interest is nociceptin (orphanin FQ), a 17-amino-acid neuropeptide identified in 1995 [18,21]. The role of the NOP receptor (previously known as opioid receptor-like 1 receptor, ORL1) in modulating nociception in the spinal cord has recently been emphasized [15,31]. Several studies have demonstrated that intrathecal nociceptin can act through NOP receptor in the spinal cord to produce antinociceptive effects in various painful conditions [3,6,9,13,14,24,26,27]. Furthermore, the antihyperalgesic effect of intrathecal nociceptin in diabetic and mononeuropathic states was suppressed by intrathecal naloxone [3]. These findings suggest that opioid receptors may also be involved in the mechanism of action of nociceptin at the spinal level. However, the roles of the spinal opioid receptor types in the actions of nociceptin remain to be determined.

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In this study, we investigated the effects of intrathecal nociceptin in a rat model of neuropathic pain following spinal nerve ligation and investigated the contribution of spinal opioid receptor types to the effect of nociceptin. In addition, the levels of opioid receptor proteins in the spinal cord were measured by Western blot analysis.

2. Materials and methods

The studies were conducted after review and approval by the Institutional Animal Care and Use Committee of Chonnam National University. Male Sprague-Dawley rats weighing 100–200 g were used. They were acclimated to the standard laboratory environment (22 °C ± 0.5 °C, with a 12-h light/dark cycle) and were given standard rat chow and tap water ad libitum.

Neuropathic pain was evoked by spinal nerve ligation (left L5 and L6), as previously described [12]. The paw withdrawal threshold in response to mechanical stimulation was measured using the up-and-down method [1]. A series of eight von Frey filaments (0.4, 0.7, 1.2, 2.0, 3.6, 5.5, 8.5, and 15 g) were vertically applied to the plantar surface of the hind paw for 5 s while the hair was bent. Rapid withdrawal or flinching of the paw was considered to be a positive response. The tests were performed in duplicate with an approximately 3-min test-free period between withdrawal responses. Average values were used for analysis. Only rats with marked allodynia (withdrawal threshold of <4 g) after spinal nerve ligation were studied.

For drug administration, a polyethylene-10 tube was placed into the subarachnoid space of the rats 7 days after spinal nerve ligation, as previously described [25]. The catheter was advanced 8.5 cm caudally through an incision in the atlanto-occipital membrane, to the lumbar enlargement. Only rats with no postsurgical motor or sensory deficits were used for further experiments. Rats with neurological deficits after catheterization were immediately euthanized with an overdose of volatile anesthetics. The behavioral studies were performed at least 5 days following intrathecal catheterization.

The drugs used in this study were purchased from Tocris Cookson, Ltd. (Bristol, UK): nociceptin, naloxone, CTOP, naltrindole and GNTI. All drugs were dissolved in distilled water. Intrathecal administration of these agents was performed using a hand-driven, gear-operated syringe pump. All drugs were delivered in a volume of 10 μl of solution, followed by an additional 10 μl of normal saline to flush the catheter.

On the day of an experiment, the rats were placed in a box with a wire mesh floor for 20 min to adapt to the environment. All experiments were carried out by an observer blind to the drug treatments.

The effects of nociceptin (1, 3, 10, 30 μg, $n = 29$) in the rats with neuropathic pain were examined. The baseline mechanical withdrawal threshold was measured before spinal nerve ligation (pre-ligated). The post-ligated baseline withdrawal threshold was measured immediately before the intrathecal delivery of nociceptin. The withdrawal threshold was determined at 15, 30, 60, 90, 120, 150 and 180 min after intrathecal administration of nociceptin.

To determine whether the effect of intrathecal nociceptin was mediated through opioid receptors, rats were pretreated with several antagonists. Each antagonist was given intrathecally 10 min before the intrathecal administration of nociceptin (30 μg) and the withdrawal threshold was determined as described above. The maximal dose of each antagonist that did not affect the post-ligated baseline threshold or cause behavioral abnormalities was determined from pilot experiments and previously published studies [8,17,30]. The antagonists included naloxone, a nonselective opioid receptor antagonist (0.3 μg, $n = 10$); CTOP, a μ opioid receptor antagonist (15 μg, $n = 10$); naltrindole, a δ opioid receptor antagonist (10 μg, $n = 10$); and GNTI, a κ opioid receptor antagonist (50 μg, $n = 10$).

Protein extracts obtained from the spinal cord were used to determine the opioid receptor protein levels of naïve ($n = 5$), spinal nerve ligation ($n = 5$) and nociceptin (30 μg, $n = 5$) delivered rats. Twelve days after spinal nerve ligation, the rats were deeply anesthetized with sevoflurane and killed by decapitation. The left dorsal spinal cord, from L5 to L6, was then quickly removed and stored at −80 °C. The spinal cord was extracted by homogenization in protein extraction solution (PRO-PREP; Intron Biotechnology, Seoul, Korea), suspended on ice for 30 min, and clarified by centrifugation at 13,000 rpm for 10 min. For the Western blot, 50 μg of protein were loaded on a 10% SDS-polyacrylamide gel in Tris–HCl and electrophoresed. The resolved proteins were electrotransferred onto a polyvinylidene difluoride membrane, which was blocked using 5% nonfat dry milk in 20 mM TBS containing 0.1% Tween 20. The membrane was incubated with polyclonal primary antibodies against μ opioid receptor (MOR; sc-15310; 1:200 dilution), δ opioid receptor (DOR; sc-9111; 1:200 dilution), or κ opioid receptor (KOR; sc-9112; 1:1000 dilution) (all from Santa Cruz Biotechnology; Santa Cruz, CA, USA), in blocking solution overnight. After the membrane was washed, immunoreactive proteins were detected by incubation with horseradish peroxidase-coupled secondary antibody diluted 1:3000 in 5% nonfat dry milk. The membrane was washed three times, and immunoreactive protein bands were visualized by electrochemiluminescence (Santa Cruz Biotechnology), which was quantified using an imaging analyzer (LAS 3000; Life-science, Fujifilm Global, Tokyo, Japan). All reagents were removed from the membrane using a stripping buffer (Pierce, WI, USA), and the membrane was reprobed with antibodies against β-actin (Santa Cruz Biotechnology). The images were analyzed using Multi gauge V3.0 software (Life-science, Fujifilm Global), and the levels of MOR, DOR and KOR were normalized to the level of β-actin.

To evaluate the behavioral changes induced by nociceptin, additional rats were examined at 5, 10, 20, 30, 40, 50, 60, 120 and 180 min after intrathecal administration of nociceptin (30 μg, $n = 5$). Motor functions were assessed by examining the righting and placing/stepping reflexes [28]. Righting reflex was evaluated by placing the rat horizontally with its back on the table, which gives rise to an immediate coordinated twisting of the body to an upright position. The placing/stepping reflex was evoked by drawing the dorsum of either hind paw across the edge of a table. Normally the rats attempt to place the paw into a position to walk. The pinna and corneal reflexes were evoked by stimulating the ear canal or cornea with string, respectively [28]. Normally the rats spontaneously shook their heads or blinked.

The data are expressed as mean ± SEM. The time response data are presented as the withdrawal threshold in grams. The dose-response data are presented as a percentage of the maximum possible effect (%MPE): %MPE = [(post-drug threshold − post-ligated baseline threshold)/(cutoff threshold − post-ligated baseline threshold)] × 100. Dose-response data were analyzed by one-way analysis of variance (ANOVA) with a Bonferroni post hoc test. Comparisons of antagonisms on the effect of nociceptin were analyzed using an unpaired t-test. Null hypotheses of no difference were rejected if P-values were less than 0.05.

3. Results

The motor function, evaluated by the righting and the place-stepping reflexes, was normal after intrathecal administration of nociceptin at the highest dose used in this study. Both pinna and corneal reflexes were also normal at the same dose of intrathecal nociceptin.