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Effects of clonidine on bilateral pain behaviors and inflammatory response in rats under the state of neuropathic pain

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ABSTRACT

This study was conducted to investigate the effects of clonidine on bilateral pain behaviors and inflammatory responses in neuropathic pain induced by partial sciatic nerve ligation (PSNL), and to better understand whether the antinociception of clonidine was related to α_2-adrenoceptor mechanisms. Rats were divided randomly into five groups: sham-operation with saline, i.p.; PSNL with clonidine (0.2 mg/kg) or saline, i.p.; PSNL with yohimbine (2 mg/kg) followed by clonidine (0.2 mg/kg), i.p.; and PSNL with naloxone (0.3 mg/kg) followed by clonidine (0.2 mg/kg), i.p. On post-operative days 1, 3, 7, 14, and 21, both ipsilateral and contralateral pain behaviors were measured. In rats receiving antagonists, bilateral behavioral changes were measured on day 14. Bilateral paw pressure threshold and paw withdrawal latencies were measured, and the extent of glial activation was dertermined by measuring macrophage antigen complex-1 (Mac-1) and glial fibrillary acidic protein (GFAP). Additionally, the levels of tumor necrosis factor α (TNF-α) and interleukin (IL)-6 were determined. PSNL induced bilateral behavioral hyperalgesia, with the ipsilateral level displaying a higher extent of behavior changes than the contralateral side. In addition, the glial activation markers and cytokine production were augmented bilaterally. Clonidine caused significant attenuation of bilateral mechanical allodynia and thermal hyperalgesia, accompanied by inhibition of glial activation and the expression of cytokines. The effects of clonidine were blocked by the α_2-adrenoceptor antagonist yohimbine and partially reversed by the μ-opioid receptor antagonist naloxone. These data suggest that the bilateral antinoceptive effects of clonidine might mediate through immunomodulation by acting on α_2-adrenoceptor in rats undergoing neuropathic pain.

1. Introduction

Nerve injury can produce neuropathic pain, a prevalent condition associated with the development of a persistent hyperalgesia and allodynia [28]. Currently, available treatments for neuropathic pain are neither adequate nor effective. In light of this, much more effort has recently been put forth to develop novel therapeutic targets and drugs for the treatment of neuropathic pain.

Amounting evidence has indicated that central neuroimmune activation and neuroinflammation played crucial roles in the generation and maintenance of chronic pain. After nerve injury, both microglia and astrocytes are activated in the spinal cord and they release pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β). Additionally, activated glia are accompanied by the release of chemical mediators including substance P and chemokines, which lead to pain hypersensitivity [4,16,27]. In addition to glia activation in the ipsilateral spinal cord, contralateral glial proliferation might also occur in association with nerve injury [12], resulting in an extension of pain to the contralateral side, which is known as mirror image pain [24].

Clonidine produces antinociception mainly through α_2-adrenoceptors in acute and chronic pain states, both in animals and humans [3,20]. It has been reported that systemic clonidine partially relieves mechanical allodynia in rodents with the peripheral nerve injury [9], and topical application has also been shown to be antinociceptive in animal studies [5]. We have previously shown that intrathecal administration of clonidine attenuated spinal neuroimmune activation in rats with existing neuropathic pain following partial sciatic nerve ligation (PSNL) [7]. However, our previous study failed to explore the involvement of α_2-adrenoceptors in the mechanism of action of clonidine on neuroimmune activation and to observe the effects of clonidine on the development of the nerve injury-induced hypersensitivity and neuroinflammation as

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well. Furthermore, although the antinociceptive property of clonidine has been well established, relatively few studies have been conducted to investigate the effects of clonidine on mirror image pain in neuropathic pain.

This study was therefore conducted to assess the role of clonidine in behavioral changes suggestive of thermal hypersensitivity and mechanical allodynia, production of the pro-inflammatory cytokines (TNF-α and IL-6), and glial activation as measured by macrophage antigen complex-1 (mac-1) for microglia and glial fibrillary acidic protein (GFAP) for astrocytes in the bilateral sides of rats during the development of pain following PSNL. In addition, the antagonists for α_2-adrenoceptor and μ-opioid receptor were used in order to better understand the sites involved in the antinociceptive properties of clonidine.

2. Materials and methods

2.1. Animals and neuropathic surgery

Male Sprague–Dawley rats weighing 200–250 g housed in a room (temperature 21–25 °C, and humidity 45–65%) on a 12-h light–dark cycle with food and water available ad libitum. Rats (n = 160) were divided randomly into five groups: sham-operation with intraperitoneal injection of saline (n = 48); PSNL with intraperitoneal injection of clonidine (0.2 mg/kg) or saline (n = 48 each group); PSNL with intraperitoneal injection of yohimbine (2 mg/kg) followed by clonidine (0.2 mg/kg) (n = 8); PSNL with intraperitoneal injection of naloxone (0.3 mg/kg) followed by clonidine (0.2 mg/kg) (n = 8). In the treatment group, a previous intraperitoneal saline injection was done before the clonidine, as control for yohimbine/clonidine and naloxone/clonidine groups. In our pilot study, the effects of yohimbine and naloxone administered alone to the rats were also investigated. All animal experiments were approved by the local ethical committee and performed in accordance with the guidelines of the Committee for Research and Ethical Issues of IASP published in PAIN®, 16 (1983) 109–110.

Under deep anesthesia with 3% isoflurane in an O_2 carrier, PSNL was performed as previously described [7]. Body temperature was maintained at approximately 37 °C by a circulating water pad during the surgery. After the surgery, animals were recovered and placed in fresh cages to be kept singly throughout the experiment.

One day after the operation, 0.2 mg/kg clonidine (Sigma–Aldrich, St Louis, MO, USA) or saline was daily given intraperitoneally 2 h prior to the behavioral testing for 7 days. The dosage of clonidine was selected for systemic administration based on the previous studies about its effects on brain TNF-α production [17]. The antinociceptive effect of clonidine demonstrated in the present study is not due to a sedative effect because the selected doses did not induce any changes in the motor coordination as evaluated by the rota rod test. In additional experiments, 2 mg/kg yohimbine or 0.3 mg/kg naloxone or saline with the same volume was administrated 30 min before clonidine each day. The investigators involved were blind to the drug given until the end of the experiments.

2.2. Mechanical threshold and thermal threshold

All behavioral experiments were conducted between 8:00 a.m. and 11:00 a.m. The day of surgery was regarded as day 0. On post-operative days 1, 3, 7, 14, and 21, both ipsilateral and contralateral pain behaviors were measured. In rats receiving antagonists, the bilateral behavioral changes were measured on day 14.

The paw pressure threshold in response to normally innocuous mechanical stimuli and the paw withdrawal latencies in response to heat stimulus were determined respectively by using an ElectroVonFrey anesthesiometer (Model 2390CE, IITC Life Science Inc.) and radiant heat (Model 390, IITC Life Science Inc.) as previously described [14]. The force (g) applied and the paw withdrawal latency were recorded.

2.3. Tissue harvest

Animals were sacrificed on days 1, 3, 7, 14, and 21 following the surgery, respectively. In the groups treated with antagonists, animals were sacrificed on day 14. All rats were deeply anesthetized, perfused intracardially with 250 ml cold heparinized saline, and then rapidly decapitated. The ipsilateral and contralateral L4–5 spinal cord tissue rostral to the injury site was removed, dissected while on ice, collected in polypropylene tubes (RNAase, DNAase, and pyrogen free), and removed quickly to liquid nitrogen for subsequent assays.

2.4. Cytokine measurement

Spinal production of TNF-α and IL-6 was quantified by Enzyme-linked immunosorbent assay (ELISA) kits for rats according to the manufacturers' instructions (Biosource USA for IL-6). Values were expressed as picogram per milligram protein.

2.5. Glial activation markers

GFAP and mac-1, markers for astrocytic and microglial activation were detected as previously described [7].

2.6. Statistical analysis

Values are expressed as means ± SEM. For behavioral data, comparisons between groups were performed using repeated measures of analysis of variance (ANOVA). The effects of PSNL surgery and drug injections on other markers were determined by two-way ANOVA followed by Bonferroni or Tamhane's T2 test based on equal variances assumed or not. Data were analyzed using SPSS for Windows 13.0 (Chicago, IL, USA). $p < 0.05$ was considered significant.

3. Results

3.1. Behavioral changes

3.1.1. Mechanical allodynia and thermal hyperalgesia

Baseline responsiveness was minimal as confirmed by von Frey testing before the surgery. After PSNL, the animals displayed a marked increase in mechanical allodynia (Fig. 1A) and thermal hyperalgesia (Fig. 1C) on day 3 until day 21 for the ipsilateral hind paw, which was maximal on day 14. In contrast, a significant difference in the behavioral response for the contralateral hind paw was not observed until day 7 (Fig. 1B and D). Overall, mechanical allodynia and thermal hyperalgesia were significantly higher in the ipsilateral (Fig. 1A and C) than in the contralateral side (Fig. 1B and D). Compared with saline, clonidine caused a significant attenuation of mechanical allodynia and thermal hyperalgesia not only in the ipsilateral side but also in the contralateral side ($p < 0.01$). The sham group did not display significant behavioral changes in either side over baseline. On day 14, when the pain behavior reached its maximum, effects of the α_2-antagonist yohimbine and the μ-opioid receptor antagonist naloxone were observed. Coadministration of yohimbine significantly prevented the reversal of clonidine on nerve injury-induced hypersensitivity (Fig. 1E and F). In addition, naloxone could also attenuate the anti-hypersensitive effect of clonidine, but to a lesser extent as compared to yohimbine.