Effect of repeated topical application of clonidine cream in a rat model of postoperative pain

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Abstract

Introduction: Activation of alpha2 adrenoceptors by clonidine produces analgesia. Few data are available regarding the analgesic effect of clonidine cream (CC). We, therefore, evaluated the analgesic effect of CC in a rat model of postoperative pain.

Methods: CC at concentrations of 30, 100, or 300 µg/g was prepared by mixing clonidine with plastibase. A postoperative pain model was prepared in halothane–anesthetized Sprague–Dawley rats by making a 1 cm–long skin incision on the plantar hindfoot. Postoperatively, rats received topical application of plastibase, 0.1 g, or clonidine at 3, 10, or 30 µg in CC, 0.1 g, to the operated paw, once a day for 6 days, on postoperative days 0 through 5 (n=6 for each group). Mechanical allodynia and thermal hyperalgesia were quantified with paw withdrawal threshold (PWT) to mechanical stimuli and paw withdrawal latency (PWL) to heat stimuli, respectively, before and after daily CC application. Antagonizing activities of intraperitoneal yohimbine at 1 mg/kg, or atropine at 0.5 mg/kg, on effects of CC at 30 µg were also evaluated.

Results: Postoperatively, PWL and PWT decreased significantly in the operated paw. Topical application of CC did not affect PWL on the operative day, but it increased PWL significantly and dose–dependently, compared with placebo, on postoperative days 1 – 4. This CC–induced increase in PWL was antagonized by yohimbine. CC did not affect PWL in the unoperated paw, and it did not affect PWT in either paw.

Conclusions: These results indicate that repeated topical application of CC attenuated thermal hyperalgesia postoperatively, presumably by activating peripheral alpha2 adrenoceptors.

Key words: Clonidine; Topical application; Alpha2 adrenergic receptor; Yohimbine; Rat postoperative pain model
INTRODUCTION

Alpha2 adrenoceptor agonists yield analgesia in acute and chronic pain states, in both animals and humans \(^{4,6,7}\). Clonidine, an alpha2 adrenoceptor agonist, is an potent antinociceptive agent \(^{10}\), and systemic, spinal and supraspinal analgesic effects of clonidine have been reported \(^{2,23}\). Intrathecal injection of clonidine produces pain relief in patients after surgery \(^{12}\), and reduces allodynia in a rat model of postoperative pain \(^{7}\). However, systematic or intrathecal clonidine often produces adverse effects, such as sedation and cardiovascular depression, thereby limiting its use as an analgesic adjuvant.

Given these dose-limiting central side effects, it may be advantageous to apply clonidine locally, at the site of pain origin. Given that alpha2 adrenergic receptors are located not only in the central nervous system but also on primary afferents \(^{19,22,24}\), topically administered clonidine may have analgesic effects. With topical treatments, one may achieve analgesic efficacy due to higher drug concentrations at the site of pain origin, without producing centrally mediated side effects \(^{21}\).

In the previous study, we showed that single topical administration of clonidine cream produced dose-dependent analgesia in a rat model of neuropathic pain \(^{17}\). However, it was ineffective in a rat model of postoperative pain. The present study was designed to evaluate whether increased doses of clonidine cream, by means of repeated topical application, produce analgesia in the rat postoperative pain model. In addition, because previous studies have suggested that not only spinal alpha2 adrenergic receptors but also spinal muscarinic receptors are involved in the analgesic effect of clonidine \(^{20}\), we evaluated whether effects of clonidine cream, if any, could be antagonized by yohimbine, an alpha2 adrenoceptor antagonist, and atropine, a muscarinic receptor antagonist.

MATERIALS AND METHODS

Surgical preparation

After obtaining approval from the Institutional Animal Care Committee of the University of Tokyo, male Sprague–Dawley rats weighing 250 – 300 g at the start of experiments were used. The animals were housed in pairs, in plastic cages containing sawdust bedding under a 12 : 12 h light–dark cycle. Food and water were available ad libitum. Experiments were conducted during the light period.

The skin incision surgery was performed aseptically under inhalational anesthesia, which was induced and maintained with 4% halothane and 2% halothane, respectively, in oxygen. As previously described \(^{5}\), a 1-cm longitudinal incision was made with a number 11 blade, through the skin and fascia of the plantar aspect of the right foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally, but the muscle origin and insertion were left intact. After hemostasis with gentle pressure, the skin was apposed with two mattress sutures of 5-0 nylon. After surgery, the animals were allowed to recover from anesthesia and surgery in their cages.

Behavioral assessments

All the behavioral tests were performed between 10 am and 5 pm. The investigator involved in the behavioral testing was blinded to treatment groups.

Thermal nociceptive latency

Thermal hyperalgesia was quantified with a rat Plantar test apparatus (7370, Ugo Basile, Comerio, Italy). Rats were placed in individual plastic boxes on the glass surface of the test apparatus, and were allowed to acclimate for 15 min. Latency to paw withdrawal (PWL) in response to an intense light focused on the hind paw was determined, as previously described \(^{14}\). The glass surface on which the animals rested
was maintained at 30°C throughout the test period. Light intensity was pre-calibrated to give baseline PWL of approximately 10 s for the unoperated hind paw. Animals were acclimated to the procedures until stable PWL values were obtained. The mean PWL values obtained from the last three measurements in each test session was used for data analysis. A cutoff time was set at 20s to avoid tissue damage.

**Mechanical nociceptive threshold**

Mechanical allodynia was quantified by application of calibrated von Frey filaments (Stoelting, Wood Dale, IL). Animals were placed on a wire floor in individual clear boxes. After accommodation to the environment for 15 min, von Frey filaments were applied vertically to the plantar surface of the hind paw and pressed to the point of bending over 2 s. This procedure was repeated 4 times for one filament. If no response was elicited, a larger filament was applied. The filaments were applied in increasing order until a brisk withdrawal or paw flinching occurred, which was considered a positive response. This withdrawal threshold was determined three times, with testing separated by 5 min, and a mean value of paw withdrawal threshold (PWT) was used for data analysis.

**Drugs**

Clonidine hydrochloride, yohimbine hydrochloride, an alpha2 adrenoceptor antagonist, and atropine sulphate, a muscarinic receptor antagonist, were obtained from Sigma Aldrich (St, Louis, MO). Clonidine cream was prepared by the Institutional Pharmacy by mixing clonidine hydrochloride with plastibase, consisting of 5% polyethylene resin and 95% liquid paraffin, as vehicle so as to make final clonidine concentrations of 30, 100 or 300 µg/g. Plastibase (vehicle) alone, 0.1 g, was used as a placebo for clonidine, at 3, 10, or 30 µg, respectively, in clonidine cream 0.1 g. Yohimbine hydrochloride, 1 mg/kg, and atropine sulphate, 0.5 mg/kg, respectively, were dissolved in normal saline, 0.3 ml, for intraperitoneal injection.

**Experimental protocol**

The animals were assigned to receive topical application of vehicle, 0.1 g, or clonidine, at 3, 10, or 30 µg (in clonidine cream 0.1 g), to the operated paw; once a day for 6 days; at 2 h postoperatively, and then at 1, 2, 3, 4, and 5 d postoperatively (n=6 for each group). PWL and PWT of both hind paws were measured; before skin incision; at 1 h before topical drug application; at 2 and 4 h after topical drug application on the operative day; and at 2 h after daily topical drug application on postoperative days 1 through 5.

Additional 4 groups of animals, respectively, were pretreated with intraperitoneal yohimbine, 1 mg/kg, or atropine, 0.5 mg/kg, at 10 min prior to topical application of vehicle, 0.1 g, or clonidine, at 30 µg (in clonidine cream 0.1 g), once a day for 6 days (n=6 for each group). PWL and PWT of both hind paws were measured at 9 time points as described above.

**Data analysis**

Results were presented as mean ± SD. Statistical analysis was carried out using repeated measures ANOVA followed by Bonferroni/Dunn test. P values less than 0.05 were considered significant, except where adjusted for multiple comparisons.

**RESULTS**

All rats maintained good health and continued to gain weight throughout the experimental period. No infection was observed in any of the animals. There was no significant difference in weight among groups.

After skin incision, PWL and PWT decreased significantly in the operated paw, indicating significant development of thermal hyperalgesia and mechanical allodynia, respectively (**Figs. 1A & 4A**). Topical application of clonidine cream did not affect PWL in the clonidine–treated
groups, compared with the vehicle–treated group, at 2 or 4 h after the topical clonidine application on the operative day, indicating that clonidine cream did not attenuate thermal hyperalgesia during the immediate postoperative period. However, topical application of clonidine, at 3 – 30 µg (in 0.1 g cream) resulted in significant dose–dependent increases in PWL, compared with the vehicle–treated group, on postoperative days 1 through 4 (Fig. 1A; p<0.05), indicating that clonidine cream attenuated thermal hyperalgesia during the early to late postoperative
period. In contrast, no significant changes in PWL were observed in the unoperated paw in any of the vehicle– or clonidine–treated groups during the observation period (Fig. 1B), indicating that clonidine cream applied to the operated paw did not produce thermal antinociception in the unoperated paw.

Systemic yohimbine, at 1 mg/kg, completely blocked the increases in PWL induced by clonidine at 30 µg in the operated paw, on postoperative days 1 through 5, indicating that the anti–hyperalgesic effect of clonidine cream was antagonized by systemic yohimbine (Fig. 2A). In contrast, systemic atropine, at 0.5 mg/kg, did not significantly block the increases in PWL, except on the postoperative day 1 (Fig. 3A).
There were no significant differences in PWT between the vehicle–treated group and any of clonidine–treated groups, in the operated or unoperated paw, during the observation period (Figs. 4A & 4B), indicating that clonidine cream did not affect mechanical allodynia in the operated paw nor mechanical nociception in the unoperated paw.

**DISCUSSION**

In the previous study, we showed that in a rat model of postoperative pain, single topical application of clonidine cream attenuated neither thermal hyperalgesia nor mechanical allodynia on the operative day \(^{17}\). Results of the present study were basically the same, regarding the lack of the analgesic effect of clonidine cream in this immediate postoperative phase. In the present study, however, repeated topical application of clonidine cream significantly attenuated thermal hyperalgesia, in a dose–dependent manner, on postoperative days 1 through 4, although it failed to reduce mechanical allodynia throughout the observation period. This anti–hyperalgesic efficacy of topical clonidine was antagonized completely by pretreatment with systemic yohimbine, an alpha2 adrenergic receptor antagonist. Such analgesic action of topical clonidine was limited to the operated paw exposed to clonidine cream, and was not seen in the unoperated paw not exposed to the compound. Taken together, clonidine cream attenuated thermal hyperalgesia during the early to late, but not immediate, postoperative period, presumably via activation of peripherally located alpha2 adrenergic receptors. The contribution of cholinergic systems to the analgesic effect of topically administrated clonidine appeared to be very small, in contrast to spinally administrated clonidine \(^{20}\), since systemic atropine blocked the analgesia induced by clonidine cream only minimally in the present study.

The postoperative pain model of rats developed by Brennan et al \(^{5}\) is considered to be very useful in simulating human postoperative pain, because this model expresses primary and secondary hyperalgesia, and allodynia, as are typically seen after surgery in patients \(^{26,27}\). Results from the present study in the rat postoperative pain model support the hypothesis that activation of alpha2 adrenergic receptors, expressed on peripheral cutaneous nociceptors, inhibit nociception. This hypothesis is supported also by previous data showing that alpha2 adrenoceptors are expressed on the dorsal root ganglion neurons \(^{19,22,24}\), that activation of alpha2 adrenoceptors inhibits the release of pronociceptive substances, such as CGRP, from capsaicin–sensitive nociceptive fibers \(^{15}\), that clonidine is a lipophilic substance which would easily penetrate the skin easily \(^{3}\), and that peripherally (i.e. topically or intra–articularly) administered clonidine and other alpha2 agonists actually elicit analgesic effects \(^{1,8,9,13}\). It is unlikely that the efficacy of topically administered clonidine cream was due to this compound diffusing into the bloodstream and acting at spinal and/or supraspinal sites, given that the antinociceptive action of topical clonidine was limited to the area exposed to the compound, confirming a peripheral site of action.

The reason why clonidine cream attenuated only thermal hyperalgesia, and failed to attenuate mechanical allodynia, was unclear. However, possibilities may include distinct mechanisms underlying mechanical allodynia and thermal hyperalgesia, differing painful intensities evoked by mechanical and thermal tests used, as experienced by the operated paw, or a combination of these possibilities \(^{25}\). For example, differential nerve block experiments showed that the signals for continuing pain and hyperalgesia to heat were carried by unmyelinated nociceptive fibers out of the skin, while touch–evoked pain was encoded by large myelinated fibers \(^{16}\). More specifically, thermal hyperalgesia, static
mechanical allodynia, and dynamic mechanical allodynia may be respectively signaled by C–, A delta–, and A beta–/capsaicin insensitive A delta– primary sensory neurons. A recent study has suggested that in the absence of nerve injury, DRG neurons of small to medium size which co–express vanilloid receptor TRPV1 and alpha2C adrenoceptors may play an important role in the analgesic effects of clonidine. When combined together, these findings may explain differential effects of clonidine cream on thermal hyperalgesia and mechanical allodynia.

Topical application of clonidine cream did not attenuate thermal hyperalgesia on the operative day, although it attenuated thermal hyperalgesia on subsequent days. The lack of anti–hyperalgesic effect on the operative day was presumably because immediately after surgery, ongoing tonic nociceptive barrages and pronociceptive effects of inflammatory substances were so large, as suggested by marked decreases in PWL on the operative day, that topical clonidine failed to block transmission of nociceptive stimuli, unlike systemic or spinal clonidine. The anti–hyperalgesic effect of clonidine cream became evident during the later period, as relief of thermal hyperalgesia spontaneously progressed with healing of the wound. Further studies are required to assess if higher doses of clonidine cream can attenuate not only thermal hyperalgesia on the operative day but also mechanical allodynia on the operative as well as postoperative days in the rat postoperative pain model, as was seen in the rat neuropathic pain model in our previous study.

In summary, we demonstrated that repeated topical application of clonidine cream produced a significant anti–hyperalgesic effect, but not anti–allodynic effect, in a rat postoperative pain model. The anti–hyperalgesic effect appeared to be mediated by peripheral alpha2 adrenoceptors. Results of the present study suggest that clonidine cream may be a useful analgesic adjunct, when applied to mild postoperative pain.

References

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