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Effects of yohimbine on naloxone-induced antinociception in a rat model of inflammatory hyperalgesia

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Abstract

Effects of the α_2-adrenoceptor antagonist yohimbine on the antinociception produced by a low dose of naloxone were examined in a rat model of carrageenan-induced inflammation. In rats receiving saline prior to naloxone injection, the low dose of naloxone (5 μg/kg, i.p.) significantly prolonged paw withdrawal latency in response to noxious thermal stimuli for both the inflamed and the non-inflamed paws 4 h after carrageenan injection (6.0 mg in 0.15 ml saline). In rats receiving yohimbine, the low dose of naloxone failed to produce prolongation of paw withdrawal latencies 4 h after carrageenan, whereas naloxone produced antinociception 7 days after carrageenan. The results suggest that noradrenergic mechanisms are involved in naloxone-induced antinociception only in the early phase of carrageenan-induced inflammation.

Keywords: Yohimbine; Naloxone; Antinociception; Inflammation

1. Introduction

Numerous studies have focused on the effects of an opioid receptor antagonist, naloxone, to show a possible role of endogenous opioid peptides in the control of pain. Naloxone has dose-dependent bidirectional effects in the control of pain in humans (Buchsbaum et al., 1977; Lasagna, 1965; Levine et al., 1979) and on the nociceptive threshold in animals (Dickenson et al., 1981; Kayser and Guilbaud, 1990; Rios and Jacob, 1983; Ueda et al., 1986; Woolf, 1980). In contrast to the expected hyperalgesic effects of high doses (mg range) of naloxone, low doses (μg range) of naloxone cause antinociceptive effects. This analgesia produced by low doses of naloxone has been investigated mainly in a rat model of experimental inflammation pain (Kayser and Guilbaud, 1990; Rios and Jacob, 1983). Although it has been hypothesized that the antinociceptive effect is mediated through putative presynaptic autoreceptors for endogenous opioid peptides, the mechanisms accounting for the antinociceptive effect induced by low doses of naloxone still remain unclear. We report here on the involvement of noradrenergic mechanisms in producing naloxone-induced antinociception in a rat model of inflammatory hyperalgesia.

2. Materials and methods

Experiments were performed on male Sprague–Dawley rats weighing 250–280 g when tested 4 h following administration of carrageenan and weighing 280–330 g when tested 7 days after carrageenan. The rats were housed in groups of 3–4 in a cage with sawdust bedding, and had free access to rat chow and water in a laboratory with a 12-h/12-h (8 AM/8 PM) light–dark cycle. Room temperature and humidity were maintained at 23 ± 0.5°C and 60%, respectively.

Carrageenan (lambda, Sigma) was used to induce unilateral inflammation. Under halothane anesthesia (2.5% in air), the rats received a subcutaneous injection of 6.0 mg carrageenan in 0.15 ml saline into the plantar surface of the left hindpaw. At this dose of carrageenan, both edema and hyperalgesia develop rapidly in the carrageenan-injected left hindpaw by 4 h and still remain at 7 days (Tsuruoka and Willis, 1996). For the present experiments,

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the rats were divided into the following two groups: (1) rats ($n = 12$) receiving the injection of carrageenan 4 h before testing of the naloxone-induced antinociception and (2) rats ($n = 12$) receiving the injection of carrageenan 7 days before testing naloxone-induced antinociception. In order to minimize discomfort of the animals under these conditions, the guidelines on ethical standards for investigations of experimental pain in animals (Zimmermann, 1983) were followed. For example, the number of animals used was kept to a minimum, and the duration of the experiment was as short as possible. The experiments were carried out in the light phase on separate groups of animals and all testing was conducted in a quiet room by the same person. The rats were tested for behavioral nociception with radiant heat stimuli. Paw withdrawal latency was determined by the method described by Hargreaves et al. (1988), which measures cutaneous hyperalgesia in response to thermal stimuli. The rats were placed on an elevated glass surface under an inverted clear plastic chamber ($13 \times 13 \times 14$ cm^3) and a radiant heat source (projection lamp: 120 V, 300 W) was positioned under the glass floor directly beneath one hindpaw. The paw withdrawal latency, to the nearest 0.01 s, was recorded using an electronic timer circuit. Heating was terminated at 15 s to avoid tissue damage if an animal failed to withdraw its paw. The paw withdrawal latency, however, was within 15 s in all animals throughout the experiments. The α_2-adrenoceptor antagonist, yohimbine (0.6, 3.0, 7.5 mg/kg, i.p., Sigma), was administered 10 min before nociceptive testing, and the effect of a low dose of naloxone (naloxone hydrochloride, 5 μg/kg, i.p., Sigma) was then examined. Paw withdrawal latency determinations were made for a period of 120 min after drug injection. In the control, saline was used for comparison with the effect of naloxone. Results are presented as the means ± S.E.M. Statistical analysis was carried out using an analysis of variance (ANOVA) for repeated measures for overall effects, with Duncan's new multiple range test for comparisons between naloxone- and saline-administrated groups. When P values were greater than 0.05, differences were not considered to be significant.

3. Results

In normal animals without inflammation, following the administration of yohimbine (3 mg/kg, i.p.), hyperalgesia was observed in all the rats tested. Paw withdrawal latencies decreased gradually for 30 min after administration and returned to the value before yohimbine during the next 30 min. The effect of yohimbine was statistically significant between 10 and 40 min after administration as compared with the effect of saline administration (Fig. 1A). Therefore, the effects of a low dose of naloxone or saline were estimated within 40 min after the administration of yohimbine.

Following the injection of carrageenan, hyperalgesia occurred in the carrageenan-injected left hindpaw with the development of edema. Four hours after carrageenan injection, paw withdrawal latencies decreased significantly compared to the value before injection. In the contralateral, uninjected paws, there was no change in either paw withdrawal latency or paw thickness. Prior to the test with a low dose of naloxone, the rats were tested for the effects of saline. The intraperitoneal administration of saline had no significant effect on paw withdrawal latencies for either the inflamed or the non-inflamed paws (Fig. 1B). When a

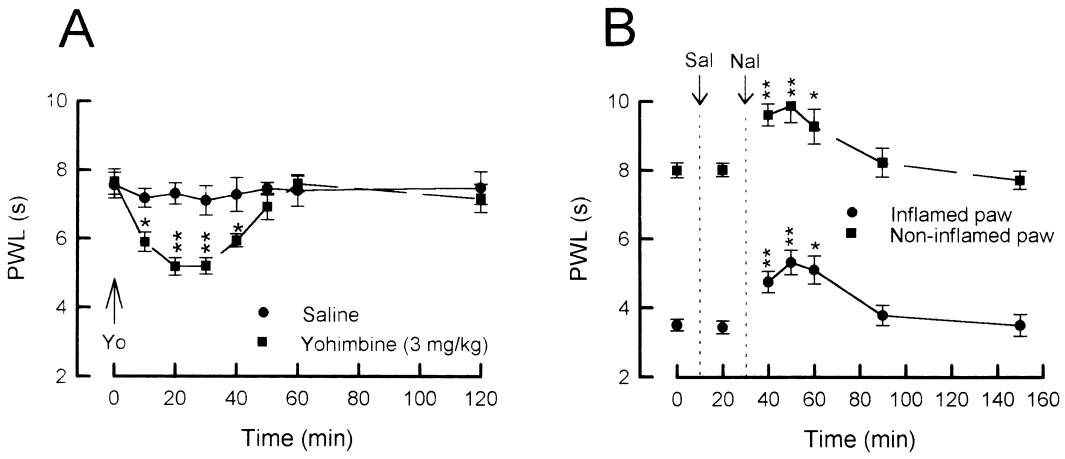

Fig. 1. (A) Time-course of changes in PWLs following the administration of yohimbine (3 mg/kg, i.p.). Data were obtained from rats given saline ($n = 14$) and rats given yohimbine ($n = 10$). The values at time zero reflect control values obtained immediately prior to administration of yohimbine. *Significant difference as compared to the value for the saline group (* $P < 0.05$, ** $P < 0.01$). (B) Effects of 5 μg/kg naloxone or saline on PWLs in rats ($n = 14$) without yohimbine, tested 4 h after carrageenan injection. Data were obtained 10 min after i.p. saline or 10, 20, 30, 60 and 120 min after i.p. naloxone. *Significant difference as compared to the value 10 min after saline administration (* $P < 0.05$, ** $P < 0.01$). PWL, paw withdrawal latency; Yo, yohimbine; Nal, naloxone; Sal, saline.

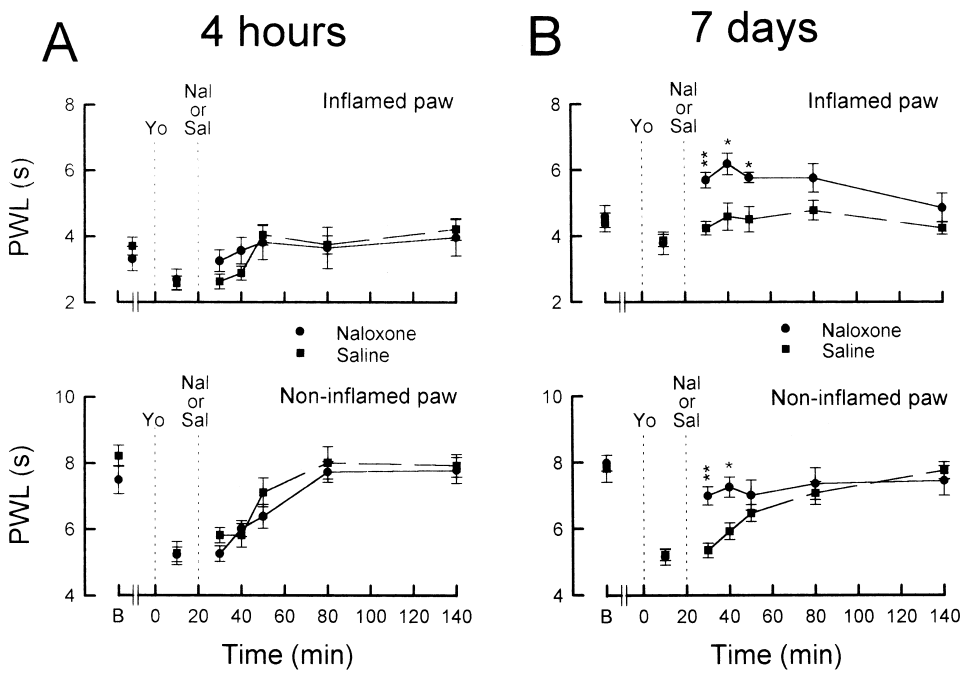

Fig. 2. Effects of yohimbine (3 mg/kg, i.p.) on naloxone-induced antinociception tested 4 h ($n = 12$) and 7 days ($n = 12$) after injection of carrageenan. Data were obtained 10 min after i.p. yohimbine or 10, 20, 30, 60 and 120 min after i.p. either naloxone or saline. $* P < 0.05$, $** P < 0.01$, compared to the value for the saline group. PWL, paw withdrawal latency; Yo, yohimbine; Nal, naloxone; Sal, saline; B, baseline latency.

low dose of naloxone was administered, paw withdrawal latencies were significantly prolonged for both the inflamed and the non-inflamed paws for 30 min following naloxone administration (Fig. 1B). In contrast, when yohimbine (3 mg/kg) was administered prior to a low dose of naloxone, the prolongation of paw withdrawal latencies following naloxone administration was not observed for either the inflamed or the non-inflamed paws. Statistical analysis of paw withdrawal latencies for rats given naloxone or saline did not reveal a significant difference between groups at comparable post-naloxone times (Fig. 2A). In the group of rats receiving carrageenan injection 7 days before, edema and the shortening of paw withdrawal latencies still remained. Systemic administration of yohimbine (3 mg/kg) produced a significant decrease of paw withdrawal latencies of both the inflamed and the non-inflamed paws. A low dose of naloxone resulted in a significant prolongation of paw withdrawal latencies for 30 min from its administration for the inflamed paw and for 20 min from naloxone administration for the non-inflamed paw, whereas saline did not produce a significant change in withdrawal latencies of either the inflamed or non-inflamed paw. There was a significant difference between rats given naloxone and those given saline at comparable post-naloxone times (Fig. 2B). Dose-dependent effects of yohimbine were examined to confirm the effect of yohimbine on naloxone-induced antinociception 4 h after the induction of inflammation (Fig. 3). In this experiment, because the effect of 0.6 mg/kg of yohimbine was statistically significant only 20 min after its administration (not shown), three doses of yohimbine were administered simultaneously with either naloxone or saline, and nociception was tested 20 min after the administration.

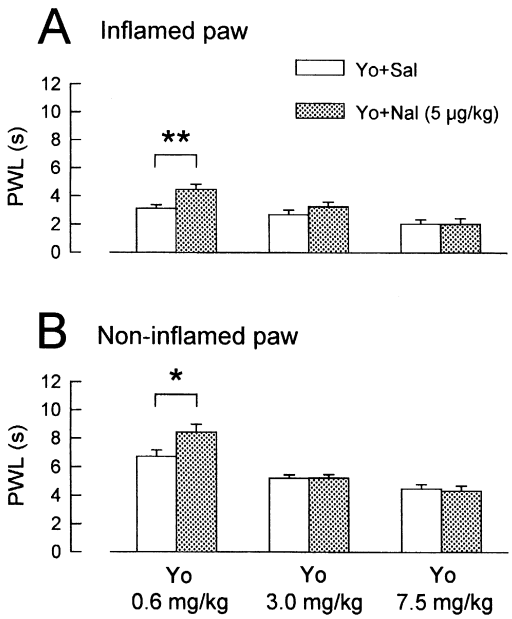

Fig. 3. Dose-dependent effects of yohimbine on naloxone-induced antinociception tested 4 h ($n = 8$) after injection of carrageenan. Yohimbine was administered simultaneously with either naloxone or saline 20 min before nociceptive testing. Data are presented for both the inflamed (A) and the contralateral non-inflamed (B) paws. $* P < 0.05$, $** P < 0.01$, significant difference between naloxone and saline groups. PWL, paw withdrawal latency; Yo, yohimbine; Nal, naloxone; Sal, saline.

With 0.6 mg/kg of yohimbine, a low dose of naloxone resulted in significant prolongation of the paw withdrawal latencies decreased by yohimbine for both the inflamed and the non-inflamed paws. After a dose of 7.5 mg/kg significant prolongation of paw withdrawal latencies following the administration of a low dose of naloxone was not seen for either the inflamed or the non-inflamed paws. This was also the case with 3.0 mg/kg of yohimbine.

4. Discussion

In rats with unilateral hindpaw inflammation induced by subcutaneous injection of carrageenan, yohimbine prevented naloxone-induced antinociception at 4 h after the induction of inflammation. In contrast, even after yohimbine, naloxone-induced antinociception was observed at 7 days, when both edema and hyperalgesia still remained. These results indicate that noradrenergic mechanisms are involved in producing naloxone-induced antinociception 4 h after the induction of inflammation and that at 7 days, the naloxone-induced antinociception is not mediated by noradrenergic mechanisms. This suggests that noradrenergic mechanisms are active only in the early phase of the inflammatory process (e.g., 4 h after the induction of inflammation), but not in the later phase of inflammation (e.g., 7 days after the induction of inflammation). In order to explain the antinociceptive effects produced by low doses of naloxone, the existence of putative presynaptic autoreceptors, responsible for the ongoing suppression of endogenous opioid peptide release, has been proposed (Bourgoin et al., 1991; Kayser et al., 1988; Ueda et al., 1986). Since it has been shown that the analgesic effects of opioids during inflammation may depend on an interaction with spinal noradrenergic pathways (Hylden et al., 1991), it seems that noradrenergic mechanisms might modify an action of presynaptic autoreceptors on the release of endogenous opioid peptides. Concerning the time-dependent action of noradrenergic mechanisms in the present study, a similar result has been reported for a descending action from the locus coeruleus in naloxone-induced antinociception. The locus coeruleus sends noradrenergic projections to the spinal cord (Clark and Proudfit, 1992; Grzanna and Fritschy, 1991; Proudfit and Clark, 1991; Westlund et al., 1983). These descending noradrenergic fibers from the locus coeruleus have been shown to be involved in both antinociception and inhibition of nociceptive activity of dorsal horn neurons (Jones, 1991; Jones and Gebhart, 1988; Margalit and Segal, 1979; West et al., 1993). In a previous study (Tsuruoka and Willis, 1998), we have shown that the locus coeruleus is involved in naloxone-induced antinociception in the early phase of inflammation and that the locus coeruleus is inactive in the later phase of inflammation. The results in the present study may support the notion that naloxone-induced antinociception in the early phase of inflammation is mediated by a descending modulation system from the locus coeruleus. In addition, naloxone-induced antinociception appears to be independent from an interaction with noradrenergic mechanisms in the later phase of inflammation. We have reported that the subnucleus reticularis dorsalis is involved in naloxone-induced antinociception during the later phase of inflammation (Tsuruoka et al., 1997). A recent study has shown that the subnucleus reticularis dorsalis is one of the supraspinal structures in the loop underlying the diffuse noxious inhibitory controls (Bouhassira et al., 1992). It is possible that naloxone-induced antinociception in the later phase of inflammation is mediated by diffuse noxious inhibitory controls.

The descending modulation system from the locus coeruleus may also be involved in a tonic descending influence on nociception during inflammation. Hyperalgesia produced by intrathecal administration of yohimbine has been observed in normal rats without inflammation, indicating the presence of a tonic descending noradrenergic inhibitory control (Sagen and Proudfit, 1984). In the present study, yohimbine produced hyperalgesic effects in both the inflamed and the non-inflamed paws 4 h and 7 days after the induction of inflammation. This indicates that the tonic descending noradrenergic modulation system is active even during the inflammatory process. A recent study has shown that peripheral inflammation activates the descending modulation system from the area of the locus coeruleus and that this activation occurs only in the early phase of inflammation, and not in the later phase (Tsuruoka and Willis, 1996). It is possible that the descending modulation system from the locus coeruleus is partly involved in the tonic descending noradrenergic influence on nociception 4 h after carrageenan injection, although the sources of tonic descending inhibition are still unclear.

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