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Effects of spinal naloxone and naltrindole on the antinociceptive action of intrathecally administered dexmedetomidine

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Abstract: Intrathecally administered alpha-2 adrenoceptor and opioid agonists are well known to exert antinociceptive effects in humans and various animals. To examine the interaction of these two groups of agents in the spinal cord, we tested the effect of the opioid antagonists naloxone or naltrindole on the antinociceptive action of an intrathecally administered alpha-2 agonist, dexmedetomidine, using a formalin test in rats. 19 groups of Sprague-Dawley rats (250–300 g) were prepared with chronic intrathecal catheters and examined for the effects of agents on the formalin test. Each group contained 6 animals. 50 μl of 5% formalin was injected subcutaneously in the plantar surface of one hind paw. For each animal, the number of spontaneous flinches, characterized by rapid and brief withdrawal of the injected paw, were counted separately at 1–2 min, 5–6 min (phase 1), and at 5-min intervals thereafter up to 60 min (phase 2). Intrathecal dexmedetomidine (1 μg) maximally depressed the behavioral changes in both phase 1 and phase 2 of the formalin test, which was antagonized by the alpha-2 adrenoceptor selective antagonist atipamezole (0.3 μg). Naloxone (0.1–10 μg) or naltrindole (1–10 μg), when coadministered with dexmedetomidine, showed a dose-dependent antagonism to the effect of dexmedetomidine, whereas naloxone, naltrindole, or atipamezole alone showed no effect on the nociceptive behavior due to formalin injection. These results indicate that the antinociceptive effect of intrathecally administered alpha-2 adrenoceptor agonists may involve opioid receptors in the spinal cord.

Key words: Spinal cord, Alpha-2 adrenoceptor, Opioid

Introduction

Intrathecal (i.t.) administration of dexmedetomidine, a highly selective alpha-2 adrenoceptor agonist, produces antinociception in rats [1–4]. Mechanisms underlying this antinociception are thought to involve presynaptic inhibition of neurotransmitter release from terminals of small-diameter afferent fibers and postsynaptic inhibition of second-order neurons in the spinal dorsal horn. Similar mechanisms are believed to mediate the effects of spinal mu and delta opioids [5]. While there is some evidence that these systems functionally interact [6], the possible interaction of dexmedetomdine with the opioid receptors remains controversial. In the present study we aimed to determine whether there is an interaction of the spinal opioid receptor system with the antinociceptive effects of spinal dexmedetomidine. For this purpose, the effects of the opioid antagonist, naloxone, or delta selective opioid antagonist, naltrindole, on the antinociceptive action of dexmedetomidine were investigated.

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Materials and methods

Intrathecal (i.t.) catheter

Following a protocol approved by the Institutional Animal Care Committee, University of California, San Diego, 19 groups of male Sprague-Dawley rats (270–320 g) were prepared with chronic catheters in the lumbar subarachnoid space [7]. Briefly, under halothane anesthesia, a PE-10 catheter was inserted through an incision in the atlantooccipital membrane and advanced, placing the tip at the level of the lumbar spinal enlargement. The catheter was externalized on the top of the skull and sealed with a piece of steel wire. The wound was closed with 3-0 silk sutures. Rats showing neurological deficits postoperatively were killed.

Formalin test

50 μl of 5% formalin was injected subcutaneously in the plantar surface of the right hind paw with a 30 G needle

under halothane anesthesia. After recovering from halothane anesthesia, the animal was then placed in a plexiglass box which permitted observation. For each animal, the number of spontaneous flinches, characterized by a rapid and brief withdrawal of the injected paw, was counted separately at 1–2 min, 5–6 min, and at 5-min intervals thereafter up to 60 min. As previously described, two distinct phases were observed: phase 1 during the 5-min interval immediately following the interplantar injection and phase 2, which began about 10 min after formalin injection. For purposes of analysis, the phase 1 and phase 2 data were examined separately. The animals were killed at this time with a lethal dose of barbiturate.

Each agonist and/or antagonist was dissolved with physiological saline and injected intrathecally 10 min before the formalin test. The volume of injected agents was always 10 µl followed by the same volume of saline flush. To see the dose-related effect of dexmedetomidine, doses of 0.03, 0.1, 0.3, and 1 µg were injected. Dexmedetomidine 1 µg was used to examine the effects of opioid and alpha-2 antagonists, because it produced a reliable just-maximal antinociceptive action on the formalin test in both phases (see Results).

In another group of rats, atipamezole 0.3 µg, a selective alpha-2 antagonist, was injected simultaneously with dexmedetomidine 1 µg.

The dose of naloxone employed was either 0.1, 1, 3, 10, 30, or 100 µg, and the dose of naltrindole was 1, 3, 10, or 30 µg. Each group contained 6 animals.

Results

Injection of formalin into the foot of the animal evoked a biphasic flinching behavior (Fig. 1). Dexmedetomidine i.t. suppressed the formalin-induced behavior dose-dependently in both phase 1 and phase 2 (Fig. 2). A dose of 1 µg produced a just-maximal inhibition in both phases (Figs. 1, 2) which was completely antagonized by coadministration of i.t. atipamezole 0.3 µg (Fig. 2). Atipamezole i.t. alone had no effect on the formalin test.

Naloxone i.t. had a partial dose-dependent antagonistic effect on the antinociceptive effect of dexmedetomidine in a dose range of 0.1–10 µg in both phases. However, the higher doses of 30 and 100 µg had a lesser effect (Fig. 3).

Naltrindole i.t. also displayed biphasic effects similar to those of naloxone (Fig. 4). Neither antagonist had a significant effect on the formalin test alone (data not shown).

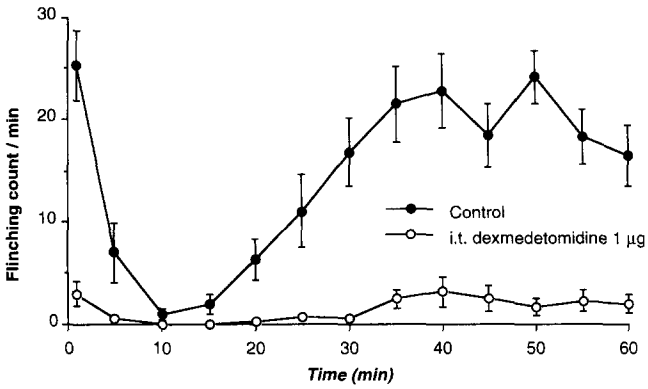

Fig. 1. Time course of flinching frequency per minute are shown. In both the control group and the group given intrathecal (i.t.) dexmedetomidine 1 µg, two distinct phases were noted. In both phases, dexmedetomidine produced submaximal suppression of the behavior

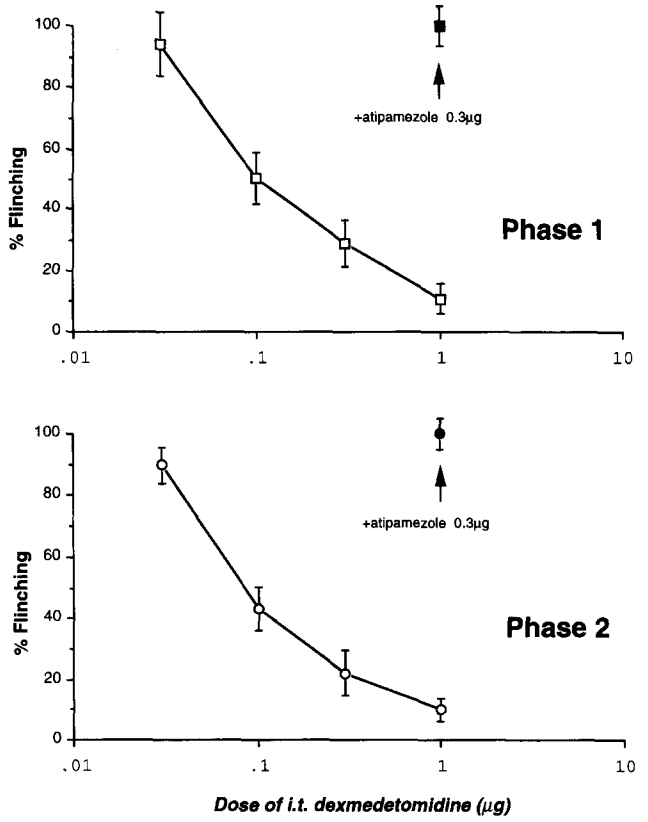

Fig. 2. Dose reponse curves of i.t. dexmedetomidine for phase 1 and phase 2. ED_{50}s are 0.14 µg and 0.12 µg for phase 1 and 2, respectively. When atipamezole 0.3 µg was coadministered with dexmedetomidine 1 µg, the antinociceptive action of dexmedetomidine was completely antagonized. Each data point represents mean ± SE from six rats

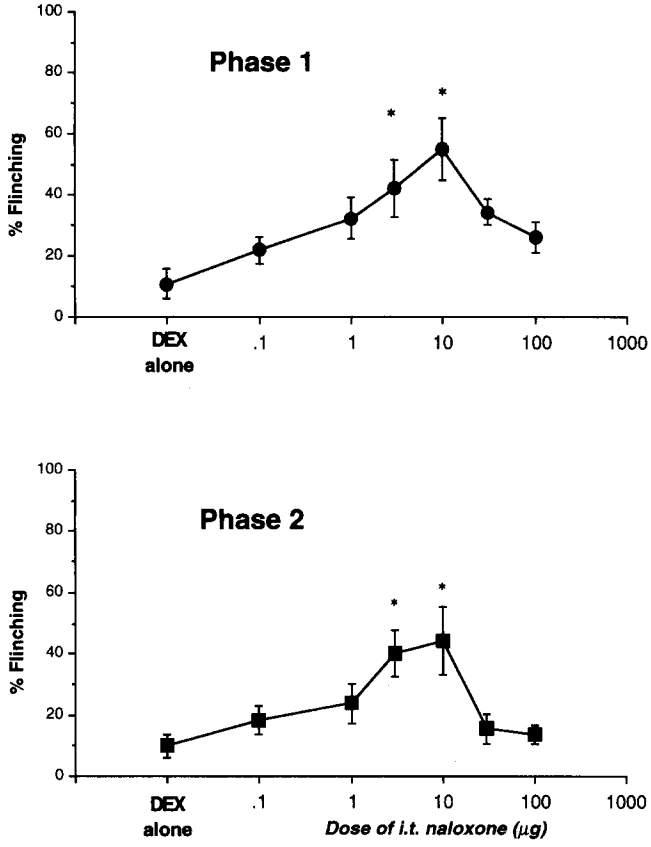

Fig. 3. The antinociceptive effect of i.t. dexmedetomidine 1 μg (*DEX*) was antagonized by i.t. naloxone, but the effect was not dose-dependent. 3 and 10 μg of naloxone showed significant antagonism (*$P < 0.05$). Naloxone had similar effects on both phases of the formalin test. Each data point represents mean ± SE from six rats

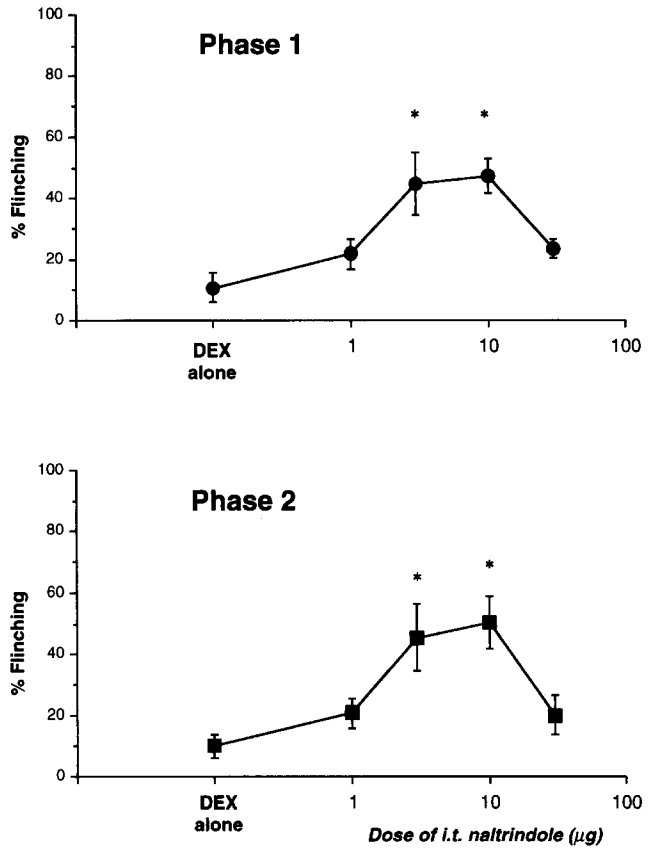

Fig. 4. Effects of the delta selective antagonist naltrindole. The effect of dexmedetomidine (*DEX*) was significantly antagonized by naltrindole 3 and 10 μg. Each data point represents mean ± SE from six rats. *Significant difference from data of DEX 1 μg

Discussion

The complete antagonism of the effect of dexmedetomidine by atipamezole confirms that the inhibitory effect of dexmedetomidine on the formalin test is mediated through alpha-2 adrenoceptors in the spinal cord. The observation that dexmedetomidine had similar suppression on both phase 1 and phase 2 responses induced with the formalin test suggests that the antinociceptive action of alpha-2 adrenoceptor agonists may be prevalent in several modalities of noxious stimuli, such as chemical stimulus in phase 1 and inflammatory stimulus in phase 2 of the formalin test. While previous studies have reported that naloxone has little effect on the antinociceptive action of spinally administered noradrenaline [8,9] in the rat, there have been several reports that the antinociceptive responses of alpha-2 agonists are antagonized by the opioid receptor antagonist naloxone. For example, Kendig et al. [10] reported that in the neonatal rat spinal cord, inhibition of the ventral root potential evoked by a dorsal root electrical stimulus was antagonized by naloxone. Loomis et al. [11] have shown that pretreatment with naloxone i.t. antagonized the effect of norepinephrine i.t. in a tail-flick test. Sullivan et al. [12] reported that the suppressive effect of dexmedetomidine on spinal convergent neuron activity evoked by an electrical stimulus was antagonized by spinal administration of naloxone. Omote et al. [13] showed that the inhibitory effect of spinal clonidine on wide dynamic range neuron activity evoked by a heat stimulus was antagonized by intravenous naloxone. Cross-tolerance studies have shown a mild asymmetric cross-tolerance between spinal morphine and norepinephrine [14] or ST-91, an alpha-2 adrenoceptor agonist [15], on the tail flick and hot plate test, respectively. Thus, in some situations, the antinociceptive effect of alpha-2 adrenoceptor agonists may interact with an opioid mechanism.

In the present study, the partial antagonism by low doses of naloxone and the delta selective antagonist naltrindole provided convergent confirmation that an opioid receptor, probably of the delta class, may modulate the activity of dexmedetomidine, a selective alpha-2 adrenoceptor agonist. The observation that the effects were not monotonic suggests several characteristics of the interaction: (a) It does not appear that the antagonism reflects an interaction of naloxone with the alpha-2 binding site or of dexmedetomidine with the opioid site. Were that to be the case, we would have anticipated a normal competitive agonist / antagonist interaction and the effects would not be biphasic. (b) Importantly, this regulation by opioid receptors did not appear to be due to the unmasking of a hyperalgesia otherwise suppressed by endogenous opioids, as the baseline formalin response in the presence of the antagonist alone at any dose was not altered.

However, the present results do not exclude the possibility that there is a modest level of ongoing opioid receptor activity in the formalin test that serves to synergize with the dose-response relationship of spinal alpha-2 adrenoceptors. The "plateau" of antagonism would suggest that once a complete blockade of the opiate receptors was achieved, the effects of the opioid antagonists would be readily surmounted. Considerable data have in fact demonstrated powerful spinal synergic interactions between opioid and alpha-2 adrenoceptor agonists [6]. The loss of antagonism at higher doses suggests that an additional mechanism must contribute to the opioid antagonists / alpha-2 agonist interaction. Again, several points may be considered: (a) At low doses, naloxone is said to have antinociceptive activities [16], but this mechanism is not likely to be relevant, as the loss of antagonism (e.g., reappearance of antinociceptive activity) in these studies occurred at the higher dose. (b) At higher doses, there would be a distribution of naloxone and naltrindole to other sites, including the periphery. It is known that naloxone has an inhibitory effect on local inflammatory pain [17]. A larger dose of naloxone could have systemic antiinflammatory effects and thereby reduce the stimulus. However, naltrindole, which is not reported to have such an effect, also showed a similar biphasic effect. (c) At high concentrations, lipid soluble agents such as naloxone can block axon conduction [18]. While this might suggest that higher doses of naloxone alone would have an antinociceptive effect, baseline changes in the formalin response were not observed.

Conclusion

In conclusion, these studies suggest that there is a mild but detectable interaction between spinal opioid (probably delta) and alpha-2 receptor agonist occupancy. The nature of this interaction is complex, and several variables must be considered to account for the biphasic interaction.

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