INVOLVEMENT OF SUPRASPINAL ALPHA-ADRENERGIC RECEPTORS IN TONIC PAIN

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ABSTRACT

The involvement of supraspinal alpha-adrenergic receptors in tonic pain was assessed in formalin-induced pain in rats. The alpha2 adrenoceptor agonist clonidine, along with yohimbine and prazosin, (X2 and (X1 receptor antagonists, were introduced intracerebroventrically (icv) and/or systemically in different doses. The data show that 1) clonidine exerts an alpha adrenergic analgesic effect, in addition to its known alpha2 role in this kind of pain, 2) icv yohimbine did not change the rat's nociception, and 3) icv prazocin also failed to alleviate the animal's nociception although both the latter drugs show analgesic activity in the formalin test when injected systemically. It can be concluded that (X1 receptors contribute significantly to adrenergic analgesia in the formalin test in supraspinal structures, by undefined nature and site(s).


INTRODUCTION

In the past two decades, there has been considerable interest in the involvement of adrenergic systems in pain and analgesia. It has been shown that noradrenergic pathways originating in the brainstem and terminating in the spinal dorsal horn are involved in descending inhibitory mechanisms of nociception.2,28 Alpha, receptor subtypes are believed to be the major adrenoceptors responsible in spinal antinociception.13, 24 Systemic administration of noradrenaline does not produce analgesia in mammals, because it cannot cross the blood-brain barrier. It was discovered that intrathecal (i.t.) injection of (X agonists induces antinociception, and i.t. (X2 antagonists antagonize antinociception produced by intrathecally administered noradrenaline which may be mediated through activation of the bulbospinal noradrenergic pathway.10, 20 However, there are few reports expressing the relative role of (X1 adrenoceptors in tail-flick or hot-plate induced pain13, 18 or the Randall-Sellito paw pressure test,4 or following electrical stimulation.21 Kuraishi et al.16 have found that i.t. prazocin in addition to i.t. yohimbine enhances substance P release in the spinal dorsal horn in response to peripheral mechano-receptive stimuli and inhibits the noradrenaline-mediated decrease in substance P release, suggesting a role for both (X1 and (X2 subtypes of adrenoceptors in spinal nociceptive modulation.

At present the detailed mechanisms of nociceptive modulation by supraspinal noradrenergic systems are not well understood, and both positive and negative properties of
these systems have been reported. In animals, antinociceptive tests vary in their sensitivities to centrally-acting analgesic drugs. It has been proven that some mechanisms involved in the perception of pain in the tail-flick test are anatomically and pharmacologically dissociable from those involved in pain processing in the formalin test.

Most of the studies concerning adrenergic analgesia have focused on spinal and supraspinal mechanisms of reflexive withdrawal from a phasic stimulus of short duration. The role of \( \alpha_1 \) and \( \alpha_2 \) adrenergic receptors in the formalin tests, a model for testing tonic pain, has been assessed by Tasker and Melzack. They administered prazocin and yohimbine, showing some \( \alpha_1 \) activity in this kind of pain. These findings confirmed the need for further studying the spinal and supraspinal structures' role in the formalin test by local injections of the above-mentioned drugs.

The present study was undertaken to investigate the nature of the alpha-adrenergic receptor subtypes involved in the supraspinal control of tonic pain. A preliminary report of these experiments has been presented in abstract form.

**MATERIALS AND METHODS**

**Animals**

Male NMRI rats weighing 250 to 300g were used for all studies. Animals were housed in groups of 4 to 5 rats per each cage at room temperature (25±3°C) with a natural light-dark cycle. The animals had free access to food and water prior to the experiments. Testing took place between 9 a.m. and 3 p.m. The animals were brought to the test lab the day before testing, and were thus adapted to the testing environment for at least 18 hours. No animal was tested more than once.

**Cannulation**

Icv infusions were performed according to the method of Popick. Briefly, the system consists of a 28-gauge stylet and a 21-gauge injection cannula, cut 1 mm longer than the guide. The unilateral guide cannula was aimed stereotaxically 1mm above the lateral ventricle (AP= -0.8, L= 1.5, H= -3.3 mm from bregma) of the rats while they were anesthetized with intraperitoneal (i.p.) sodium pentobarbital (65 mg/kg). The guide was attached to the skull with 3 stainless steel screws and dentate cement. A stylet was introduced in the cannula to prevent its obstruction.

**Formalin test**

The formalin test was used according to the modifications described by Cohen et al. The rats were observed in a formalin test box of clear plexiglas, 32x32x32 cm in size. A mirror was positioned at a 45° angle below the floor of the test box, allowing an unobstructed view of the animal's paw.

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**Fig. 1.** The effects of different doses of clonidine (5, 10, 20 \( \mu \text{g} \)-icv) on formalin-induced pain. The error bars represent S.E.M. (n=6 in each dose) (P<0.005).

**Fig. 2.** The effects of different doses of yohimbine (5, 10, 20 \( \mu \text{g} \)-icv) on formalin-induced pain. The error bars represent S.E.M. (n=6 in each dose).
Fig. 3. The effects of clonidine (20 µg-icv) injected 30 minutes after yohimbine (0.5, 1 mg/kg-ip) on tonic pain. The error bars represent S.E.M. (n= 5 in each dose) - yoh (0.5 mg/kg) & clo (20 µg).

Fig. 4. The effects of different doses of prazocin (5, 10, 20 µg-icv) on formalin-induced pain. The error bars represent S.E.M. (n= 6 in each dose).

Drugs
Four to five days after surgery the stylets were removed while the rats were gently restrained and the drug was slowly injected through an internal cannula (28g) extending 1mm beyond the guide cannula. All drugs were injected icv in a volume of 5µL. Sixty seconds after drug infusion, the injection cannula was removed and the stylet was replaced in the guide cannula. icv injections were given 1 min. before the actual testing (in the formalin test). Clonidine was dissolved in water and given in doses of 5, 10 and 20 µg per rat (n= 5). Yohimbine and prazosin were dissolved in water and administered in doses of 5, 10 and 20 µg per rat (n= 5). Control animals (n= 10) were injected with the same volume of saline. The experiments with clonidine, yohimbine (n= 18) and prazosin (n= 15) were performed in separate groups of animals. Control groups received saline. I.p. injections were carried out 40 min. before the formalin test.

Histology
The animals were killed after the experiments by an overdose of sodium pentobarbital. Cerebro-ventricular injection sites were marked by injection of 0.5% methylene blue solution under the same conditions as those of the experiment. The site was located by cutting frontal sections of the brain at the level of the cannula and locating the dye position by a light microscope.

Statistics
Behavioral data were analyzed by analysis of variance (ANOVA), and by Student's t-test when the analysis was restricted to two means. The difference between groups was considered statistically significant when P<0.05.

RESULTS
The icv injection of 5µg clonidine, an α₂ agonist, induced analgesia, especially 10 to 20 minutes after formalin injection (Fig. 1). Injection of 10 and 20 µg of clonidine also increased the analgesia in a dose-dependent manner...
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Fig. 5. The effect of clonidine (20 μg-icv) injected 30 minutes after prazocin (0.5, 1 mg/kg-ip) on tonic pain (n=5).
-○- praz (0.5 mg/kg) + clo (20 μg)
-●- praz (1 mg/kg) + clo (20 μg)

Fig. 5. The effect of clonidine (20 μg-icv) injected 30 minutes after prazocin (0.5 and 1 mg/kg ip) (Fig. 5). The result was a long-lasting analgesia which still existed 70 minutes after formalin injection (P<0.005). The control group suggests that i.p. prazocin at these doses can show analgesic activity by itself on tonic pain (Fig. 6), but the group receiving clonidine in addition showed a greater degree of analgesia.

DISCUSSION

Adrenergic sedation and antinociception are complex phenomena, involving both spinal and supraspinal sites, particularly the locus coeruleus and other midbrain and medullary areas. Most animal studies on adrenergic analgesia have concentrated on spinal and supraspinal mechanisms of reflexive withdrawal from a phasic stimulus, but many research works emphasize that different neural mechanisms underlie tonic versus phasic pain. One report compared the effects of 8 irritants, namely acetic acid, carrageenan, formalin, kaolin, platelet-activating factor, mustard oil, seroton in and yeast. This study strongly supports the use of formalin as a noxious stimulus in tonic pain research.

Because of the above mentioned information and the fact that the detailed mechanisms of the role of the supraspinal adrenergic system in tonic pain is still not well understood, we tried to evaluate the central alpha-adrenergic receptors involved in this kind of pain in the current study.

Icv administration of norepinephrine has been reported to produce analgesia, using phasic pain measurements. Therefore at first we introduced clonidine, a potent α2-receptor agonist, at different doses in the lateral cerebroventricular area. Clonidine produces a wide variety of effects. These include an antihypertensive action, alleviation of the opiate-withdrawal syndrome, antinociception and sedation. Clonidine is extremely potent as an antinociceptive agent. Compared to morphine, clonidine often shows greater antinociceptive potency, sometimes by a factor of more than 50. Clonidine has been shown to exhibit antinociceptive activity against a wide variety of noxious stimuli such as chemical irritants (e.g., formalin, acetylcholine, acetic acid), heat, pressure and electrical stimuli, but its exact site of action is unknown. In the present study, the icv administration of different doses of clonidine was employed. The dose-related attenuation in nociception seen in our work following icv injection of this drug, supports earlier observations of its peripheral and central α2-adrenergic effect on phasic pain. This raises the possibility that its central analgesic effect may be mediated through an α2-receptor subtype. However, a number of recent reports have shown that clonidine produces behavioral effects, including antinociception, in vivo, also by an α2-receptor mechanism. Therefore, blockade of α2-receptors with systemic yohimbine was used to detect clonidine's effect on α2-
receptors. Interestingly, it was shown that in spite of the blocked α₂-receptors, clonidine could still exert its antinociceptive effect in the formalin test, but the degree of analgesia was less than that of the same dose of the drug applied alone. We conclude that clonidine has α₂-adrenergic effects in addition to α₁ in this type of pain. The question of the anatomical location of the appropriate α₂-receptors has not been answered to date. Furthermore, these results are consistent with the classical model of adrenergic subtypes in which α₁-receptors are located postsynaptically on effector organs and α₂-receptors are located on noradrenaline pathway terminals and regulate noradrenaline release.¹⁷

**REFERENCES**


