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Antinociception Induced by Amitriptyline and Imipramine Is Mediated by α_{2A}-Adrenoceptors

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ABSTRACT—The involvement of α_2-adrenoceptors in the antinociception induced by the tricyclic antidepressants amitriptyline and imipramine was investigated in mice by using the hot-plate and abdominal constriction tests. The antinociception produced by amitriptyline (15 mg/kg, i.p.) and imipramine (15 mg/kg, i.p.) was prevented by reserpine (2 mg/kg, i.p.) and yohimbine (3 – 10 mg/kg, i.p.) but not by naloxone (1 mg/kg, i.p.), atropine (5 mg/kg, i.p.), CGP 35348 (100 mg/kg, i.p.) and prazosin (1 mg/kg, i.p.). On the basis of the above data, it can be postulated that amitriptyline and imipramine exerted their antinociceptive effect by activation of α_2-adrenoceptors. Administration of the α_{2A}-adrenoceptor antagonist BRL 44408 (1 mg/kg, i.p.) prevented amitriptyline and imipramine antinociception, whereas the $\alpha_{2B/C}$-adrenoceptor antagonist ARC 239 (10 mg/kg, i.p.) was ineffective. These data indicate that the enhancement of the pain threshold produced by amitriptyline and imipramine is mediated by activation of α_{2A}-adrenoceptors. Neither tricyclic antidepressants nor the antagonists used impaired mouse performance evaluated by the rota-rod and hole-board tests.

Keywords: Analgesia, Amitriptyline, Imipramine, α_{2A}-Adrenoceptor, Tricyclic antidepressant

The tricyclic antidepressants have been used for many years to suppress certain types of pain in humans, including diabetic neuropathy, postherpetic neuralgia, headaches, arthritis, chronic back pain, cancer pain and phantom limb pain (1, 2). Tricyclic antidepressants are also analgesics in laboratory animals (3 – 6). The analgesic effect of tricyclic antidepressant drugs seems to be independent of their antidepressant activity since the doses used for analgesia are lower than those considered effective in the treatment of depression (6).

It has been suggested that amitriptyline, imipramine, nortriptyline, clomipramine and doxepine exert their antinociceptive properties through an inhibition of serotonin and noradrenaline reuptake in the central nervous system (7, 8). Selective inhibitors of serotonin reuptake, such as zimelidine and fluoxetine, have been demonstrated to be only weakly effective as analgesics in humans (9, 10). By contrast, maprotiline and desipramine, selective inhibitors of noradrenaline reuptake, showed potent analgesic activity (11, 12).

Activation of the adrenergic system produces a wide variety of effects, including antinociception, which is believed to be mediated by α_2-adrenoceptors (13, 14). Clonidine, an agonist of α_2-adrenoceptors, is extremely potent as an antinociceptive agent, showing equal or greater potency than morphine in rodents (15). Clonidine has been shown to exhibit antinociceptive activity against a wide variety of noxious stimuli such as chemical irritants, heat, pressure and electrical stimuli (13, 16). Antinociception induced by clonidine appears to be mediated by α_2-adrenoceptors within the central nervous system since yohimbine, an α_2-adrenoceptor antagonist, inhibits clonidine analgesia (13). On the other hand, prazosin, an α_1-adrenoceptor antagonist, does not affect clonidine enhancement of the pain threshold (17, 18). Central α_2-adrenoceptors are localized not only postsynaptically, but also presynaptically, as inhibitory receptors on non-adrenergic neurons (heteroreceptors) and on the terminals and dendrites of noradrenergic neurons themselves (autoreceptors) (19 – 21). It has been suggested that the antinociceptive properties of α_2 agonists reflect activation of sites postsynaptic to noradrenergic pathways (22). Furthermore, it has been postulated that central α_{2A}-adrenoceptor subtypes play a major role in the control of nociceptive responses (23 – 25). This hypothesis has been confirmed by experiments in which dexmedetomidine (α_{2A} agonist) had potent antinociceptive effects in wild-type control mice and in mice with inactivating mutations of the α_{2B} and α_{2C} genes, but was ineffective in the D79N mutant mice, a mutant strain equivalent of a knockout line for the α_{2A}-adrenoceptor subtype (26).

In view of the above considerations, in the present work,

we have investigated the role played by α_2-adrenoceptors in the antinociception induced by amitriptyline and imipramine in mice. We have also attempted to determine which α_2-adrenoceptor subtype is involved in the control of nociceptive responses by the above mentioned tricyclic antidepressants.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (23 – 30 g) from the Morini (San Polo d'Enza, Italy) breeding farm were used. Fifteen mice were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1°C with a 12-h light /dark cycle, light on at 7 a.m. All experiments were carried out according to the guidelines of the European Community Council for Experimental Animal Care.

Hot-plate test

The method adopted was described by O'Callaghan and Holtzman (27). Mice were placed inside a stainless steel container, which was set thermostatically at 52.5 ± 0.1°C in a precision water-bath from KW Mechanical Workshop, Siena, Italy. Reaction times (s) were measured with a stopwatch before and 15, 30, 45 and 60 min after treatment. The endpoint used was the licking of the fore or hind paws. Those mice scoring less than 12 and more than 18 s in the pretest were rejected (30%). An arbitrary cut-off time of 45 s was adopted.

Abdominal constriction test

Mice, 15 min after treatment, were injected i.p. with a 0.6% solution of acetic acid (10 ml/kg), according to Koster et al. (28). The number of stretching movements was counted for 10 min, starting 5 min after acetic acid injection.

Rota-rod test

The apparatus consisted of a base platform and a rotating rod of 3-cm diameter with a non-slippery surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus, up to 5 mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught et al. (29). Those mice scoring less than 3 and more than 6 falls in the pretest were rejected (20%). The performance time was measured before and 15, 30 and 45 min after treatment.

Hole-board test

The hole-board test setup utilizes a 40 cm square plane with 16 flush-mounted cylindrical holes (diameter of 3 cm) distributed 4 by 4 in an equidistant, grid-like manner. The mice were placed in the center of the board one by one and left to move about freely for a period of 5 min each. Two photoelectric beams, crossing the plane from mid-point to mid-point of opposite sides, thus dividing the plane into 4 equal quadrants, automatically signaled the movement of the animals on the surface of the plane. Miniature photoelectric cells, in each of the 16 holes, recorded the exploration of the holes (head plunging activity) by the mice. The test was performed 30 min after treatment.

Drugs

Atropine sulfate, yohimbine hydrochloride, baclofen and amitriptyline were purchased from Sigma (Milan, Italy); CGP 35348 (3-aminopropyl-diethoxymethyl-phosphinic acid) and reserpine were purchased from Ciba Geigy (Basel, Switzerland); naloxone hydrochloride, imipramine hydrochloride, clonidine hydrochloride, prazosin hydrochloride, BRL 44408 (2-(2*H*-(1-methyl-1,3-dihydroisoindole)methyl)-4,5-dihydroimidazole) were purchased from RBI (Milan, Italy); ARC 239 ((2-(2-(4-*o*-methoxyphenyl) piperazine-1-yl)-ethyl)-4,4-dimethyl-1,3 (2*H*,4*H*)-isoquinolinedione) were purchased from Neuroscience Institute (Geneva, Switzerland); morphine hydrochloride were purchased from USL 10/D (Florence, Italy); D-amphetamine was purchased from De Angeli (Milan, Italy).

Drugs were dissolved in isotonic (NaCl 0.9%) saline solution, with the exception of reserpine which was dissolved in a 20% solution of ascorbic acid, immediately before use. All antagonists were injected simultaneously with the analgesic drugs except reserpine which was administered twice 48 and 24 h before the test. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml/kg by intraperitoneal (i.p.) injection.

Statistics

All experimental results are given as the mean ± S.E.M. Analysis of variance (ANOVA), followed by Fisher's Protected Least Significant Difference (PLSD) procedure for post-hoc comparison, was used to verify significance between two means. Data were analyzed with the StatView software for the Macintosh (1992). P values of less than 0.05 were considered significant.

RESULTS

The administration of naloxone (1 mg/kg, i.p.), atropine (5 mg/kg, i.p.) and CGP 35348 (100 mg/kg, i.p.) did not modify the antinociception induced by amitriptyline

(15 mg/kg, i.p.) and imipramine (15 mg/kg, i.p.) in the mouse hot-plate test (Fig. 1). Reserpine, administered at the dose of 2 mg/kg, i.p., twice 48 and 24 h prior to the test, prevented the antinociceptive effect of both amitriptyline and imipramine (Fig. 1).

Yohimbine dose-dependently reduced the enhancement of the pain threshold induced by both tricyclic antidepressants investigated in the mouse hot-plate test. Yohimbine at the dose of 1 and 3 mg/kg, i.p., respectively, reduced and prevented the amitriptyline-induced antinociception, whereas at 0.1 mg/kg, i.p., it was completely ineffective (Fig. 2). Also imipramine antinociception was prevented by yohimbine even though higher doses (10 mg/kg, i.p.) were needed. In fact yohimbine at the dose of 3 mg/kg, i.p. did not modify imipramine analgesic activity (Fig. 3).

Yohimbine reduced the antinociception induced by clonidine (0.125 mg/kg, i.p.) in a dose-dependent manner (0.01 – 10 mg/kg, i.p.) as illustrated in Fig. 4. At the

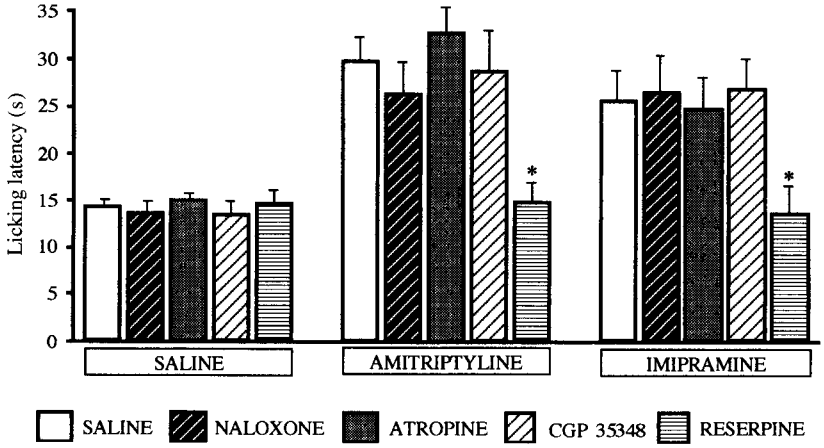

Fig. 1. Effect of naloxone (1 mg/kg, i.p.), atropine (5 mg/kg, i.p.), CGP 35348 (100 mg/kg, i.p.) and reserpine (2 mg/kg, i.p.) on antinociception induced by amitriptyline (15 mg/kg, i.p.) and imipramine (15 mg/kg, i.p.) in the mouse hot-plate test. Naloxone, atropine, CGP 35348 were injected simultaneously with the other drugs. Reserpine was injected twice 48 and 24 h before test. Nociceptive responses were recorded 30 min after injection. *P<0.01, in comparison with the corresponding group not treated with antagonists. Each column represents the mean of at least 12 mice.

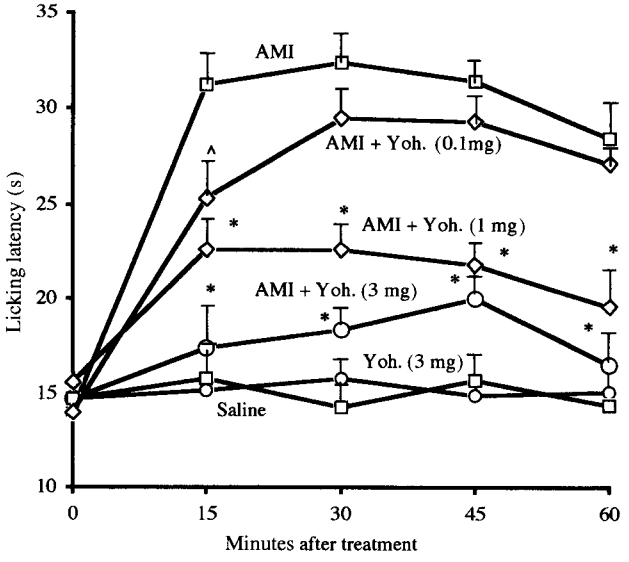

Fig. 2. Dose-response relationship of yohimbine on antinociception induced by amitriptyline (15 mg/kg, s.c.) in the mouse hot-plate test. Each point represents the mean of at least 10 mice. Yohimbine (Yoh.) and amitripyline (AMI) were administered simultaneously. ^P<0.05, *P<0.01, in comparison with mice not treated with yohimbine.

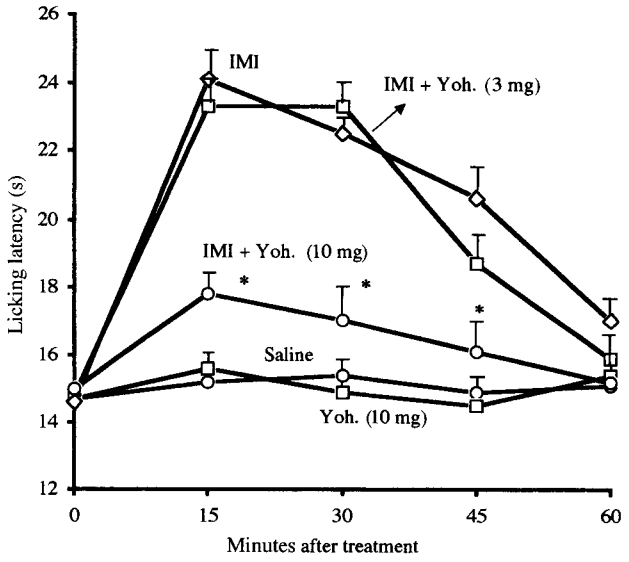

Fig. 3. Dose-response relationship of yohimbine on antinociception induced by imipramine (15 mg/kg, s.c.) in the mouse hot-plate test. Each point represents the mean of at least 10 mice. Yohimbine (Yoh.) and imipramine (IMI) were administered simultaneously. *P<0.01, in comparison with mice not treated with yohimbine.

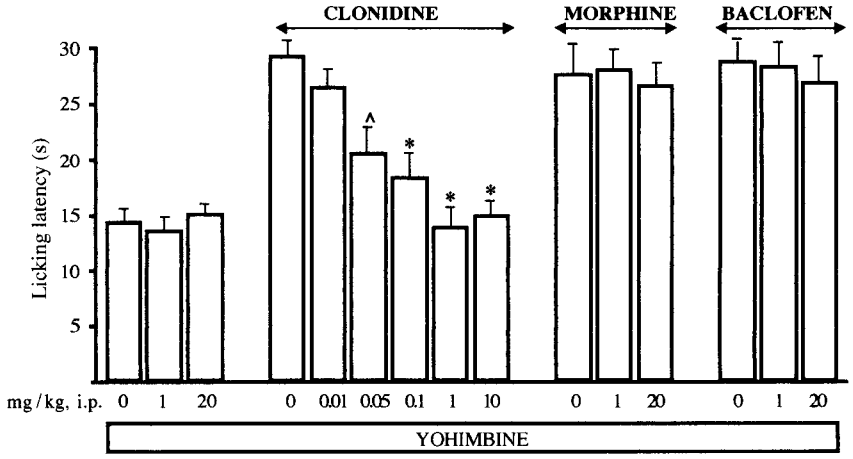

Fig. 4. Effect of yohimbine on antinociception induced by clonidine (0.125 mg/kg, i.p.), morphine (8 mg/kg, i.p.) and baclofen (4 mg/kg, i.p.) in the mouse hot-plate test. Yohimbine was administered simultaneously with the other drugs. Nociceptive responses were recorded 30 min after injection. ^P<0.05, *P<0.01, in comparison with the corresponding yohimbine untreated mice. Each column represents the mean of at least 10 mice.

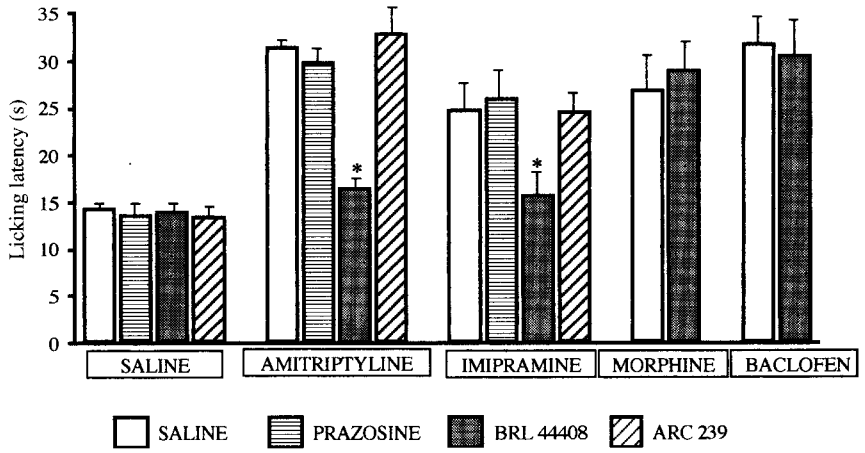

Fig. 5. Effect of prazosine (1 mg/kg, i.p.), BRL 44408 (1 mg/kg, i.p.) and ARC 239 (10 mg/kg, i.p.) on antinociception induced by amitriptyline (15 mg/kg, i.p.) and imipramine (15 mg/kg, i.p.) in the mouse hot-plate test and lack of effect by BRL 44408 on morphine and baclofen antinociception under the same experimental conditions. Prazosine, BRL 44408 and ARC 239 were administered simultaneously with the other drugs. Nociceptive responses were recorded 30 min after injection. *P<0.01, in comparison with the corresponding group not treated with antagonists. Each column represents the mean of at least 12 mice.

highest doses used at which a complete prevention of clonidine antinociception was reached (1 – 10 mg/kg, i.p.), this compound selectively prevented only analgesia mediated by α_2-adrenoceptors since it did not modify antinociception induced by morphine (8 mg/kg, i.p.) and baclofen (4 mg/kg, i.p.) (Fig. 4). Furthermore, a twofold higher dose of yohimbine (20 mg/kg, i.p.) remained ineffective in preventing non-α_2-adrenergic antinociception (Fig. 4).

The amitriptyline and imipramine analgesic effect was prevented by the α_{2A}-adrenoceptor antagonist BRL 44408 (1 mg/kg, i.p.), whereas it was modified neither by the α_1-adrenoceptor antagonist prazosin (1 mg/kg, i.p.) nor by the $\alpha_{2B/C}$-adrenoceptor antagonist ARC 239 (10 mg/kg, i.p.) in the mouse hot plate test (Fig. 5). The same results were obtained by using the mouse abdominal constriction test (Fig. 6).

When given alone, neither yohimbine (Figs. 2 – 4), BRL 44408 nor ACR 239 (Figs. 5 and 6) modified the pain threshold of mice.

The motor coordination of mice treated with amitriptyline (15 mg/kg, s.c.), imipramine (16 mg/kg, s.c.), yohimbine (3 mg/kg, i.p.), prazosin (1 – 2 mg/kg, i.p.), BRL 44408 (1 mg/kg, i.p.) and ARC 239 (10 mg/kg, i.p.) was evaluated by using the rota-rod test (Table 1), while their spontaneous activity was investigated by using the hole-board test (Fig. 7). The rota-rod performance of mice

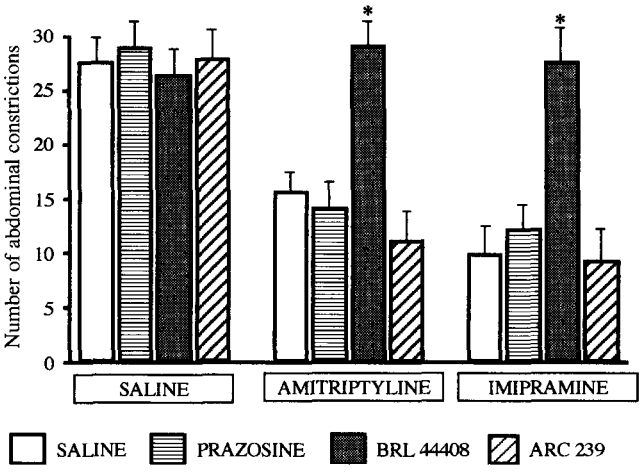

Fig. 6. Effect of prazosine (1 mg/kg, i.p.) BRL 44408 (1 mg/kg, i.p.) and ARC 239 (10 mg/kg, i.p.) on antinociception induced by amitriptyline (1 mg/kg, i.p.) and imipramine (20 mg/kg, i.p.) in the mouse abdominal constriction test. Each column represents the mean of at least 8 mice. All drugs were administered simultaneously. Nociceptive response was recorded 30 min after administration. *P<0.01, in comparison with saline-treated mice.

treated with the above-mentioned compounds was not impaired in comparison with saline-treated mice (Table 1). The number of falls by control animals progressively decreased at every measurement since the mice learned how to balance on the rotating rod. The spontaneous motility and exploratory behaviour of mice were not modified by treatment with yohimbine, prazosin, BRL 44408 and ARC 239 as revealed by the hole-board test (Fig. 7). D-Amphetamine (2 mg/kg, i.p.) was used as the reference drug.

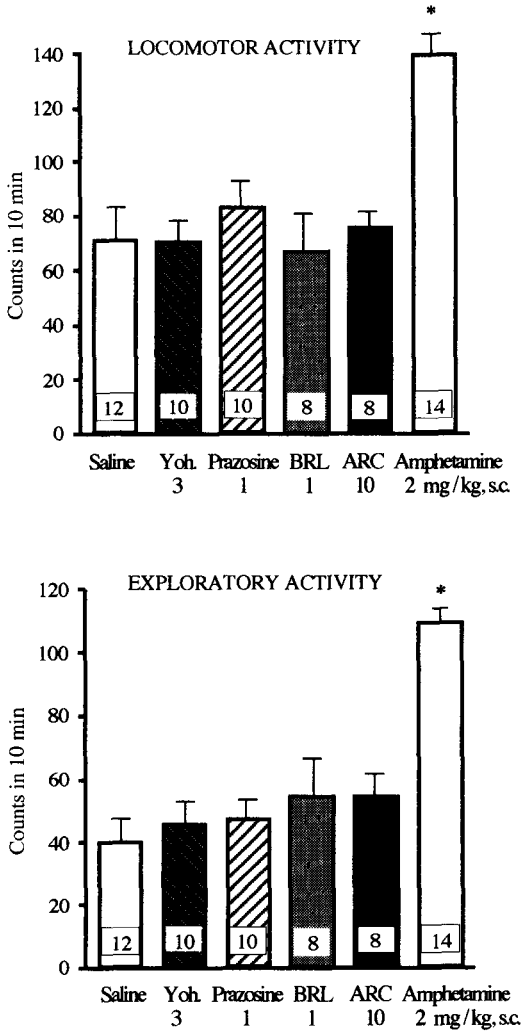

Fig. 7. Lack of effect by yohimbine, prazosine, BRL 44408 and ARC 239 in the mouse hole-board test in comparison with amphetamine. *P<0.01, in comparison with saline-treated mice.

Table 1. Effect of amitriptyline, imipramine, yohimbine, prazosine, BRL 44408 and ARC 239 in mouse rota-rod test

Treatment	Dose	Number of falls in 30 s			
		Before treatment	After treatment 15 min	After treatment 30 min	After treatment 45 min
Saline	10 ml/kg, i.p.	3.1 ± 0.3	1.8 ± 0.3*	1.4 ± 0.2*	0.8 ± 0.2*
Amitriptyline	15 mg/kg, i.p.	3.2 ± 0.5	1.9 ± 0.3*	1.5 ± 0.5*	1.3 ± 0.5*
Imipramine	15 mg/kg, i.p.	3.4 ± 0.4	2.3 ± 0.3*	1.2 ± 0.4*	0.8 ± 0.3*
Yohimbine	10 mg/kg, i.p.	2.9 ± 0.2	1.7 ± 0.3*	1.1 ± 0.5*	1.1 ± 0.5*
Prazosine	1 mg/kg, i.p.	3.2 ± 0.4	2.1 ± 0.3*	1.3 ± 0.4*	1.2 ± 0.4*
Prazosine	2 mg/kg, i.p.	3.1 ± 0.3	3.4 ± 0.6	3.0 ± 0.4	2.7 ± 0.4
BRL 44408	1 mg/kg, i.p.	3.3 ± 0.2	2.5 ± 0.4	1.8 ± 0.5*	1.4 ± 0.3*
ARC 239	10 mg/kg, i.p.	3.0 ± 0.3	1.9 ± 0.3*	1.1 ± 0.4*	0.8 ± 0.2*

Each value represents the mean of 10 mice. *P<0.01, in comparison with the corresponding pretest values.

DISCUSSION

The present results indicate that α_2-adrenoceptors play an important role in the mechanism of analgesic action of the tricyclic antidepressants amitriptyline and imipramine. In particular, α_{2A} appears to be the α_2-adrenoceptor subtype involved in this action.

Pretreatment with the monoamine store depletor reserpine prevented the antinociception induced by amitriptyline and imipramine. Furthermore, the administration of yohimbine, an α_2-adrenoceptor antagonist, inhibited the enhancement of the pain threshold produced by both tricyclic antidepressants investigated. Amitriptyline and imipramine induce an enhancement of both catecholamines and serotonin levels in the synaptic cleft, but these data indicate that the adrenergic neurotransmitter system is mainly involved in the mechanism of analgesic action of these two compounds. Reserpine is also a histamine store depletor. However, an involvement of the histaminergic system can be excluded since the administration of the selective histamine synthesis inhibitor *R*-α-methyl-histamine (20 mg/kg, i.p.) did not modify amitriptyline- and imipramine-induced antinociception (data not shown).

It has been observed that the antinociception induced by activation of the adrenergic system is mediated by α_{2A}-adrenoceptors (14). To validate the hypothesis of the activation of the adrenergic system by the two tricyclic antidepressants, we investigated the α_2-adrenoceptor subtype involved in amitriptyline and imipramine antinociception. The administration of the α_{2A}-adrenoceptor antagonist BRL 44408 (30 – 33), but not the $\alpha_{2B/C}$-adrenoceptor antagonists ARC 239 and prazosin (34, 35), prevents the enhancement of the pain threshold induced by the two tricyclic antidepressants. The α_2-adrenoceptor subtype responsible for the induction of antinociception by amitriptyline and imipramine, therefore, belongs to the α_{2A} subtype. Prazosin is not only an $\alpha_{2B/C}$-adrenoceptor antagonist, but it is also an α_1-adrenoceptor antagonist. Its lack of effect also indicates that α_1-adrenoceptors are not involved in the mechanism of analgesic action of amitriptyline and imipramine.

It has been reported that the analgesic effect of amitriptyline and clomipramine could be related to an activation of the endogenous opioid system since these compounds potentiate the effect of morphine and increased hypothalamic β-endorphin concentrations (36). However, these data were not confirmed by studies of Ventafridda et al. (37) and Hwang and Wilcox (38). Furthermore, in our experimental conditions, the opioid receptor antagonist naloxone was unable to prevent amitriptyline and imipramine antinociception, ruling out the hypothesis of an opioid mechanism of action for the tricyclic antidepressants investigated. Other neurotransmitter systems are not involved in amitriptyline and imipramine antinociception since the $GABA_B$ antagonist CGP 35348 and the muscarinic antagonist atropine are all unable to prevent the effect of the two tricyclic antidepressants.

The doses and administration schedules of the abovementioned antagonists, naloxone, CGP 35348 and atropine, are ideal for preventing antinociception induced, respectively, by the opioid agonist morphine (39), the $GABA_B$ agonist baclofen (40) and the muscarinic agonist oxotremorine (41). Similarly, the doses of yohimbine able to prevent amitriptyline and imipramine analgesia are selective for preventing antinociception induced by activation of α_2-adrenoceptors since opioid and GABAergic antinociception is not modified.

In these experimental conditions, neither the α_2-adrenoceptor antagonist yohimbine, nor the α_{2A}-adrenoceptor antagonist BRL 44408 and $\alpha_{2B/C}$-adrenoceptor antagonist ACR 239 modify the pain threshold of mice in comparison with control animals. The lack of effect of these antagonists agrees with results of studies in which these compounds did not modify the nociceptive threshold against both thermal (hot-plate) and chemical (writhing) noxious stimuli (42). We can, therefore, exclude that the prevention of amitriptyline and imipramine antinociception is due to a hyperalgesic effect of the α_2-adrenoceptor antagonists used.

Analgesia induced by amitriptyline (15 mg/kg, i.p.) and imipramine (15 mg/kg, i.p.) in the mouse hot-plate and abdominal constriction tests is obtained without any visible change in the normal behaviour of animals as demonstrated in rota-rod experiments in which no impairment of mouse rota-rod performance is observed. Similarly, the α-adrenoceptor antagonists used were administered at doses that do not modify the animals' behaviour as revealed by the rota-rod and hole board tests.

In conclusion, the present data demonstrate that antinociception induced by both amitriptyline and imipramine involves the activation of α_{2A}-adrenoceptors.

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