J Anesth (2001) 15:154–158

Journal of
Anesthesia

Antinociceptive efficacy of antidepressants: assessment of five antidepressants and four monoamine receptors in rats

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Abstract

Purpose. For assessment of the antinociceptive potency of antidepressants, we compared the antinociceptive effects of serotonin selective reuptake inhibitors (SSRIs) and classical tricyclic antidepressants (TCAs) in rats. We also attempted to elucidate the monoamine receptor subtypes predominantly involved in the antinociceptive effect of antidepressants.

Methods. Male Wistar rats received SSRIs (sertraline, fluvoxamine, and citalopram) or TCAs (imipramine and desipramine) intraperitoneally, and the reaction time until pain response in the hot plate test and licking time in the formalin test were measured 60 min later. We also observed the effects of prazosin (an α_1 antagonist), WB-4101 (a selective α_{1A} antagonist), yohimbine (an α_2 antagonist), WAY-100635 (a selective 5-HT_{1A} antagonist), and ketanserin (a 5-HT_2 antagonist), which were simultaneously administered with imipramine or desipramine, on the antidepressant-induced antinociceptive effect in the formalin test.

Results. In the hot plate test, desipramine, 20 mg·kg^{-1}, but not imipramine or sertraline, produced a significant increase in reaction time. In the formalin test, desipramine and imipramine produced significant reductions in the licking time at over 5 mg·kg^{-1} and at over 10 mg·kg^{-1}, respectively. These reductions were nearly complete at 20 mg·kg^{-1}. On the other hand, both SSRIs induced significant reductions in the licking time only at 20 mg·kg^{-1}. Prazosin, WB-4101, and ketanserin significantly antagonized the antinociceptive effect of 10 mg·kg^{-1} of imipramine. However, imipramine-induced antinociception was not affected by yohimbine and WAY-100635. Prazosin and ketanserin also significantly suppressed antinociception by 5 mg·kg^{-1} of desipramine.

Conclusion. These findings suggest that classical TCAs are likely to have a therapeutic advantage over SSRIs for pain control. In addition, it is likely that central α_1 adrenoceptors and 5-HT_2 receptors are predominantly involved in imipramine- and desipramine-induced antinociception.

Key words Selective serotonin reuptake inhibitor (SSRI) · Tricyclic antidepressant · Antinociception · Pain · Rat

Introduction

Antidepressant agents have antinociceptive and analgesic effects in humans and animals [1–3]. Therefore, in pain clinics these agents are preferentially used for control of pain, such as postherpetic neuralgia, diabetic neuropathy and thalamic pain [4–7]. Recently, serotonin selective reuptake inhibitors (SSRIs) have become more frequently prescribed for control of pain, since they are generally safer than classical tricyclic antidepressants (TCAs) [8–10]. However, the antinociceptive effects of SSRIs have not been well evaluated in comparison with those of TCAs [2,11].

Most antidepressants, including TCAs and SSRIs, are potent inhibitors of the reuptake of the monoamines norepinephrine and serotonin (5-HT) at neuronal terminals [12,13]. Therefore, although the site of analgesic action of antidepressants remains unclear, stimulated monoamine transmission that results from increased levels of monoamines in synaptic clefts is presumed to change pain thresholds and induce antinociception. However, which monoamine receptors (or receptor subtypes) are responsible for their analgesic effects is still controversial [3,11,14].

Therefore, in the present study, we compared the potency of antinociceptive effects in SSRIs (sertraline, fluvoxamine, and citalopram) and classical TCAs (imipramine and desipramine) by the hot plate test and the formalin test after single administration to rats and assessed the antinociceptive potency of antidepressants. In addition, we attempted to elucidate the monoamine receptor subtypes ($\alpha_{1,2}$ and 5-$HT_{1,2}$) predominantly involved in the antinociceptive effect of antidepressants by using specific antagonists of these receptors.

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Received: November 15, 2000 / Accepted: March 14, 2001

Materials and methods

Animals

We used 287 male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 210 ± 2 g (mean ± SEM). The rats were housed at 20°–24°C in a humidity-controlled room under a 12/12 h light/dark cycle (lights on at 6:00 A.M., off at 6:00 P.M.). The rats were allowed to adapt to the animal room for at least 3 days prior to use and given free access to food and water. The ambient temperature during testing was 20°–24°C. The animals were brought to the test room at least 1 h before testing. We did not use the same rats for multiple experiments. All procedures were in strict accordance with the NIH Guide for Care and Use of Laboratory Animals and were approved by our Animal Care and Use Committee.

Hot plate test

Animals were placed in an open Plexiglas box (30 cm × 30 cm × 34 cm) on a hot plate (EC-1200, Iuchi Seieido, Osaka, Japan), which was thermostatically maintained at 55°C. According to the method of Schreiber et al. [15], the reaction time (hind paw licking or jumping) of each animal was measured as the pain response at 60 min after drug injection. A maximal latency of 40 s was chosen in order to avoid damage to the animals. Thus, when the reaction time reached more than 40 s, we regarded the reaction time as 40 s.

Formalin test

A 50 µl volume of 2% formalin [16] was injected subcutaneously into the plantar surface of the right hind paw with a 27-G needle, and the animal was immediately placed in an open Plexiglas box (30 cm × 30 cm × 34 cm). We measured the total time that the animal spent licking the injected paw from 10 to 30 min after formalin injection. For evaluation of the antinociceptive effect of antidepressants, the formalin test was more sensitive than the hot plate test (see Results). Therefore, in most experiments we used the formalin test.

Drugs

An antidepressant was intraperitoneally injected simultaneously with or without a monoamine receptor antagonist 60 min before measurement of the reaction time in the hot plate test or the formalin injection. Desipramine HCl (Sigma, St. Louis, MO, USA), imipramine HCl (Wako Pure Chemical Industries, Osaka, Japan), sertraline HCl (donated by Pfizer, Groton, CT, USA), fluvoxamine maleate (donated by Solvay Pharmaceuticals, Weesp, The Netherlands), and citalopram HBr (donated by ZERIA Pharmaceutical, Tokyo, Japan) at 5–20 $mg \cdot kg^{-1}$ were used as antidepressants. Prazosin HCl at 0.5–1 $mg \cdot kg^{-1}$, yohimbine HCl at 0.5 $mg \cdot kg^{-1}$ (Sigma), WB-4101 HCl at 1 $mg \cdot kg^{-1}$, WAY-100635 maleate at 1 $mg \cdot kg^{-1}$, and ketanserin tartrate at 1 $mg \cdot kg^{-1}$ (RBI, Natick, MA, USA) were used as selective α-adrenergic or serotonergic receptor antagonists. The doses of the antagonists were based on those reported previously in studies with rats [17–20]. We randomized the daily choice of tested drug and its dose.

Table 1. Antinociceptive activity of antidepressants after single intraperitoneal administration in the hot plate test

Drug	Dose ($mg \cdot kg^{-1}$)	Reaction time (s)
Vehicle		8.9 ± 1.0
Sertraline	5	10.6 ± 1.5
	10	11.5 ± 2.1
	20	12.0 ± 1.6
Desipramine	5	9.9 ± 0.6
	10	11.2 ± 1.1
	20	24.5 ± 4.0**
Imipramine	5	9.3 ± 0.9
	10	10.5 ± 1.1
	20	18.1 ± 4.7

Values are means ± SEM ($n = 7$ each)
** $P < 0.01$ versus vehicle

Statistics

The results were expressed as means ± SEM. The statistical significance of differences between experimental data was analyzed by analysis of variance (ANOVA) followed by the Bonferroni/Dunn post hoc test. A P value < 0.05 was considered to indicate statistical significance.

Results

In the hot plate test, only desipramine, 20 $mg \cdot kg^{-1}$, produced a significant increase in reaction time (Table 1). Imipramine or sertraline did not produce a significant increase in reaction time.

In the formalin test, sertraline, fluvoxamine, citalopram, desipramine, and imipramine produced a significant reduction in the time that the animals spent licking the injected paw (Table 2). Antinociceptive effects of desipramine were observed at over 5 $mg \cdot kg^{-1}$ and those of imipramine at over 10 $mg \cdot kg^{-1}$, whereas antinociceptive effects of sertraline, fluvoxamine, and citalopram were observed only at 20 $mg \cdot kg^{-1}$. Desipramine and imipramine at doses of 20 $mg \cdot kg^{-1}$ nearly abolished the formalin-induced nociceptive response (92.0%, and 95.2% reduction, respectively). On the other hand, the antinociceptive effects of sertraline,

Table 2. Antinociceptive activity of antidepressants after single intraperitoneal administration in the formalin test

Drug	Dose ($mg \cdot kg^{-1}$)	Licking time (s)
Vehicle		163.0 ± 13.8
Sertraline	5	143.4 ± 12.1
	10	96.4 ± 24.2
	20	65.0 ± 21.5**
Fluvoxamine	5	159.0 ± 18.9
	10	126.9 ± 14.5
	20	91.4 ± 15.7*
Citalopram	5	159.7 ± 13.4
	10	142.7 ± 7.3
	20	86.4 ± 12.0**
Desipramine	5	68.4 ± 20.0**
	10	59.4 ± 15.3**
	20	13.0 ± 7.2**
Imipramine	5	150.0 ± 10.3
	10	41.0 ± 13.4**
	20	7.9 ± 7.5**

Values are means ± SEM ($n = 7$ each)
* $P < 0.05$ and ** $P < 0.01$ versus vehicle

Table 3. Antinociceptive activity of monoamine receptor antagonists after single intraperitoneal administration in the formalin test

Drug	Dose ($mg \cdot kg^{-1}$)	Licking time (s)
Vehicle		163.0 ± 13.8
Prazosin	0.5	129.6 ± 21.6
	1	139.4 ± 11.5
WB-4101	1	223.3 ± 26.0
Yohimbine	0.5	220.1 ± 17.4
WAY-100635	1	218.4 ± 8.4
Ketanserin	1	166.9 ± 29.7

Values are mean ± SEM ($n = 7$ each)

fluvoxamine, and citalopram were partial even at $20 mg \cdot kg^{-1}$ (60.1%, 43.9%, and 47.0% reduction, respectively).

Prazosin, a nonselective α_1 antagonist, $0.5 mg \cdot kg^{-1}$, and WB-4101, a selective α_{1A} antagonist, $1 mg \cdot kg^{-1}$, significantly antagonized suppression of the licking time by imipramine, $10 mg \cdot kg^{-1}$ (Fig. 1). Yohimbine, an α_2 antagonist, $0.5 mg \cdot kg^{-1}$, or WAY-100635, a selective 5-HT_{1A} antagonist, $1 mg \cdot kg^{-1}$, did not alter the antinociceptive effect of imipramine, $10 mg \cdot kg^{-1}$. However, the antinociceptive of imipramine effect was nearly completely antagonized by ketanserin, a 5-HT_2 antagonist, $1 mg \cdot kg^{-1}$.

Prazosin, $1 mg \cdot kg^{-1}$, significantly antagonized suppression of licking time by desipramine, $5 mg \cdot kg^{-1}$ (Fig. 2). Ketanserin, $1 mg \cdot kg^{-1}$, also nearly completely antagonized desipramine-induced antinociception.

No single antagonist had a significant effect on licking time in the formalin test (Table 3). Apparent inhibition of ambulatory activity was not observed after most drug treatments except for $20 mg \cdot kg^{-1}$ of sertraline, citalopram, desipramine, and imipramine, which appeared to induce sedation to some degree.

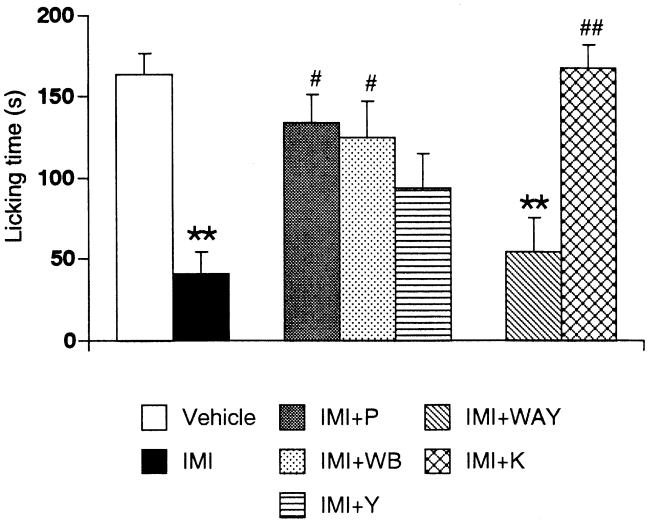

Fig. 1. Effect of monoamine receptor antagonists on the antinociceptive effect of imipramine. Imipramine (IMI $10 mg \cdot kg^{-1}$) was intraperitoneally injected simultaneously with α-adrenoceptor antagonists (P, prazosin $0.5 mg \cdot kg^{-1}$; WB, WB-4101 $1 mg \cdot kg^{-1}$; and Y, yohimbine $0.5 mg \cdot kg^{-1}$) or with 5-HT receptor antagonists (WAY, WAY-100635 $1 mg \cdot kg^{-1}$; and K, ketanserin $1 mg \cdot kg^{-1}$). Values are means ± SEM ($n = 7$ each). ** $P < 0.01$ versus vehicle. # $P < 0.05$, ## $P < 0.01$ versus IMI

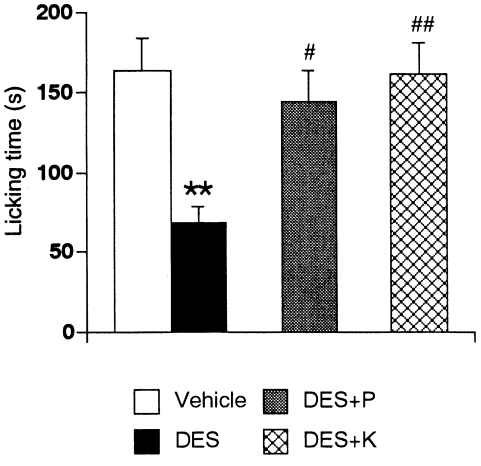

Fig. 2. Effect of monoamine receptor antagonists on the antinociceptive effect of desipramine. Desipramine (DES $5 mg \cdot kg^{-1}$) was intraperitoneally injected simultaneously with an α_1-adrenoceptor antagonist (P, prazosin $1 mg \cdot kg^{-1}$) or with a 5-HT_2 receptor antagonist (K, ketanserin $1 mg \cdot kg^{-1}$). Values are means ± SEM ($n = 7$ each). ** $P < 0.01$ versus vehicle. # $P < 0.05$, ## $P < 0.01$ versus DES

Discussion

In this study, classical TCAs (imipramine and desipramine) showed a more potent antinociceptive effect than SSRIs (sertraline, fluvoxamine, and citalopram) in the formalin test. The antinociceptive effects of imipramine and desipramine were markedly antagonized by an α_1 antagonist, prazosin, and by a specific 5-HT_2 antagonist, ketanserin. However, an α_2 antagonist, yohimbine, and a 5-HT_{1A} selective antagonist, WAY-100635, did not affect imipramine-induced antinociception.

The formalin test and the hot plate test are well-known nociception tests by which the potency of analgesic agents can be easily determined [14,21,22]. In the present study, the dose of imipramine, desipramine, or sertraline inducing antinociception was smaller in the formalin test than in the hot plate test. These results indicate that the nociceptive effect observed in the formalin test may be more sensitive and useful than the hot plate test for evaluation of the antinociceptive effect of antidepressants in rats. Our findings confirm previous studies reporting a greater effectiveness of TCAs in tests using a chemical stimulus (formalin test or writhing test with acetic acid or phenylbenzoquinone) than in tests using a thermal stimulus (hot plate test or tail flick test) [11,22] and an electrical stimulus [14].

In this study, classical TCAs (imipramine and desipramine) had a more potent antinociceptive effect than SSRIs in the formalin test. Lund et al. [21] have also reported potent in vivo efficacy of desipramine over zimelidine (SSRI) according to the hot plate and the formalin tests. Thus, classical TCAs are likely to have a therapeutic advantage over SSRIs for pain control. Coquoz et al. [2] reported that the subjective analgesic effects of desipramine and fluvoxamine in healthy volunteers were comparable, but the objective effects on the spinal R-III reflex were greater after desipramine treatment. However, there are only a few clinical studies on the therapeutic efficacy of SSRIs for patients with chronic pain, and their results seem to be controversial [1,7,23].

In vitro studies have demonstrated that the inhibition constant (K_i) of imipramine (41 nM) for blockade of 5-HT uptake into rat brain synaptosomes is greater than those of sertraline (3.4 nM), fluvoxamine (7 nM), and citalopram (1.3 nM) [12,13], suggesting a lower affinity of imipramine for the 5-HT uptake site. Desipramine is known to be a specific inhibitor of norepinephrine reuptake. Taken together with these in vitro data, our finding that possible inhibition of norepinephrine reuptake induces more potent antinociception than that of 5-HT reuptake suggests that central adrenoceptors are more dominantly involved in an antidepressant-induced antinociceptive effect than 5-HT receptors. In preliminary experiments, we also confirmed that other norepinephrine specific reuptake inhibitors, nisoxetine and maprotiline, markedly inhibited formalin-induced nociception.

In our study the antinociceptive effects of imipramine were markedly antagonized by an α_1 antagonist, prazosin, and a specific α_{1A} antagonist, WB-4101, but not by an α_2 antagonist, yohimbine. Prazosin also significantly antagonized desipramine-induced antinociception. These data suggest that α_1, especially α_{1A} adrenoceptors, which are known to be rich in the central nervous system [24], play a dominant role in antidepressant-induced antinociception. Our results are supported by the findings of Ansuategui et al. [17] that the antinociceptive effects of clomipramine were closely related to α_1 adrenoceptors, but not to α_2 adrenoceptors. It is widely known that stimulation of α_2 adrenoceptors, for example by clonidine, can induce an antinociceptive effect. However, in our investigation, α_2 adrenoceptors seem to be less involved in the antinociceptive effects of antidepressants than α_1 adrenoceptors.

On the other hand, the antinociceptive effects of imipramine were completely antagonized by a specific 5-HT_2 antagonist, ketanserin, but were not affected by a 5-HT_{1A} selective antagonist, WAY-100635. These data indicate involvement of 5-HT_2 but not 5-HT_{1A} receptors in imipramine-induced antinociception. Surprisingly, the antinociceptive effects of a norepinephrine-specific reuptake inhibitor, desipramine, were markedly antagonized by a specific 5-HT_2 antagonist, ketanserin. These results suggest that imipramine and desipramine, despite a difference in their specificity to monoamine transporters, produce antinociception commonly via activation or potentiation of both noradrenergic and serotonergic neurotransmission mediated by α_1 receptors and 5-HT_2 receptors. According to previous reports [25], 5-HT_2 antagonists (pirenperone and ritanserin) were effective in producing a significant reduction in the antinociception induced by repeated naloxone treatment, whereas ketanserin [18] produced a reduction of swim analgesia in a tail-flick test.

However, in this study, it remains unclear why a 5-HT_2 specific antagonist, ketanserin, as well as α_1 antagonists, could almost completely antagonize the antinociceptive effect of imipramine and desipramine, and how and where adrenergic transmission via the α_1 receptor interacts with serotonergic transmission via the 5-HT_2 receptor in the analgesic pathway after antidepressant treatment. The antinociceptive activity of the antidepressants is suggested to be probably indirect in part and dependent on critical levels of free 5-HT and norepinephrine at the receptor sites in the central nervous system [26]. Therefore, adrenergic and serotonergic systems are likely to work together via α_1 and 5-HT_2

receptors, not simply in an independent manner, but by a complicated interaction in the antidepressant-induced analgesic pathway.

In conclusion, classical TCAs had a more potent antinociceptive effect than SSRIs on the late phase in the formalin test. These findings suggest that classical TCAs are likely to have a therapeutic advantage over SSRIs for pain control. In addition, central α_1 adrenoceptors and 5-HT_2 receptors are predominantly involved in an antidepressant-induced antinociceptive effect. However, the pharmacological and anatomical interactions between α_1 adrenoceptors and 5-HT_2 receptors in antinociception by antidepressants still remain unclear.

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