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The Effect of a Selective α_2-Adrenoceptor Antagonist on Pain Behavior of the Rat Varies, Depending on Experimental Parameters

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KAUPPILA, T., E. JYVÄSJÄRVI, M. M. HÄMÄLÄINEN AND A. PERTOVAARA. *The effect of a selective alpha$_2$-adrenoceptor antagonist on pain behavior of the rat varies, depending on experimental parameters.* PHARMACOL BIOCHEM BEHAV **59**(2) 477–485, 1998—Effects of atipamezole, an α_2-adrenoceptor antagonist, in various acute pain tests were studied in the rat. Atipamezole (at doses $\geq$0.1 mg/kg IP) and idazoxan, another α_2-adrenoceptor antagonist (2.5 mg/kg, IP), increased licking latency in the hot-plate test. Bilateral administration of atipamezole (10 μg) into the locus coeruleus did not increase licking latency in the hot-plate test. Medetomidine (an α_2-adrenoceptor agonist; 1–3 mg/kg) or repeated pre-exposures to the testing apparatus reversed the effect of atipamezole (1.5 mg/kg) in the hot-plate test. Atipamezole also increased the latency to mechanically induced licking/biting response at a dose of 1.5 mg/kg, but not at lower doses. In the heat-induced tail-flick test, in contrast, atipamezole at doses of 0.1 and 1.5 mg/kg produced a medetomidine-reversible decrease of response latencies. This facilitation of the tail-flick response disappeared if the intensity of the heat stimulus was high. At a dose range from 0.03 to 1.5 mg/kg atipamezole did not significantly alter the paw withdrawal latency to noxious mechanical stimulation, nor pain behavior in the formalin test. Responses to nociceptive spinal dorsal horn neurons were not modulated by atipamezole (1 mg/kg) in anesthetized spinalized rats. The results indicate that an α_2-adrenoceptor antagonist may have variable effects in behavioral pain tests, depending on habituation of the experimental animals to the testing conditions, the dose of the drug, the type of behavioral response and the submodality or the intensity of the noxious test stimulus. The atipamezole-induced changes in pain behavior observed in this study may rather be explained due to action on motor expression of pain than due to modulation of nociception. © 1998 Elsevier Science Inc.

α_2-Adrenoceptor Antinociception Atipamezole Idazoxan Medetomidine Pain<Rat

α_2-ADRENOCEPTOR antagonists' ability to reverse α_2-adrenoceptor–mediated antinociception is well known [e.g., see (21,29,32)]. However, when administered alone, their actions in acute pain tests show great variety. Systemically administered yohimbine has been reported to shorten response latencies in the tail-flick test in the rat when administered intraperitoneally (19). On the other hand, systemically administered idazoxan did not alter paw withdrawal latency to noxious thermal stimulus in the rat (17). Neither did atipamezole administered at a systemic dose of 3 mg/kg decrease mechanically induced paw withdrawal or tail-flick latencies in the rat (18). Furthermore, Dennis and co-workers (9) reported that yohimbine had no effect on the spinally mediated tail-flick response, but it reduced supraspinally mediated pain behavior, licking of the paws, in the formalin and the hot-plate tests in the rat. This facilitatory effect of yohimbine on the hot plate-induced licking, later confirmed by other groups (5,36), was reversed with propranolol, an unselective β-adrenoceptor antagonist (9) and also with repeated exposures to the hot-plate apparatus before the testing (36). In mice, idazoxan did not increase hot plate-induced paw licking (29). Thus, the results of the experiments concerning the effects of α_2-adrenoceptor antagonists on pain behavior are partially contradictory.

One explanation to the variability in the α_2-adrenoceptor antagonist-induced actions in various pain tests is the fact that these drugs may mediate some of their behavioral effects via

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other receptor types as α_2-adrenoceptors (9,46). Furthermore, α_2-adrenoceptor antagonists induce emotional stress in the rat (2,16,25) and humans (6,23). Stress-induced suppression in the hot-plate behavior is reversed by naloxone, an opiate antagonist, suggesting an opioidergic mechanism in mediating this type of hypoalgesia (7).

Diazepam, a benzodiazepine agonist, also reduces opiate-induced antinociception in the hot-plate test (38). Diazepam reduces yohimbine-induced anxiety in humans without effects on various physiological or biochemical indicators of noradrenergic activity (6). Thus, diazepam provides a tool that helps to dissociate anxiety-induced effects from specific antinociceptive effects of a drug that increases anxiety and modulates pain behavior.

In the present study we studied whether the conflicting results regarding the effect of α_2-adrenoceptor antagonists on behavioral pain responses could be explained by differences in experimental parameters. As an α_2-adrenoceptor antagonist we used atipamezole, which is more selective in binding to α_2-adrenoceptors than the older antagonists yohimbine and idazoxan (39,46). In behavioral rat studies atipamezole has usually been administered at doses >0.3 mg/kg (e.g., (18,25, 39,40,47), and at these doses it reverses α_2-adrenoceptor–induced sedation (10,26,34,46) and antinociception (26,32). However, at doses >0.1 mg/kg atipamezole tends to loose its selectivity, as shown by a prazosin-reversible rise in blood pressure in the pithed rat (46). Whether atipamezole itself facilitates or suppresses pain is also of clinical importance, because it is commonly used in veterinary medicine as an antianesthetic agent (20), and it may be used in human patients also (23,24). The specific aim of the present study was to find out whether the effect of atipamezole on pain behavior varies depending on the behavioral response studied (spinally vs. supraspinally mediated response), the submodality of noxious stimulation (thermal, mechanical, chemical), the dose level of atipamezole, or on some other experimental parameter such as habituation to the test situation or test stimulus intensity. To study the putative receptor types involved in the behavioral effects of atipamezole, we tried to reverse the observed atipamezole-induced changes in pain behavior with various agonists and antagonists acting on adrenoceptors, benzodiazepine- or opioid-receptors. For comparison, the effect of atipamezole on responses to nociceptive spinal dorsal horn neurons was also determined.

METHOD

Animals

Naive male Han–Wistar rats were used (age 5–6 months, weight 370–540 g, six per cage, 12 L:12 D cycle, light phase 0600–1800 h, humidity 35–50%, food ad lib). The experiments were approved by the Institutional Ethical Committee of the University of Helsinki and the municipal government of Uusimaa, Finland.

Formalin Test

To study the effects of atipamezole on supraspinally mediated pain behavior induced by noxious chemical stimulation, five groups of four rats were used. Atipamezole, a selective α_2-adrenoceptor antagonist (0.03, 0.3, or 1.5 mg/kg, 0.5 ml/kg; Farmos Group, Orion Ltd., Finland (39,46)), or saline was administered intraperitoneally 20 min before the testing. At the beginning of the test, 0.05 ml of 5% formalin was injected subcutaneously into the plantar site of one hind paw (42). Pain behaviour was scored continuously throughout the 3-min observation periods for 30 min according to the proportion of the time the paw was held up and licked (grade 3), held fully elevated (grade 2), partially weight bearing (grade 1), or fully weight bearing (grade 0).

Tail Pinch Test

To study the effects of atipamezole (0.03, 0.1, 0.3, or 1.5 mg/kg) on supraspinally mediated pain behavior induced by noxious mechanical stimulation, six groups of six rats were used. The drug or saline was applied as described earlier. Twenty minutes later a hemostatic clamp was applied to the tail and the latency to the biting or licking of the tail and/or clamp was measured (14,32). The clamp was removed after 60 s if no response was observed.

Mechanically Induced Hindlimb Withdrawal Reflex

To study the effects of atipamezole (0.03, 0.3, or 1.5 mg/kg) on a spinally mediated reflex induced by noxious mechanical stimulation, four groups of 6–18 rats were used. For testing we used an analgesymeter (Ugo Basile, Varese, Italy) as originally described earlier (35). The training of the rats for the immobilization by the experimenter's hand was started in the morning of the testing day. To keep their hind paws in the test apparatus, the rats were first adjusted to the machine by "sham testing" them without any load for 15–20 times. Once the rat was repeatedly able to hold the paw in the test apparatus for the maximum time of the test (18 s, i.e, the time that the machine needed to increase the load from zero to the maximum) it was tested twice with a load. The mean of the loads that caused the withdrawal of the paw served as a predrug threshold. Then the rat was trained again without a load as described above. Once the rat was again able to hold the paw for 18 s in the apparatus, atipamezole or saline was administered and the rat was tested twice 20 min later as described earlier to obtain a postdrug threshold. The difference between pre- and postdrug thresholds was then used in further calculations.

Tail-Flick Test

To study the effects of atipamezole (0.03, 0.1, 0.3, or 1.5 mg/kg) on a heat-induced nocifensive tail reflex, eight groups of four to six rats were used. Also, medetomidine (0.01 mg/kg; Farmos Group, Orion Ltd., Finland), an α_2-adrenoceptor agonist (21,48), was used to reverse the effect of atipamezole. The drugs or saline were administered as described above. In the tail-flick test, the rat was immobilized with a clear Plexyglas cylinder, and radiant heat was applied to the tail until the tail was withdrawn (8). Three different stimulus intensities were used. The stimuli were adjusted so that the baseline latencies of the tail-flick response in untreated animals were 1–2 s, 4–6, or 7–9 s at high, intermediate, and low stimulus intensity, respectively. These baseline latencies covered the range that has been used in previous studies on this subject (see the Discussion section). Testing took place 20 min after injections. A tail-flick testing device (Socrel Model DS20, Ugo Basile, Varese, Italy) electronically measured the latency from the onset of heating to the first movement of the tail. The tail flick was tested two times at 2-min intervals. A 3-s cutoff was imposed if no response was observed at the highest intensity of the stimulus, whereas at moderate and low stimulus intensities the cutoff was 12 s and 20 s, respectively. With these cutoff times tissue injury could be avoided according to our preliminary

experiments. With the highest stimulus intensity, only the effect of the highest atipamezole dose (1.5 mg/kg) was tested. Skin temperature at the base of the tail was measured during the experiment with a thermocouple.

Hot-Plate Test

The effects of atipamezole on supraspinally mediated pain behavior was evaluated using the hot-plate test. Atipamezole (0.03–1.5 mg/kg), idazoxan (another α_3-adrenoceptor antagonist; 2.5 mg/kg) or saline control was administered IP 20 min before the testing in a volume of 0.5 ml/kg. In the hot-plate test the rat was placed on a copper plate whose temperature was maintained at 54°C. The latencies to lick of the hind and fore paws were separately measured (3,9,13). To avoid burns of the paws, a 30-s cutoff time was imposed. In four saline- and four atipamezole-treated (1.5 mg/kg IP) rats we also measured the latency to a response consisting of shaking or repeated kicking of the hind leg as described earlier (30).

To study the contribution of various receptors to the atipamezole-induced changes in pain behavior in the hot-plate test, atipamezole was injected subcutaneously at a dose of 1.5 mg/kg. The testing was performed 20 min after the injection of atipamezole as described above. Licking latency of the hind paws was considered to be the response measured. Prazosin-HCl (Orion Ltd., Finland, 1 mg/kg) as an aqueous solution (46) was given IP 15 min prior to atipamezole injection (43). Propranolol-HCl (Orion Ltd., Finland, 4 and 8 mg/kg) was diluted in saline and it was injected IP 5 min before atipamezole (9). Naloxone (Du Pont, USA) 1 and 10 mg/kg, diluted in saline) was also injected IP 5 min before atipamezole injection (7) as well as diazepam 0.5 and 2.0 mg/kg (diluted in saline, Orion Ltd., Finland) (38). Physiological saline served as a control (0.5 ml/kg IP 5 min before the injection of atipamezole). An α_2-adrenoceptor agonist medetomidine was also used. It was given IP at doses of 1 and 3 mg/kg 5 min before atipamezole administration. Because the potency of medetomidine may vary depending on the site of administration [IP vs. SC (21)], the dose of 1 mg/kg was also tested with SC administration. The test with medetomidine 3.0 mg/kg was repeated with 15 additional rats to compare the effects of systemically administered atipamezole (1.5 mg/kg + saline, $n = 5$), with atipamezole + medetomidine (1.5 mg/kg + 3.0 mg/kg, respectively, $n = 6$) and with medetomidine (3.0 mg/kg + saline, $n = 4$) in the hot-plate test.

To study the effects of repeated exposures to the test environment on the paw-licking latency eight rats were used. For 8 consecutive days the rats were exposed to the testing room and 30 min later they were placed into the testing chamber for 90 s (36). The temperature of the testing device was at the room level (22–23°C). At the ninth day the testing was performed as described above after the administration of atipamezole (1.5 mg/kg SC) or saline. The shaking or repeated kicking latency of the hind limb was also measured as described above.

To study the effects of administration of atipamezole into the locus coeruleus eight rats were used. They were prepared and housed as described earlier (34). Briefly, under pentobar-

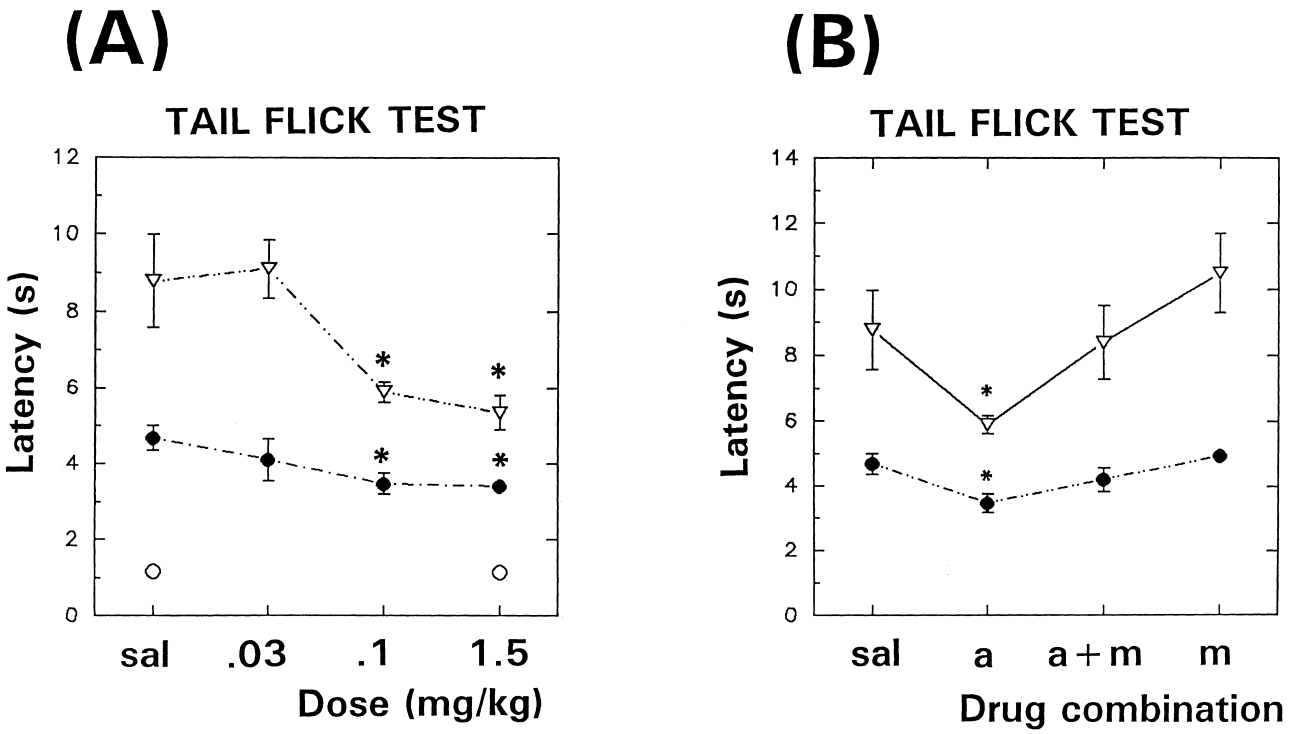

FIG. 1. (A) Atipamezole dose dependently decreases the latency of the heat-induced tail-flick reflex when the stimulus intensity is low (open triangles) or intermediate (filled circles) but not when the stimulus intensity is high (open circles). In both high intensity groups $n = 6$. In intermediate and low intensity groups $n = 6$, except at the dose of 0.03 and 1.5 mg/kg of atipamezole $n = 4$. (B). The decrease of tail-flick latency induced by atipamezole (= a; 0.1 mg/kg; $n = 6$) is reversed by medetomidine (= a + m; $n = 4$) at a dose that alone had no significant effect (= m; $n = 4$). In both graphs, sal = saline control ($n = 6$). The vertical error bars represent ± SEM. *$p < 0.05$ (ref: corresp. sal-group).

bital anesthesia (50 mg/kg) the rats were placed in a stereotaxic frame and injection sites located according to the atlas of Paxinos and Watson (31). The desired injection sites were in the locus coeruleus (AP −0.7 mm, ML ±1.3 mm, DV 2.8 mm). A pair of 22-gauge stainless steel guide cannulae were lowered into a position 2 mm dorsal of the desired injection site. The cannulae were fixed into the scull using a dental screw and dental cement. After the operation the rats received G-penicillin and they were allowed to recover in individual cages for 1 week.

The injection was made bilaterally as described earlier (34) with a 10 μl Hamilton syringe through a 27-gauge cannula protruding 2 mm beyond the tip of the guide cannula. The injection volume was 1.0 μl and the total bilateral dose of atipamezole was 10 μg (corresponding to a systemic dose of 27 μg/kg). This dose of atipamezole in the locus coeruleus is enough to reverse the sedative effect induced by systemically administered medetomidine in the rat (34). The testing was performed 5 min after injections as described earlier. After the tests the rats were sacrificed and the sites of the injections were verified histologically.

Electrophysiological Recordings

To study the effect of atipamezole on nociceptive responses to spinal dorsal horn neurons, the animals were initially anesthetized with pentobarbital (50 mg/kg IP) and then placed in a standard stereotaxic frame. The level of anesthesia was frequently monitored by observing the size of the pupils, the general muscle tone and responses to noxious pinching. Supplemental doses (20 mg/kg) were administered as required. The rats were spontaneously breathing and the body temperature was kept within physiological range with a homeothermic blanket. The peripheral vascularization was checked by considering the color of the ears and extremities.

A laminectomy was performed at the level of the vertebrae T12–L2, the dura removed, and a pool of skin formed and filled with warm mineral oil. Two spinal clamps, one distal and one rostral to the lumbar laminectomy, were used to stabilize the preparation. An additional laminectomy was performed at the midthoracic level through which the spinal cord was cut.

Spinal unit activity was recorded extracellularly with lacquer-coated tungsten microelectrodes (tip impedance 5–10 MOhm at 1 kHz) using standard techniques. The amplified and filtered signal was fed through an amplitude window discriminator to a rate monitor and timed counter. The discharge rate and integrated spike activity counts were observed on a digital storage oscilloscope screen and hardcopies were printed for off-line analysis.

During the search for spinal units, the glabrous skin of the hindpaw was repeatedly stimulated with a brush. After a neuron responding to brushing was found, its receptive field characteristics were determined using calibrated monofilaments and a feedback-controlled Peltier thermode (LTS-3 Stimulator, Thermal Devices Inc., Golden Valley, MN). The brush-driven neurons were classified as wide-dynamic range (WDR) neurons, if they gave differential responses to thermal stimulation within nociceptive range (46–54°C) (49). WDR neurons also gave differential responses to low- vs. high-intensity mechanical stimuli (2–46 g). Other types of neurons were not further considered in this study. Only neurons that were consid-

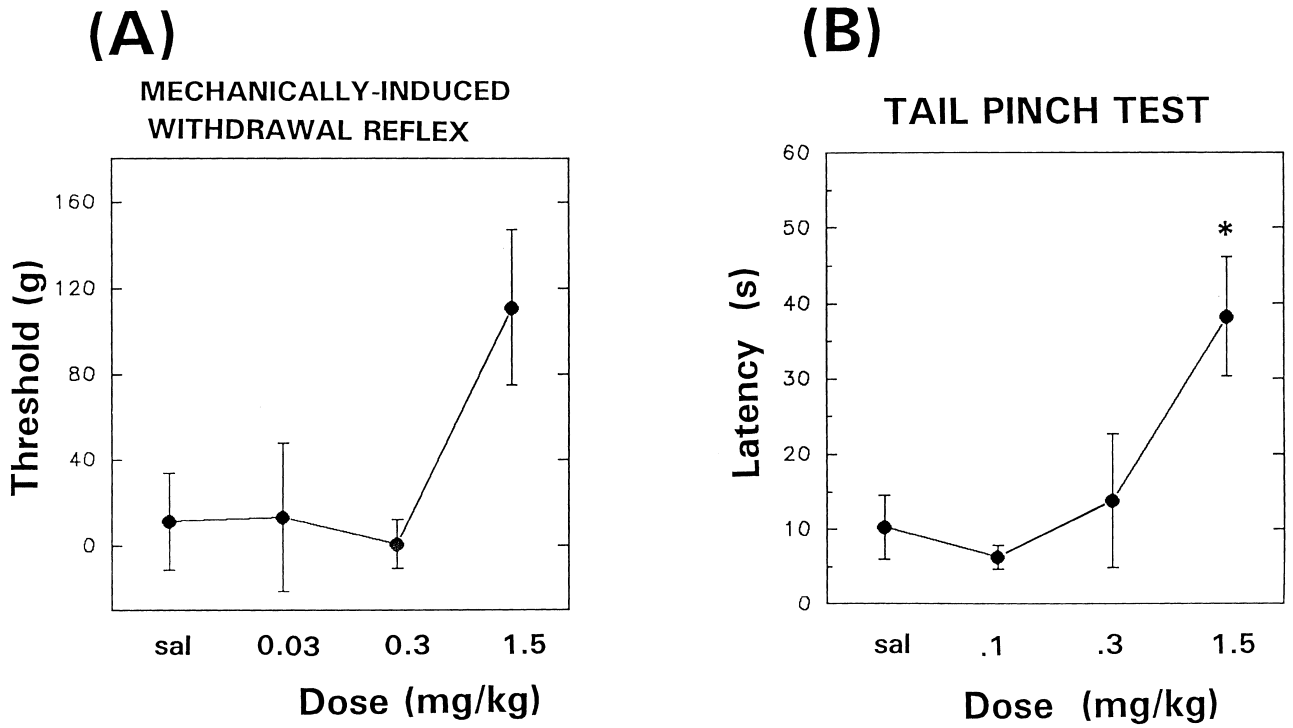

FIG. 2. (A) Effect of atipamezole on a mechanically induced hindlimb withdrawal reflex was short of statistical significance. In the abscissa, dose atipamezole. sal = saline control. In the ordinate: postdrug threshold—predrug threshold. The vertical error bars represent ± SEM (n = 6–18). (B) Atipamezole dose dependently increases response latency to tail pinch. *$p < 0.05$ (ref.: saline-treated rats).

ered to be in the spinal dorsal horn according to the recording depth from the cord surface (<1000 μm) were included (49).

When assessing drug effects, first the predrug responses to a noxious mechanical stimulus (46–164 g) and to a noxious heat stimulus (52°C) were determined. Then atipamezole was administered at a dose of 1 mg/kg SC. Postdrug responses to noxious mechanical and heat stimuli were determined 15 min following the administration of atipamezole. Only one neuron was studied in each animal. At the termination of the experiment, an overdose of pentobarbital was given to kill the animal.

Statistical Methods

Kruskall–Wallis analysis of variance followed by Mann–Whitney *U*-test was used for statistical analysis of behavioral data. Wilcoxon's paired test was used in assessing differences in the same animal. Neurophysiological data were analyzed using paired *t*-test. $p < 0.05$ represented a statistically significant difference.

RESULTS

Effect of Atipamezole on Nocifensive Spinal Reflexes

Heat-induced tail-flick reflex. Atipamezole produced a dose-dependent decrease in the latency of the heat-induced tail-flick reflex (Fig. 1A). The atipamezole-induced decrease of the tail-flick latency was dependent on the intensity of the heat stimulus. The higher the stimulus intensity, the smaller the atipamezole-induced latency decrease. The atipamezole-induced decrease of tail-flick latency was reversed by medetomidine at a dose (0.01 mg/kg) that alone did not produce any significant effects on the tail-flick latency (Fig. 1B). Atipamezole, at the dose of 0.1 mg/kg, which produced a significant decrease in the tail-flick latency, did not produce a significant change in the tail skin temperature.

Mechanically induced hindlimb withdrawal reflex. Atipamezole (0.03–1.5 mg/kg) did not produce any significant change in the threshold of a mechanically induced hindlimb withdrawal reflex (KW = 6.313, $p = 0.097$, Kruskal–Wallis; Fig. 2A).

Effect of Atipamezole on Supraspinally Mediated Pain Behavior

Tail-pinch test. In the tail-pinch test the response to pinch was significantly delayed with an IP dose of 1.5 mg/kg of atipamezole but not at lower doses (Fig. 2B).

Formalin test. Atipamezole (0.03–1.5 mg/kg) did not change pain scores in the formalin test as indicated by overlapping standard error values between various experimental groups (Fig. 3).

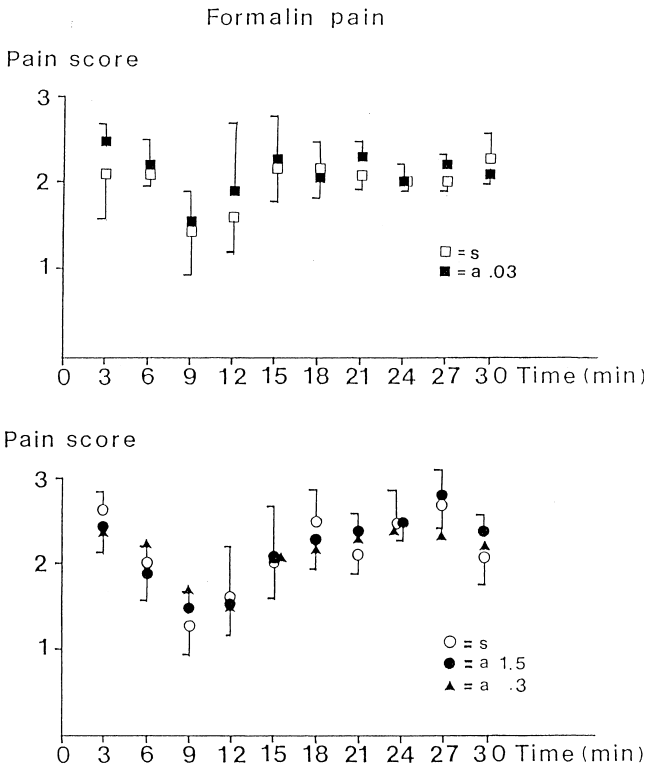

FIG. 3. Atipamezole (= a) does not alter formalin-induced pain behavior (upper figure 0.03 mg/kg vs. saline = s; lower figure 0.3 and 1.5 mg/kg vs. saline). Brackets represent the SEM. To maintain clarity in the lower figure the SEM values of the rats receiving 0.3 mg/kg of atipamezole are not shown (the SEM values were overlapping with those of other groups). Formalin was administered at time point 0.

Hot-plate test. In the hot-plate test, atipamezole significantly increased the licking latency of the hind paw at doses ⩾0.1 mg/kg IP (Fig. 4A). Idazoxan (2.5 mg/kg IP, $n = 4$) also increased the hind paw-licking latency from 12.4 ± 2.1 s (mean ± SEM; saline, $n = 4$) to 28.3 ± 1.3 s ($U = 0$, $p < 0.05$, Mann–Whitney test). Administration of atipamezole in the locus coeruleus had no significant effect in the hot-plate test. The hind paw-licking latency was 7.5 ± 2.9 s ($n = 4$) after administration of saline and 7.1 ± 1.5 s ($n = 4$) after administration of atipamezole (10 μg) in the locus coeruleus.

The licking latency of the fore paw was slightly shorter than the licking latency of the hind paw in saline-treated rats ($T = 0$, $p < 0.05$, Wilcoxon's test). Atipamezole had only a marginal effect on the licking latency of the fore paw (Fig. 4B). There was also a qualitative difference between saline and atipamezole-treated rats in the forepaw licking. While saline-treated rats tended to lift the paw into the mouth changing their balance from one foot to another, atipamezole treated rats just left the paw down, bowed towards it, and licked it.

Unlike the licking latency, the shaking or kicking latency of the hind paw was not increased by atipamezole (1.5 mg/kg) (6.5 ± 1.1 s, $n = 4$) when compared with saline treated rats (7.0 ± 0.8 s, $n = 4$).

Modulation of the Atipamezole-Induced Latency Increase by Various Receptor-Specific Agents

Subcutaneously administered atipamezole (1.5 mg/kg) induced an increase in the hind paw-licking latency from the baseline latency of 15 ± 4 s to the cutoff latency of 30 s in all rats. This atipamezole-induced prolongation of the licking latency was not decreased by saline, naloxone (1 or 10 mg/kg), diazepam (0.5 or 2.0 mg/kg), prazosin (1 mg/kg), or propranolol (4 or 8 mg/kg) in any rat (Table 1). However, medetomidine at the dose of 1.0 mg/kg SC, but not IP, significantly attenuated the atipamezole-induced (1.5 mg/kg) prolongation of the licking latency (Table 1).

Effect of Habituation

While all atipamezole-treated (1.5 mg/kg) rats with no preexposures to the testing apparatus had hind paw-licking latencies exceeding the cutoff (30 s), the atipamezole-treated rats

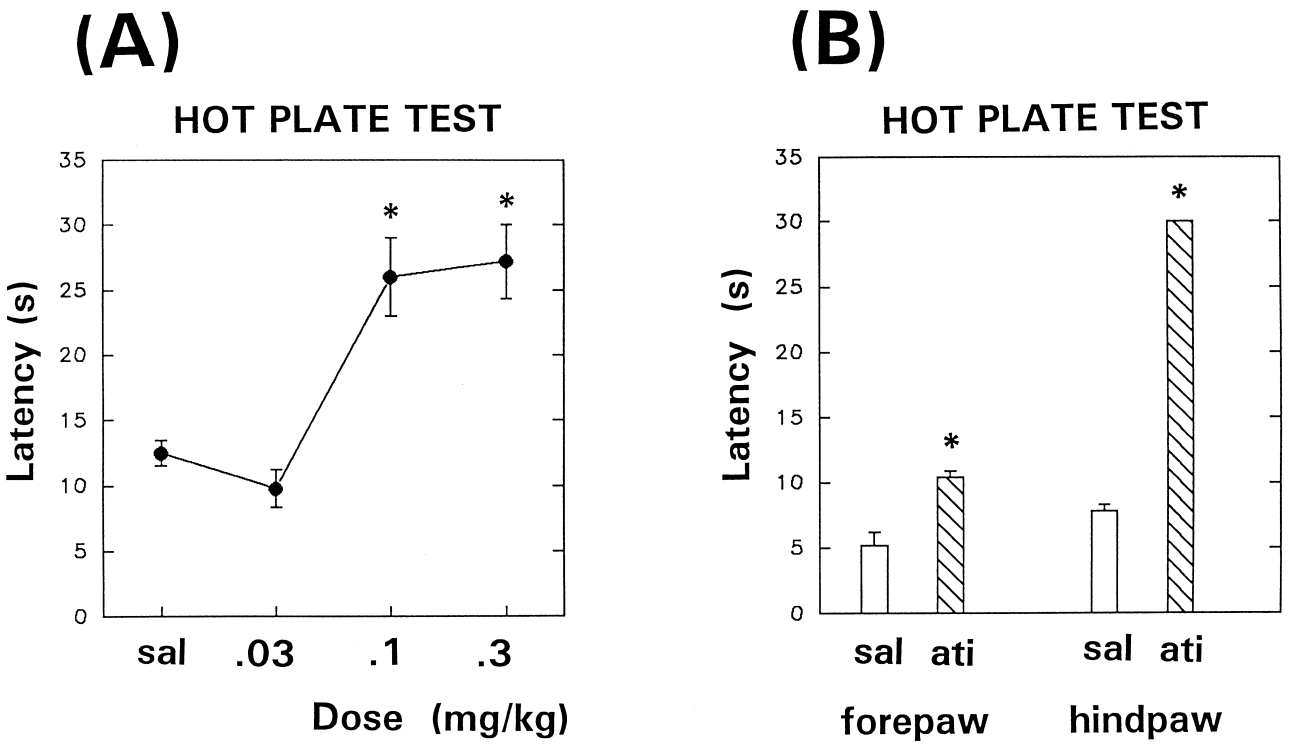

FIG. 4. (A) Atipamezole dose dependently increases latency of hindpaw licking in the hot-plate test. (B) Atipamezole (= ati; 1.5 mg/kg) increases latency of hindpaw licking more than that of forepaw licking. *$p < 0.05$ (ref.: corresponging saline group). Following atipamezole (1.5 mg/kg), the latency of hindpaw licking is significantly more increased than the latency of forepaw licking ($p < 0.05$).

with preexposures to the testing apparatus had considerable shorter hind paw-licking latencies (18.0 ± 8.0 s, $n = 4$). The difference in hind paw-licking latency of the saline-treated rats (8.5 ± 2.6 s, $n = 4$) and atipamezole-treated ones was no more statistically significant following habituation. Also, there was no difference in the shaking/kicking latency between the habituated atipamezole-treated rats and the saline-treated rats (6.4 ± 1.3 s and 6.8 ± 0.7 s, respectively; $n = 4$).

TABLE 1

EFFECTS OF DIFFERENT DRUGS ON THE ATIPAMEZOLE-INDUCED PROLONGATION OF THE HINDPAW LICKING LATENCY

	Licking latency	(mean ± SE)
Saline + saline	15 ± 4 s	$n = 4$
Ati + saline	30 ± 0 s*	$n = 4$
Ati + med (1 mg/kg i.p.)	30 ± 0 s*	$n = 4$
Ati + med (1 mg/kg s.c.)	25 ± 5	$n = 4$
Ati + med (3 mg/kg i.p)	18 ± 6 s	$n = 4$
Ati + propanolol (4 or 8 mg/kg)	30 ± 0 s*	$n = 4$
Ati + prazosin (1 mg/kg)	30 ± 0 s*	$n = 4$
Ati + diazepam (0.5 or 2 mg/kg)	30 ± 0 s*	$n = 4$
Ati + naloxone (1 or 10 mg/kg)	30 ± 0 s*	$n = 4$

*$p < 0.05$ (ref: saline+saline-group). Ati = atipamezole (1.5 mg/kg sc), med = medetomidine

Neurophysiological Results

Atipamezole, at the dose of 1 mg/kg, did not produce any significant change in the mechanically or thermally evoked responses to nociceptive spinal dorsal horn (WDR) neurons in spinalized rats ($n = 4$; Fig. 5). Spontaneous activity of spinal dorsal horn WDR neurons was 1.9 ± 1 Hz before atipamezole and 1.5 ± 0.7 Hz following atipamezole.

DISCUSSION

Atipamezole-Induced Changes in Hot-Plate Behavior

Hind paw licking induced by noxious heat was suppressed by systemic administrations of atipamezole, which observation is in accordance with previously published results with other α_2-adrenoceptor antagonists yohimbine (5,9,36) and idazoxan (5). Interestingly, hindpaw licking, which is considered the most relevant index of pain in the hot-plate test (3,13), was significantly more suppressed by atipamezole than fore paw licking. According to previous studies, intrathecal administrations of atipamezole (41), idazoxan (41), or yohimbine (4,41,45) have not produced increases in hot-plate latencies. Together, these observations suggest that supraspinal structures are involved in the α_2-adrenoceptor antagonist-induced prolongation of hot-plate latencies. Furthermore, our results suggest that of the supraspinal structures the locus coeruleus is not a critical site for mediating the α_2-adrenoceptor antagonist-induced effects in the hot-plate test.

In line with an earlier yohimbine study (36), the atipamezole-induced increase in the hind paw-licking latency could be attenuated by habituating the rats properly to the testing situ-

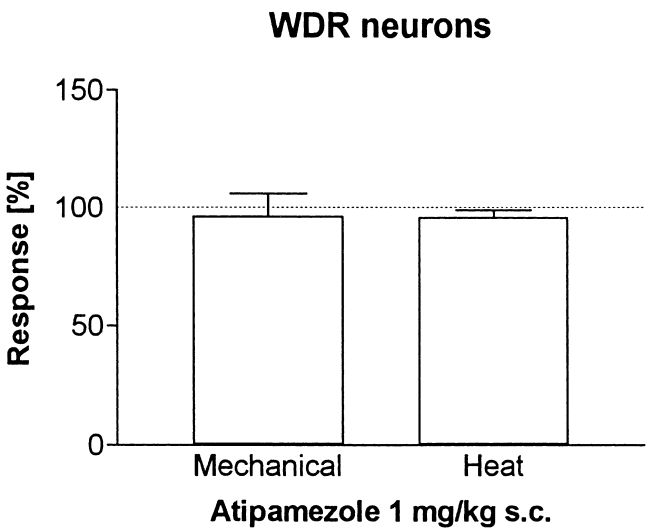

FIG. 5. Atipamezole (1 mg/kg SC) did not produce any change in the mechanically or thermally induced nociceptive responses to spinal dorsal horn WDR neurons. 100% = the corresponding response before atipamezole. The vertical bars represent SEM ($n = 4$).

ation. Interestingly, in another previous study yohimbine increased paw-licking latency also in those rats whose habituation was inhibited with naloxone (37). This suggests that the α_2-adrenoceptor antagonist-induced suppression of hot-plate behavior is at least partly independent of the opioidergic mechanisms activated by novelty of the experimental environment.

The atipamezole-induced increase in the hot-plate licking latency might be due to several reasons, one of which is antinociception. However, several findings of the present study do not support the hypothesis that atipamezole had a "true" antinociceptive action in the hot-plate test. The identical shaking and kicking latency of the hind paw in atipamezole- and saline-treated rats in the hot plate suggests that atipamezole may rather induce a selective suppression in licking behavior than "true" antinociception or analgesia in accordance with the suggestion by Carter (5). Also, when administered at identical doses as in the hot-plate test, atipamezole had no antinociceptive effect in the heat-induced tail-flick test or in the formalin test (however, the suppression of responses to noxious mechanical stimulation of the tail by atipamezole at the dose of 1.5 mg/kg). Importantly, atipamezole, at the dose of 1 mg/kg, did not modulate mechanically or thermally induced nociceptive responses to spinal dorsal horn WDR neurons that are considered important for the relay of pain (49). According to these findings, it might be more appropriate to use the term α_2-adrenoceptor antagonist-induced 'altered pain behavior' (5) than the term 'hypoalgesia' (9,36) to define the atipamezole-induced effects in the hot-plate test. Moreover, α_2-adrenoceptor antagonists do induce emotional changes (25). Emotional influences (1) as well as motor effects are important confounding factors to be considered whenever performing behavioral pain studies in awake animals (15).

Receptor Mechanisms Underlying Atipamezole-Induced Changes in Hot-Plate Behavior

In line with previous results (38,46), benzodiazepine or α_1-adrenergic systems did not interfere with the action of atipamezole on the licking behavior in the hot-plate test, as indicated by current results obtained using diazepam and prazosin, respectively. Neither did the opiate μ-receptor antagonist naloxone interfere with the effects of atipamezole, which is in agreement with previous results obtained on the interaction of less selective α_2-adrenoceptor antagonists with naloxone (7). Lack of reversal by propranolol suggests that β-adrenoceptors were not involved in mediating the action of atipamezole, whereas according to earlier reports β-adrenoceptors were involved in the actions of yohimbine in the hot-plate test (9). Only medetomidine, an α_2-adrenoceptor agonist, reversed the effects of atipamezole in the hot-plate test, suggesting a major involvement of α_2-adrenoceptors in the atipamezole-induced prolongation of hot-plate latencies. Although atipamezole acts via α_2-adrenoceptors at the dose of 0.1 mg/kg (46), which dose increased the heat-induced paw licking in the present experiments, we cannot exclude the possibility that atipamezole and medetomidine exerted part of their effects via some other receptor type (e.g., imidazole receptors).

Atipamezole-Induced Changes in the Tail-Flick Test

In contrast to the suppression of supraspinally mediated nocifensive behavior induced by the hot plate, atipamezole facilitated the spinally mediated tail-flick response to noxious heat. In previous studies α_2-adrenoceptor antagonists have had variable effects on the heat-induced tail-flick reflex. It is noteworthy that in experiments reporting a decrease of the tail-flick latency by α_2-adrenoceptor antagonists, the baseline tail-flick latency has been rather long (4.5–6.0 s), independent of the route of drug administration (19,45). However, in experiments reporting lack of effect by α_2-adrenoceptor antagonists on the tail-flick latency, the baseline latency has been short (2.1–3.0 s), also independent on the route of drug administration (4,9,18,33). In the present study atipamezole produced a decrease in tail-flick latencies only when the intensity of the heat stimulus was low or intermediate, and consequently, the baseline tail-flick latency long (4.7–8.8 s). The atipamezole-induced tail-flick facilitation was reversed by medetomidine at a low dose that alone had no effect on the tail-flick latency. This finding suggests that the facilitation was due to an action on α_2-adrenoceptors. Moreover, the lack of atipamezole-induced facilitation of heat-evoked responses to nociceptive neurons of the spinal dorsal horn suggests that the atipamezole-induced facilitation of the tail-flick reflex was due to action on motor neurons.

Effect of Atipamezole on Responses Induced by Noxious Mechanical Stimulation

In the tail-pinch test, a high dose of atipamezole (1.5 mg/kg) increased the latency of the supraspinally mediated biting response, whereas a spinally mediated hindlimb withdrawal reflex induced by noxious mechanical stimulation was not significantly suppressed. In a previous study high doses of idazoxan increased vocalization thresholds to mechanical pinch, whereas yohimbine did not (27). It should be noted that although atipamezole at low or intermediate doses (up to 0.3 mg/kg) has not changed locomotor performance (11,12,25,39), the high dose of atipamezole (1.5 mg/kg) required to suppress the tail pinch-induced biting is enough to decrease motor activity as determined in a test of exploratory behavior (25). Thus, the atipamezole-induced suppression of responses to noxious mechanical stimulation might be due to a general change in motor behavior. A putative agonistic action of ati-

pamemezole on α_2-adrenoceptors should also be considered when the high dose of 1.5 mg/kg is used (46). Interestingly, it was recently shown that following inflammation atipamezole may attenuate enhanced withdrawal responses to mechanical test stimulation at a rather low dose (0.1 mg/kg; (28)). Thus, the present results obtained under physiological conditions may not apply to some pathophysiological conditions.

Effect of Atipamezole in the Formalin Test

Atipamezole as idazoxan previously (44) did not alter formalin-induced pain scores. However, systemically and intrathecally administered yohimbine has decreased pain behavior in the formalin test (9,22). With thermal and mechanical test stimulation a supraspinally mediated pain response, unlike a spinally mediated nocifensive reflex, was suppressed with atipamezole. This differs from the lack of atipamezole-induced effect on supraspinal responses to noxious chemical (formalin) stimulation. This difference in the effect of atipamezole on supraspinally mediated behavior induced by various submodalities of noxious stimulation indicates that the involvement of supraspinal mechanisms per se is not the only factor that determines whether atipamezole suppresses responses or not.

CONCLUSIONS

The results of this study indicate that the effect of atipamezole, an α_2-adrenoceptor antagonist, on nocifensive behavior varies from facilitation to suppression depending on several experimental parameters. Whether atipamezole has a suppressive, facilitatory or no effect on nocifensive behavior depends on the submodality of the noxious test stimulation, habituation of the experimental animals to the testing situation, the type of behavioral response (whether it involves spinally or supraspinally mediated behavior), the dose of atipamezole, and the intensity of the noxious test stimulation. It should also be noted that atipamezole induced effects in various behavioral pain tests may rather reflect changes in motor behavior than in nociception. This conclusion is further supported by the present electrophysiological results indicating that atipamezole did not produce any change in the responses to nociceptive spinal dorsal horn neurons. Selective changes in pain-induced behavioral responses (e.g., suppression of paw licking) may be obtained at low or intermediate doses ($\leqslant$0.3 mg/kg) of atipamezole, whereas following high atipamezole doses (1.5 mg/kg) changes in pain-induced behavior may be due to a general suppression of motor performance. The present results, together with some earlier findings (9), indicate that one should be cautious when making conclusions on antinociceptive effects of adrenergic agents on the basis of one pain test only.

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