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5-HT_{1A} Agonists Induce Central Cholinergic Antinociception

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GALEOTTI, N., C. GHELARDINI AND A. BARTOLINI. *5-HT_{1A} agonists induce central cholinergic antinociception.* PHARMACOL BIOCHEM BEHAV **57**(4) 835–841, 1997.—The antinociceptive effects of the 5-HT_{1A} agonists buspirone [3 mg/kg intraperitoneally (IP)], gepirone (3–6 mg/kg IP), and 8-OH-DPAT [3–5 mg/kg IP; 1–3 μg per mouse intracerebroventricularly (ICV)] were examined in mice by using the hot-plate (thermal stimulus) and abdominal constriction (chemical stimulus) tests. Buspirone, gepirone, and 8-OH-DPAT produced significant antinociception, which was prevented by atropine (5 mg/kg IP), the ACh depletor hemicholinium-3 (1 μg per mouse ICV), and the 5-HT_{1A} antagonist NAN 190 (0.5 μg per mouse ICV), but not by naloxone (1 mg/kg IP), the $GABA_B$ antagonist CGP 35348 (100 mg/kg IP), and pertussis toxin (0.25 μg per mouse ICV). NAN 190, which totally antagonized buspirone, gepirone, and 8-OH-DPAT antinociception, did not modify the analgesic effect of morphine (5 mg/kg subcutaneously). In the antinociceptive dose range, none of the $5HT_{1A}$ agonists impaired mouse performance evaluated by rota-rod and hole board tests. On the basis of these data, it can be postulated that buspirone, gepirone, and 8-OH-DPAT exert an antinociceptive effect mediated by a central amplification of cholinergic transmission. © 1997 Elsevier Science Inc.

8-OH-DPAT Buspirone Gepirone 5-HT_{1A} receptors ACh Cholinergic neurotransmission Antinociception Analgesia

THE nonbenzodiazepine anxiolytic drugs buspirone, gepirone, and 8-OH-DPAT are agonists of the serotoninergic 5-HT_{1A} receptors in the central nervous system. Serotonin 5-HT_{1A} sites are localized predominantly on the axon terminals of serotoninergic neurons. They function as autoreceptors (14), and their stimulation leads to the disinhibition of the cholinergic system that is tonically inhibited by tryptaminergic control (11). Bianchi et al. (4) demonstrated that the full 5-HT_{1A} agonist 8-OH-DPAT was able to increase ACh release from the cerebral cortex of freely moving guinea pigs. Recent studies have shown that buspirone, given systemically, produces antinociception in several pain tests in rats (12). Giordano and Rogers (13) reported that buspirone-induced analgesia may be a nonopioid, adrenally mediated co- and/or epi-phenomenon to core hypothermia evoked by 5-HT_{1A} receptor agonism. Recently, it was reported that sumatriptan, another 5-HT_{1A} agonist, was able to induce antinociception in mice and rats through a cholinergic mechanism (3).

It has long been known that the direct and indirect activation of the cholinergic system produces analgesia in both animals (1,2,9,16–18,21,23,24) and humans (20). Because sumatriptan is endowed with cholinergic antinociceptive properties and 8-OH-DPAT enhances ACh release, the goals of the present study were to explore whether other 5-HT_{1A} agonists, such as gepirone and 8-OH-DPAT, were able to increase the pain threshold in mice and then investigate whether a cholinergic mechanism underlies 5-HT_{1A} antinociception. Moreover, we examined whether such antinociception is mediated via $G_{i/o}$ proteins.

METHODS

Animals

Male Swiss albino mice (23–30 g) from the Morini breeding farm were used. Fifteen mice were housed per cage, and the cages were placed in the experimental room 24 h before

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the test for acclimatization. The animals were kept at 23 ± 1°C with a 12 L:12 D cycle (lights on at 0700 h), with food and water ad lib. Animals were randomly assigned to a control (saline solution) or a treated group (5-HT_{1A} agonists). Both groups received a pretreatment consisting of injection of saline or one of the following antagonists: atropine, hemicholinium-3 (HC-3), CGP-35348, or NAN 190. All the antagonists were injected 15 min before treatment, with the exception of HC-3 and CGP 35348, which were administered, respectively, 5 h and 5 min before treatment. For pertussis toxin (PTX) experiments, mice were randomly assigned to a vehicle (water solution containing 0.01 M sodium phosphate buffer, pH 7.0, with 0.05 M sodium chloride) or a PTX group (0.25 μg per mouse). Naive animals did not receive any pretreatment, whereas vehicle and PTX groups received a single intracerebroventricular (ICV) injection on day 0.

All animals used were drug-naive. At least six mice per group were employed. Four different tests were performed by using separate groups of animals. All experiments were carried out according to the guidelines of the European Community Council. Furthermore, the experimental protocol was approved by the Ethical Committee of the Department of Pharmacology of Florence.

Hot Plate Test

The method adopted was described by O'Callaghan and Holtzman (26). Mice were placed inside a stainless steel container, thermostatically set at 52.5 ± 0.1°C in a precision water bath from CW Mechanical Workshop, Siena, Italy. Reaction times (s) were measured with a stopwatch, and each animal was tested before pretreatment (pretest) and 15, 30, and 45 min after treatment. The endpoint used was the licking of the fore or hind paws. Those mice scoring below 12 s or over 18 s in the pretest were rejected. An arbitrary cutoff time of 45 s was adopted, after which the animals were immediately removed from the hot plate. Following a single pretreatment with vehicle or PTX, the antinociceptive effect of 5-HT_{1A} agonists was tested 11 days later. PTX was injected 11 days before the experiment because we had previously observed that the analgesic effects of some drugs, such as baclofen, were prevented by PTX only 11 days after administration (8). Because the ADP ribosylation of $G_{i/o}$ proteins produced by PTX is irreversible, we waited 11 days after pretreatment before performing the hot plate test to rule out the possibility that a lack of antagonism could be due to allowing too short a time for obtaining the PTX-induced inactivation of $G_{i/o}$ proteins.

Abdominal Constriction Test

The test was performed in the mice according to Koster et al. (22). Mice were injected intraperitoneally (IP) with a 0.6% solution of acetic acid (10 ml kg^{-1}). 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, 3 cm in diameter and 30 cm long, with a nonslippery surface. The rod was placed at a height of 15 cm above the base. Six disks divided the rod into five equal sections; thus, up to five mice could be simultaneously tested on the apparatus. Rotation speed of the rod was 16 rpm. 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. (31). Performance was measured before and 15, 30, and 45 min after treatment.

Hole Board Test

The hole board test consists in a 40-cm-square plane with 16 flush-mounted cylindrical holes (diameter 3 cm) distributed 4 by 4 in an equidistant, gridlike manner. Mice were placed one at a time on the center of the board and left to move about freely for a period of 5 min. Two electric eyes, crossing the plane from midpoint to midpoint of opposite sides, thus dividing the plane into four equal quadrants, automatically signaled the movement of the animals on the surface of the plane. Miniature photoelectric cells in each of the 16

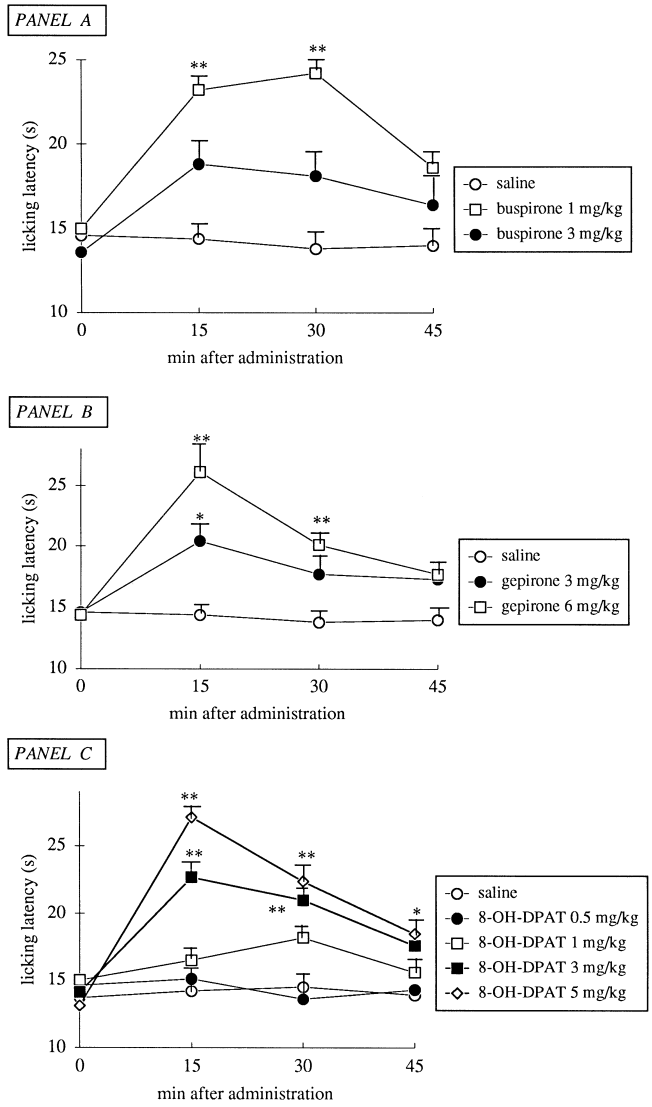

FIG. 1. Dose–response curves of buspirone (A), gepirone (B), and 8-OH-DPAT (C) after IP administration in the mouse hot plate test. The number of mice ranged between 6 and 15, with the exception of the saline-treated controls ($n = 25$). Vertical lines represent SEM. $^*p < 0.05$ and $^{**}p < 0.01$ vs. saline-treated mice.

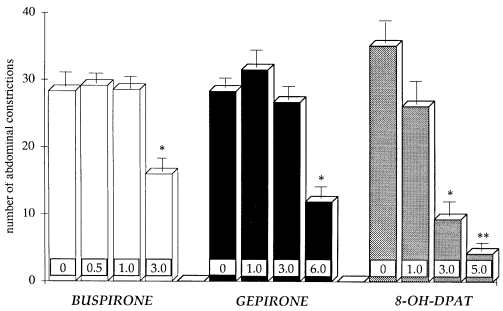

FIG. 2. Dose–response curves of buspirone, gepirone, and 8-OH-DPAT in the mouse acetic acid abdominal constriction test. Buspirone, gepirone, and 8-OH-DPAT were administered IP 10 min before the test. The dose of the analgesic compound administered is reported in each column. The number of mice ranged between 6 and 16. Vertical lines represent SEM. *$p < 0.01$ and **$p < 0.001$ vs. saline-treated mice.

holes recorded exploration of the holes (head plunging activity) by the mice.

Drugs

The following drugs were used: gepirone, buspirone, 8-OH-DPAT hydrobromide (2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene), NAN 190 hydrobromide [1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine], and PTX (all from RBI); atropine sulphate (from Sigma); hemicholinium-3 hydrobromide (HC-3), CGP 35348 (3-aminopropyldiethoxymethyl-phosphinic acid), and reserpine (all from Ciba-Geigy); sodium chloride and glacial acetic acid (both from Merck); and morphine (from U.S.L. 10/D, Florence). All drugs, with the exception of PTX, were dissolved in isotonic saline solution (0.9% NaCl) immediately before use. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml/kg by the subcutaneous (SC) or IP route. Intracerebroventricular administration was performed under ether anaesthesia using isotonic saline as the solvent, according to the method described for mice by Haley and McCormick (15). Substances were injected in the necessary dose dissolved in 5 μl. To ascertain the exact point into which the drugs were administered, some mice were injected ICV with 5 μl of India ink (diluted 1:10) and their brains were examined macroscopically after sectioning.

Statistical Analysis

Results are given as the mean ± SEM; analysis of variance, followed by Fisher's PLSD procedure for post hoc comparison, was used to verify significance between two means. Probability (p) values of less than 0.05 were considered significant. Data were analyzed by using StatView for the Macintosh (1992).

RESULTS

Buspirone, gepirone, and 8-OH-DPAT induced a significant increase in the pain threshold in the mouse hot plate (Fig. 1; Table 1) and abdominal constriction (Fig. 2) tests. In the mouse hot plate test, antinociception from buspirone (3 mg/kg IP), gepirone (3–6 mg/kg IP), and 8-OH-DPAT (3–5 mg/kg IP) reached a maximum after 15 min, persisted for up to 30 min, and then diminished 45 min after injection (Fig. 1A–C). The antinociceptive effect of 8-OH-DPAT was still present after ICV injection in the range of doses of 0.5–3 μg per mouse, as reported in Table 1. The dose–response curves of buspirone, gepirone, and 8-OH-DPAT on the abdominal constriction test are illustrated in Fig. 2. The abdominal constriction test was performed 15 min after administration of buspirone, gepirone, and 8-OH-DPAT (i.e., the time of the maximum effect of the analgesics). The doses of the above-mentioned compounds effective in the abdominal constriction test were the same as those that were effective in the hot-plate test, with the exception of gepirone, which showed analgesic properties only at 6 mg/kg IP (Fig. 2).

Figure 3 shows that buspirone, gepirone, and 8-OH-DPAT antinociception was completely prevented by the antimuscarinic agent atropine (5 mg/kg IP), the choline uptake blocker HC-3 (1 μg per mouse ICV), and the 5-HT$_{1A}$ antagonist NAN 190 (0.5 μg per mouse ICV). The antagonists were all injected 15 min before the analgesic compounds, with the exception of HC-3, which was administered 5 h before the analgesic test. Conversely, no modification in buspirone, gepirone, or 8-OH-DPAT antinociception was obtained by pretreating the mice with the opioid antagonist naloxone (1 mg/kg IP), the GABA$_B$ antagonist CGP 35348 (100 mg/kg IP), or pertussis toxin (0.25μg per mouse ICV), as shown in Table 2. Furthermore, 8-OH-DPAT antinociception was not prevented by pretreatment with reserpine at a dose of 2 mg/kg IP injected twice (48 h and 24 h) before the test in the mouse abdominal constriction test (controls = 31.1 ± 2.0 s; 8-OH-DPAT = 13.1 ± 3.4 s; 8-OH-DPAT + reserpine = 29.3 ± 2.1 s). The doses of nalox-

TABLE 1

DOSE–RESPONSE CURVE OF 8-OH-DPAT VIA ICV INJECTION IN THE MOUSE HOT PLATE TEST

Treatment (μg per mouse ICV)	Licking Latency (s)			
	Before Treatment	After Treatment		
		1min	30 min	4min
Saline	13.9 ± 0.6	15.0 ± 0.5	14.9 ± 0.7	14.0 ± 0.5
8-OH-DPAT (0.1)	14.± 0.4	16.2 ± 0.9	14.0 ± 1.1	15.3 ± 0.9
8-OH-DPAT (0.5)	13.9 ± 0.7	20.2 ± 1.0*	20.6 ± 1.1*	17.0 ± 1.0
8-OH-DPAT (1.0)	14.2 ± 0.6	23.1 ± 1.8**	21.4 ± 1.3*	20.3 ± 1.4*
8-OH-DPAT (3.0)	13.1 ± 0.5	26.1 ± 2.1**	23.4 ± 1.9**	19.± 1.7*

The number of mice ranged between 8 and 10. *$p < 0.0$and **$p < 0.01$ vs. saline-treated mice.

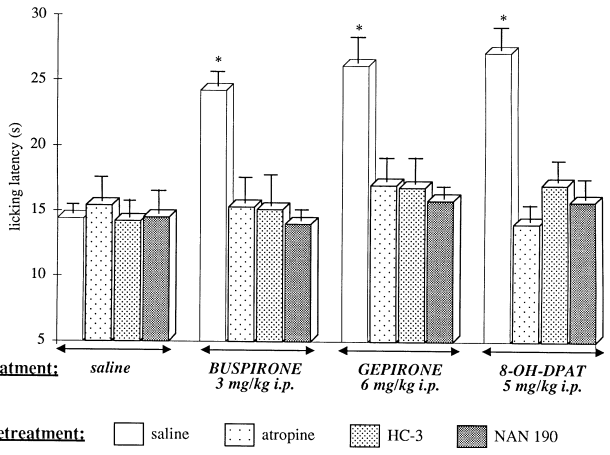

FIG. 3. Antagonism by atropine (5 mg/kg IP), HC-3 (1 μg per mouse ICV), and NAN 190 (0.5 μg per mouse ICV) of buspirone-, gepirone-, and 8-OH-DPAT-induced antinociception in the mouse hot plate test. Atropine and NAN 190 were injected 15 min before analgesic treatment, and HC-3 was injected 5 h before analgesic treatment. Licking latency values were recorded 15 min after buspirone, gepirone, or 8-OH-DPAT administration. The number of mice ranged between 8 and 12. Vertical lines represent SEM. *$p < 0.01$ vs. saline/saline-treated mice.

one and CGP 35348 used were those able to completely antagonize antinociception induced by, respectively, morphine (5 mg/kg SC) and baclofen (4 mg/kg SC). Figure 4 illustrates that doses of 0.1 mg/kg IP and 0.5 μg per mouse ICV of NAN 190 were needed to completely antagonize the antinociception induced by the 5-HT_{1A} agonist 8-OH-DPAT (5 mg/kg IP) but did not interfere in any way with morphine (5 mg/kg SC) evoked analgesia.

Finally, it should be noted that buspirone, gepirone, and 8-OH-DPAT elicited their antinociceptive effects without changing general behaviour or motor coordination as revealed by the rota-rod test, wherein the above-mentioned drugs did not increase the number of falls from the rotating rod (Table 3). Furthermore, buspirone, gepirone, and 8-OH-DPAT did not produce any modification of spontaneous motility or inspection activity as revealed by the hole board test (Fig. 5).

DISCUSSION

The 5-HT_{1A} agonists buspirone (30), gepirone (30), and 8-OH-DPAT (5) were able to induce dose-dependent antinociception in mice. The increase of the pain threshold was detected by using both thermal (hot plate test) and chemical (abdominal constriction test) stimuli. Antinociceptive doses were devoid of any other modification of animal behaviour; motor coordination, evaluated by the rota-rod test, and spontaneous motility and inspection time, evaluated by the hole board test, were not modified by buspirone, gepirone, or 8-OH-DPAT administration at analgesic doses. The 5-HT_{1A} antagonist NAN 190 (6) completely prevented buspirone, gepirone, and 8-OH-DPAT analgesia, suggesting the involvement of this 5-HT receptor subtype in their antinociceptive mechanisms. This prevention of antinociception by NAN 190 was obtained by both peripheral (IP) and central (ICV) administration, providing evidence that the site of analgesic action is located in the central nervous system (CNS). This hypothesis was confirmed by the fact that 8-OH-DPAT, when injected ICV, showed antinociceptive properties, and the intensity of this effect is comparable to that obtained after IP administration.

The antinociception produced by the 5-HT_{1A} agonists was found to be dependent on a cholinergic activation, because

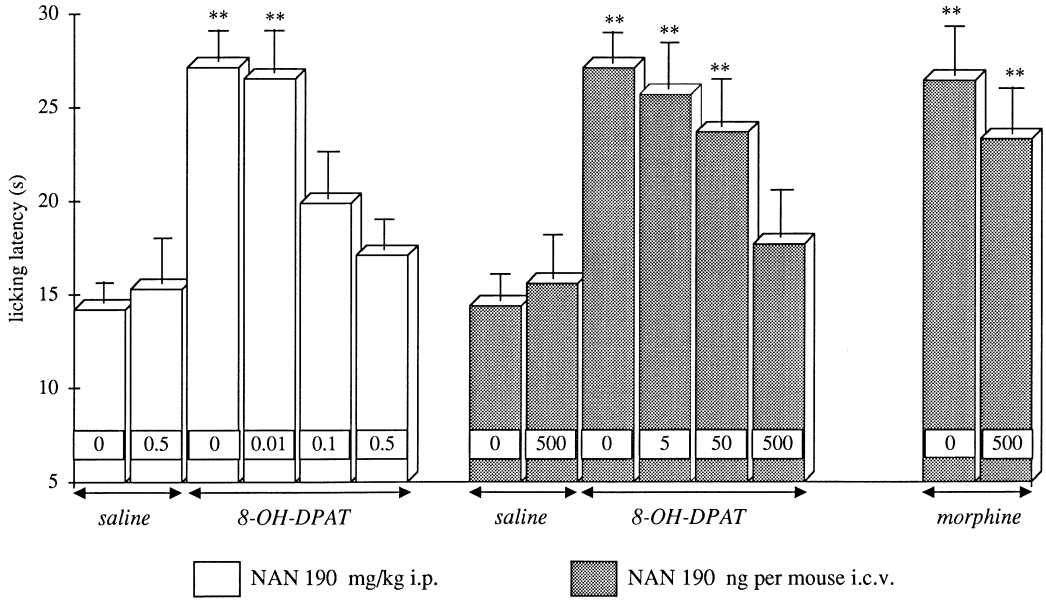

FIG. 4. Dose–response curve of NAN 190 via IP and ICV administration for antinociception induced by 8-OH-DPAT (5 mg/kg IP) in the mouse hot plate test. NAN 190 was injected 15 min before analgesic treatment. Licking latency values were recorded 15 min after 8-OH-DPAT administration. Morphine (5 mg/kg SC) was used as the nonserotoninergic reference drug. The dose of NAN 190 administered is reported in each column. The number of mice ranged between 6 and 18, with the exception of the saline/saline-treated group ($n = 31$). Vertical lines represent SEM. *$p < 0.01$ vs. saline/saline-treated mice.

TABLE 2

LACK OF EFFECT OF NALOXONE, CGP 35348, AND PTX ON BUSPIRONE-, GEPIRONE-, AND 8-OH-DPAT-INDUCED ANTINOCICEPTION IN THE MOUSE HOT PLATE TEST

		Licking Latency (s)			
			After Treatment		
Pretreatment	Treatment	Before Treatment	15 min	30 min	45 min
Saline	Saline	14.6 ± 0.6	14.4 ± 0.7	13.8 ± 0.6	14.0 ± 0.3
	Buspirone	15.0 ± 0.4	23.2 ± 1.2**	24.2 ± 0.8**	18.6 ± 0.9
	Gepirone	14.4 ± 0.5	26.1 ± 2.2**	20.1 ± 0.9**	17.7 ± 1.1
	8-OH-DPAT	13.1 ± 0.6	27.1 ± 2.0**	22.4 ± 1.1**	18.5 ± 1.1*
Naloxone	Saline	13.7 ± 0.7	12.8 ± 1.3	14.1 ± 1.7	13.8 ± 1.9
	Buspirone	15.1 ± 0.9	23.4 ± 2.4**	21.3 ± 1.8*	16.5 ± 1.0
	Gepirone	14.7 ± 0.8	24.2 ± 2.7**	18.1 ± 1.4	17.9 ± 1.4
	8-OH-DPAT	13.2 ± 0.7	27.5 ± 1.8**	21.3 ± 2.0	16.7 ± 1.3
CGP 35348	Saline	13.8 ± 0.7	14.0 ± 0.7	14.4 ± 0.8	14.8 ± 0.7
	Buspirone	14.0 ± 1.0	27.4 ± 2.1**	21.2 ± 2.1**	18.3 ± 1.6
	Gepirone	14.3 ± 1.2	28.4 ± 1.9**	24.0 ± 3.9**	22.0 ± 1.5*
	8-OH-DPAT	14.6 ± 0.9	25.4 ± 2.2**	18.3 ± 2.4	14.3 ± 1.9
PTX	Saline	13.5 ± 1.0	13.6 ± 0.8	14.1 ± 0.7	13.2 ± 0.9
	Buspirone	15.1 ± 0.6	25.7 ± 1.9**	23.4 ± 2.9**	20.2 ± 1.8*
	Gepirone	17.3 ± 0.7	23.2 ± 3.0**	22.7 ± 2.0**	20.5 ± 1.0*
	8-OH-DPAT	14.8 ± 0.5	25.8 ± 2.5**	23.2 ± 1.2**	20.1 ± 2.4*

Naloxone (1 mg/kg IP) and CGP 35348 (2.5 μg per mouse ICV) were injected 5 min before buspirone (3 mg/kg IP), gepirone (6 mg/kg IP), or 8-OH-DPAT (5 mg/kg IP), whereas PTX (0.25 μg per mouse ICV) was administered 11 days before the test. The number of mice ranged between 8 and 10, with the exception of the saline-treated group ($n = 43$). *$p < 0.05$ and **$p < 0.01$ vs saline-treated mice.

this analgesia was antagonized by the muscarinic antagonist atropine and by the ACh depletor HC-3. The prevention of buspirone, gepirone, and 8-OH-DPAT antinociception by ICV administration of HC-3 further supports the hypothesis that the analgesic site of action of these compounds is located in the CNS; moreover, the HC-3 antagonism suggests that the analgesic drugs investigated act through a presynaptic mechanism, facilitating cholinergic transmission. In agreement with this interpretation, antinociception induced by direct muscarinic agonists (e.g., McN-A-343) was not decreased after ICV administration of HC-3 (2). Furthermore, it has been demonstrated that 8-OH-DPAT, by activating serotonergic autoreceptors, is able to increase ACh release from the cerebral cortex of freely moving guinea pigs (4). The prevention of buspirone, gepirone, and 8-OH-DPAT antinociception produced by the 5-HT$_{1A}$ antagonist NAN 190 suggests, moreover, that the central serotoninergic system, through 5-HT$_{1A}$ receptors, exerts an excitatory tone on the cholinergic system. The activation of the central cholinergic system is, therefore, fundamental for buspirone, gepirone, and 8-OH-DPAT antinociception. A large difference exists between the analgesia induced in animals by buspirone, gepirone, and 8-OH-DPAT and that induced by direct muscarinic agonists and cholinesterase inhibitors. In fact, whereas 5-HT$_{1A}$ agonists produce antinociception without any visible side effect, the direct muscarinic agonists and the cholinesterase inhibitors provoke, simultaneously with the analgesic effect, a clear cholinergic symptomatology (tremors, sialorrhoea, diarrhoea, lacrimation, etc.).

TABLE 3

LACK OF IMPAIRMENT OF PERFORMANCE BY BUSPIRONE, GEPIRONE, AND 8-OH-DPAT ADMINISTRATION IN THE MOUSE ROTA-ROD TEST

		Number of Falls in 30 s			
			After Treatment		
Treatment (mg/kg IP)	n	Before Treatment	15 min	30 min	45 min
Saline	8	4.2 ± 0.4	3.1 ± 0.4	2.1 ± 0.3	0.9 ± 0.2
Buspirone (3)	8	3.9 ± 0.4	3.7 ± 0.5	3.3 ± 0.7	2.5 ± 0.7
Gepirone (6)	8	2.9 ± 0.7	1.4 ± 0.4	1.0 ± 0.2	0.9 ± 0.3
8-OH-DPAT (5)	8	3.9 ± 0.3	2.8 ± 0.3	1.3 ± 0.3	0.8 ± 0.2

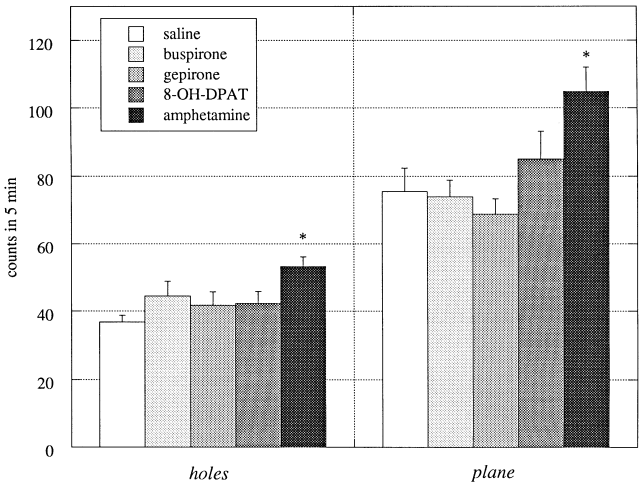

FIG. 5. Effects of buspirone (3 mg/kg IP), gepirone (6 mg/kg IP), and 8-OH-DPAT (5 mg/kg IP), in comparison with D-amphetamine (1 mg/kg SC), in the mouse hole board test. Buspirone, gepirone, 8-OH-DPAT, and D-amphetamine were injected 15 min before the test. The number of mice ranged between 7 and 10. Vertical lines represent SEM. *$p < 0.01$ vs. saline-treated mice.

The involvement of the opioid and GABAergic systems in the buspirone, gepirone, and 8-OH-DPAT analgesic mechanism of action can be ruled out because naloxone, in agreement with Giordano and Rogers (13), and the $GABA_B$ blocker CGP 35348 did not modify the antinociception induced by the three 5-HT_{1A} agonists. The doses and administration schedules of the above-mentioned drugs were ideal for selectively and completely preventing antinociception induced, respectively, by morphine (10) and the $GABA_B$ agonist baclofen (25). Giordano and Roger (13) supposed that the antinociceptive effect of buspirone in rats was related to 5-HT_{1A}-induced hypothermia through a nonopioid adrenally mediated mechanism. In our experimental conditions, this hypothesis was not confirmed. In fact, the three compounds examined, in the same range of doses, have been demonstrated to be effective not only in a thermal test (hot plate), in which hypothermia could lead to a misinterpretation of the data, but also in a chemical test (abdominal constriction), indicating that 5-HT_{1A}-induced hypothermia and antinociception were not related. The adrenergic hypothesis for the antinociception induced by 5-HT_{1A} agonists can be ruled out, because pertussis toxin, which inactivates $G_{i/o}$ proteins, at doses able to prevent noradrenaline (19) and clonidine (28) antinociception, was not effective in preventing buspirone, gepirone, or 8-OH-DPAT analgesia. Moreover, pretreatment of mice with the monoamine store depletor reserpine did not produce any antagonism of the 5-HT_{1A} agonists 8-OH-DPAT and sumatriptan (3). The doses and administration schedule of reserpine were able to prevent antinociception induced by the antidepressant drugs clomipramine and amitriptyline (7). The lack of antagonism by pretreatment with pertussis toxin further supports the hypothesis of a cholinergic mechanism underlying buspirone-, gepirone-, and 8-OH-DPAT-induced antinociception. In fact, PTX pretreatment was able to prevent opioid (27), catecholaminergic, GABAergic (19), histaminergic (8), and purinergic (29) analgesia but not muscarinic antinociception (8).

In summary, these data indicate that the 5-HT_{1A} agonists buspirone, gepirone, and 8-OH-DPAT are endowed with central antinociceptive properties by potentiating endogenous cholinergic activity.

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REFERENCES

1. Bartolini, A.; Galli, A.; Ghelardini, C.; Giotti, A.; Malcangio, M.; Malmberg-Aiello, P.; Zucchi, P. L.: Antinociception induced by systemic administration of local anaesthetics depends on a cholinergic mechanism. Br. J. Pharmacol. 92:711–721; 1987.
2. Bartolini, A.; Ghelardini, C.; Fantetti, L.; Malcangio, M.; Malmberg-Aiello, P.; Giotti, A.: Role of muscarinic receptor subtypes in central antinociception. Br. J. Pharmacol. 105:77–82; 1992.
3. Bartolini, A.; Ghelardini, C.; Figini, M.; Nicolodi, M.; Sicuteri, F.: The antimigraine drug sumatriptan induces central antinociception through a cholinergic mechanism. Can. J. Physiol. Pharmacol. (suppl. IUPHAR):409; 1994.
4. Bianchi, C.; Siniscalchi, A.; Beani, L.: 5-HT_{1A} agonists increase and 5-HT_3 agonists decrease acetylcholine efflux from the cerebral cortex of freely-moving guinea-pigs. Br. J. Pharmacol. 101: 448–452; 1990.
5. Björk, L.; Backlund Höök, B.; Nelson, D. L.; Andén, N. E.; Hacksell, U.: Resolved *N,N*-dialkylated 2-amino-8-hydroxytetralins: Stereoselective interactions with 5-HT_{1A} receptors in the brain. J. Med. Chem. 32:779–783; 1989.
6. Claustre, Y.; Rouquier, L.; Serrano, A.; Bénavidés, J.; Scatton, B.: Effect of the putative 5-HT_{1A} receptor antagonist NAN-190 on rat brain serotoninergic transmission. Eur. J. Pharmacol. 204: 71–77; 1991.
7. Galeotti, N.; Ghelardini, C.; Bartolini, A.: Involvement of the serotoninergic system in the analgesic effect of tricyclic antidepressants. Behav. Pharmacol. 6(suppl. 1):20; 1995.
8. Galeotti, N.; Ghelardini, C.; Follegatti, F.; Bartolini, A.: Effect of PTX on morphine, diphenhydramine, baclofen and physostigmine induced analgesia. Fund. Clin. Pharmacol. 10:191; 1996.
9. George, R.; Haslett, W. L.; Jendel, D. J.: The central action of metabolite of tremorine. Life Sci. 1:361–363; 1962.
10. Ghelardini, C.; Malmberg-Aiello, P.; Giotti, A.; Malcangio, M.; Bartolini, A.: Investigation into atropine-induced antinociception. Br. J. Pharmacol. 101:49–54; 1990.
11. Gillet, G.; Ammor, S.; Fillion, G.: Serotonin inhibits acetylcholine release from rat striatum slices: Evidence for a presynaptic receptor-mediated effect. J. Neurochem. 45:1687–1691; 1985.
12. Giordano, J.; Rogers, L. V.: Antinociceptive effects of the novel anxiolytic buspirone in three pain tests in rats. Pain 39:109–113; 1989.
13. Giordano, J.; Rogers, L. V.: Putative mechanisms of buspirone-induced antinociception in the rat. Pain 50:365–372; 1992.
14. Gozlan, H.; El Mestikawy, S.; Pichat, L.; Glowinski, J.; Hamon, M.: Identification of presynaptic serotonin autoreceptors using a novel ligand [^{3}H]-PAT. Nature 305:140–142; 1983.
15. Haley, T. J.; McCormick, G. L.: Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. J. Pharmacol. Chemother. 12:12–15; 1957.
16. Harris, L. S.; Dewey, W. L.; Howes, J.; Kennedy, J. S.; Pars, H.: Narcotic antagonist analgesics: Interaction with cholinergic system. J. Pharmacol. Exp. Ther. 169:17–22; 1969.
17. Hendershot, L. C.; Forsaith, J.: Antagonism of the frequency of

phenylquinone-induced writhing in the mouse by weak analgesics and nonanalgesics. J. Pharmacol. Exp. Ther. 125:237–240; 1959.
18. Herz, A.: Wirkungen des Arecolins auf das Zentralnervensystem. Arch. Exp. Pathol. Pharmakol. 242:414–429; 1962.
19. Hoehn, K.; Reid, A.; Sawynok, J.: Pertussis toxin inhibits antinociception produced by intrathecal injection of morphine, noradrenaline and baclofen. Eur. J. Pharmacol. 146:65–72; 1988.
20. Hood, D. D.; Eisenach, J. C.; Tuttle, R.: Phase I safety assessment of intrathecal neostigmine methylsulphate in humans. Anesthesiology 82:331–343; 1995.
21. Ireson, J. D.: A comparison of the antinociceptive action of cholinomimetic and morphine-like drugs. Br. J. Pharmacol. 40:92–101; 1970.
22. Koster, R.; Anderson, M.; De Beer, E. J.: Acetic acid for analgesic screening. Fed. Proc. 18:412; 1959.
23. Lenke, D.: Narkosepotenzierende und Analgetische Wirkung von 1,4-dipyrrolidino-2-butin. Exp. Pathol. Pharmacol. 234:35–45; 1958.
24. Lentz, T. L.; Liley, L.; Michaelson, U.: Some actions of anticholinergic drugs. Br. J. Pharmacol. 32:156–162; 1969.
25. Malcangio, M.; Ghelardini, C.; Giotti, A.; Malmberg-Aiello, P.; Bartolini, A.: CGP-35348, a new $GABA_B$ antagonist, prevents antinociception and muscle-relaxant effect induced by baclofen. Br. J. Pharmacol. 103:1303–1308; 1991.
26. O'Callaghan, J. P.; Holtzman, S. G.: Quantification of the analgesic activity of narcotic antagonists by a modified hot-plate procedure. J. Pharmacol. Exp. Ther. 192:497–505; 1975.
27. Parenti, M.; Tirone, F.; Giagnoni, G.; Pecora, N.; Parolaro, D.: Pertussis toxin inhibits the antinociceptive action of morphine in the rat. Eur. J. Pharmacol. 124:357–359; 1986.
28. Sanchez-Blazquez, P.; Jarzon, J.: Cholera toxin and pertussis toxin on opioid- and &agr;<inf>2-mediated supraspinal analgesia in mice. Life Sci. 48:1721–1727; 1991.
29. Sawynok, J.; Reid, A.: Role of G-proteins and adenylate cyclase in antinociception produced by intrathecal purines. Eur. J. Pharmacol. 156:25–34; 1988.
30. Traber, J.; Glaser, T.: 5-HT-1A receptor-related anxiolytics. Trends Pharmacol. Sci. 8:432–437; 1987.
31. Vaught, J.; Pelley, K.; Costa, L. G.; Sether, P.; Enna, S. J.: A comparison of the antinociceptive responses to GABA-receptor agonists THIP and baclofen. Neuropharmacology 24:211–216; 1985.