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Role of 5-HT_4 Receptors in the Mouse Passive Avoidance Test[1]

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ABSTRACT

The effects of the administration of different 5-HT_4 receptor antagonists (SDZ 205557, GR 125487) and 5-HT_4 receptor agonists (BIMU 1, BIMU 8) on memory processes were evaluated in the mouse passive avoidance test. The administration of SDZ 205557 (10 mg kg^{-1} i.p.) and GR 125487 (10 mg kg^{-1} i.p.) immediately after termination of the training session produced an amnesic effect. BIMU 1 (20 mg kg^{-1} i.p.) and BIMU 8 (30 mg kg^{-1} i.p.), administered 20 min before the training session, prevented the 5-HT_4 receptor antagonist-induced amnesia. In the same experimental conditions BIMU 1 (10 mg kg^{-1} i.p.; 25 μg/mouse intracerebroventricularly) and BIMU 8 (30 mg kg^{-1} i.p.; 30 μg per mouse intracerebroventricularly) prevented scopolamine (1 mg kg^{-1} i.p.) and dicyclomine (2 mg kg^{-1} i.p.) amnesia and, at the dose of 10 and 30 mg kg^{-1} i.p. respectively, prevented amnesia induced by exposure to a hypoxic environment. At the highest effective doses, none of the drugs impaired motor coordination, as revealed by the rota rod test, or modified spontaneous motility and inspection activity, as revealed by the hole board and Animex tests. The 5-HT_3 antagonist ondansetron (0.1–1 mg kg^{-1} i.p.) was unable to prevent scopolamine-, 5-HT_4 antagonist- and hypoxia-induced amnesia. These results suggest that the modulation of 5-HT_4 receptors plays an important role in the regulation of memory processes. On these bases, the 5-HT_4 receptor agonists could be useful in the treatment of cognitive deficits although 5-HT_4 receptor antagonists may represent pharmacological tools for investigation of new potential antiamnesic drugs.

The regulation of synaptic plasticity, which is fundamental in learning and memory, involves long-term modulation of ion-channel activity. Stimulation of 5-HT_4 receptors may initiate this process by activation of adenylate cyclase. It has been reported that stimulation of 5-HT_4 receptors increases intracellular cyclic AMP levels in mouse colliculi neurones (Dumuis *et al.*, 1988), guinea pig hippocampus (Bockaert *et al.*, 1990) and human prefrontal cortex (Monferini *et al.*, 1993) which then modulates the activity of protein kinase A (Fagni *et al.*, 1992; Ansanay *et al.*, 1995). In rat hippocampus and in mouse colliculi neurones, activation of protein kinase A closes potassium channels. Therefore, reduced after-hyperpolarization may increase neuronal excitability and ultimately neurotransmitter release (Ansanay *et al.*, 1995). Even short exposures (1–2 sec) to 5-HT_4 receptor agonists induce long-lasting (2 hr) inhibition of inward potassium currents.

Early studies demonstrated the ability of metoclopramide, a weak 5-HT_4 receptor agonist, to prevent dicyclomine-induced amnesia in the mouse passive avoidance test (Galeotti *et al.*, 1993). Fontana *et al.* (1994), using RS66331, a 5-HT_3 receptor antagonist and 5-HT_4 receptor agonist, have reported ameliorative effects in the rat spatial memory test in which this compound reverted performance deficit produced by atropine as well as cognitive impairment of aged rats when given alone. This findings are corroborated by experiments using more selective 5-HT_4 receptor agonists. Administration of RS67333, a highly selective 5-HT_4 receptor agonist, suppresses the rat performance deficit induced by atropine in the Morris water maze, an effect also reversed by the selective 5-HT_4 receptor antagonist RS 67532 (Fontana *et al.*, 1997). Furthermore, (R)-zacopride, a weak 5-HT_4 receptor agonist with low affinity for 5-HT_3 receptors, exerts marked procognitive activity (Barnes *et al.*, 1990). However, the mechanism underlying this effect of (R)-zacopride is not completely clear, considering the lack of effect of the more selective 5-HT_4 receptor agonist (S)-zacopride (Barnes *et al.*, 1990).

BIMU 1 and BIMU 8 are two benzimidazolones endowed with 5-HT_4 receptor agonistic and 5-HT_3 receptor antagonis-

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ABBREVIATIONS: i.c.v., intracerebroventricular; BIMU 1, (endo-N-(8-methyl-8-azabicyclo[3.2.1]-oct-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazol-1 carboxamide hydrochloride); BIMU 8, (endo-N-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1H-benzimidazol-1 carboxamide hydrochloride); SDZ 205557, (2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino) ethyl ester hydrochloride); GR 125487, [1-[2(methylsufonyl)amino]ethyl]-4-piperidinyl] methyl-5-fluoro-2-methoxy-1H-indole-3-carboxylate hydrochloride; 5-HT, 5-hydroxytryptamine.

tic properties (Dumuis *et al.*, 1989, 1991; Turconi *et al.*, 1990, 1991). Recent findings suggested that these compounds have also affinity for σ_2 receptors (Bonhaus *et al.*, 1994; Weatherspoon *et al.*, 1997). BIMU 1 showed about 1000-fold lower affinity for other serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}), dopamine (D_1 and D_2) and muscarinic (M_1, M_2, M_3 and M_4) receptors (Bonhaus *et al.*, 1993). Furthermore, the compounds labeled SDZ 205557 and GR 125487 have been reported as selective 5-HT_4 receptor antagonists (Buchheit *et al.*, 1991; Gale *et al.*, 1994). By using both the above-mentioned 5-HT_4 receptor agonists (BIMU 1 and BIMU 8) and antagonists (SDZ 205557 and GR 125487), the role of the 5-HT_4 receptors in the modulation of learning and memory processes was investigated in different passive avoidance paradigms.

Methods

Animals. Male Swiss albino mice (23–30 g) from Morini (San Polo d'Enza, Italy) were used. The mice were housed 15 per cage. The cages were placed in the experimental room 24 hr before the test for adaptation. The animals were fed a standard laboratory diet and tap water *ad libitum* and kept at 23 ± 1°C with a 12-hr 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.

Passive-avoidance test. The test was performed according to the step-through method described by Jarvik and Kopp (1967). The apparatus consists of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. Mice, as soon as they entered the dark compartment, received a punishing electrical shock (0.5 mA, 1 sec). The latency times for entering the dark compartment were measured in the training test and after 24 hr in the retention test. For memory disruption, mice were either exposed to a hypoxic environment (5%O_2 in water-saturated nitrogen) for 8 min up to 30 sec before passive avoidance training or i.p. injected with amnesic drugs. BIMU 1, BIMU 8, ondansetron, physostigmine and piracetam were injected 20 min before the training session while scopolamine, dicyclomine, GR 125487 and SDZ 205557 were injected immediately after termination of the training session. The maximum entry latency allowed in the retention session was 120 sec. The deficit in passive avoidance performance was expressed as the difference (in seconds) between retention and training latencies.

Hole board test. The hole board test uses a 40 cm square plane with 16 flush-mounted cylindrical holes (diameter 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 four 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.

Rota rod test. The apparatus consists of a base platform and a rotating rod of 3-cm diameter with a non-skid surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus up to five mice were tested simultaneously on the apparatus, with a rod-rotation 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 sec, according to Vaught *et al.* (1985). Performance time was measured before and 15, 30 and 45 min after s.c. administration of the drugs.

Spontaneous activity meter (Animex). Locomotor activity in mice was quantified using an Animex activity meter Type S (LKB, Farad, Sweden) set to maximum sensitivity. Mice were placed on the top of the Animex activity meter and each movement produced a signal due to variation in inductance and capacity of the apparatus resonance circuit. These signals were automatically converted to numbers. On the day of the experiment the mice were treated and then the cage, containing five mice, was put on the measurement platform. Activity counts were made every 15 min for 45 min starting immediately after injection of the drug. Because of the arbitrary scale adopted to quantify movements, drug-treated mice were always compared with saline-treated ones.

i.c.v. injection technique. i.c.v. administration was performed under ether anesthesia with isotonic saline as a solvent, according to the method described by Haley and McCormick (1957). During anesthesia, mice were grasped firmly by the loose skin behind the head. A hypodermic needle (0.4-mm external diameter) attached to a 10-μl syringe was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse, where 5 μl of drug were then administered. The injection site was 1 mm to the right or left from the midpoint on a line drawn through to the anterior base of the ears. Injections were performed randomly into the right or left ventricle. To ascertain that the drugs were administered exactly into the cerebral ventricle, some mice were injected with 5 μl of 1:10 diluted India ink and their brains were examined macroscopically after sectioning. The accuracy of the injection technique was evaluated and the percentage of correct injections was 95.

Drugs. The following drugs were used: BIMU 1, BIMU 8, GR 125487, ondansetron (Boehringer Ingelheim, Milan, Italy), SDZ 205557 prepared in the Department of Pharmaceutical Sciences of University of the Florence according to the method described by Romanelli *et al.* (1993); D-amphetamine (De Angeli, Florence, Italy); scopolamine hydrobromide, physostigmine hemisulphate, piracetam (Sigma); dicyclomine hydrochloride (Le Petit).

Drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use. Drug concentrations were prepared so that the necessary dose could be administered in a volume of 5 μl/mouse by i.c.v. injection and 10 ml kg^{-1} by i.p. injection.

Statistical analysis. All experimental results are given as the means ± S.E.M. Analysis of variance, followed by Fisher's protected least significant difference procedure for *post hoc* comparison, was used to verify significance between two means. Data were analysed with the StatView software for the Macintosh (1992). $P < .05$ were considered significant.

Results

Amnesic effect of 5-HT_4 antagonists. The dose response curves for SDZ 205557 (1–20 mg kg^{-1} i.p.) and GR 125487 (0.1–20 mg kg^{-1} i.p.) in the mouse passive avoidance test are reported in figure 1. The two compounds, injected immediately after the training session, produced deficits in passive-avoidance behavior. This effect was dose-dependent and statistical significance was reached at the dose of 10 mg kg^{-1} i.p. Higher doses of SDZ 205557 and GR 125487 were not investigated because the doses of 10 and 20 mg kg^{-1} i.p. produced the same degree of behavioral impairment. The maximum amnesic effect obtained was of the same intensity of that produced by scopolamine (1 mg kg^{-1} i.p.) and dicyclomine (2 mg kg^{-1} i.p.), used as reference drugs (fig. 1).

Prevention of deficits in passive avoidance behavior by 5-HT_4 agonists. The deficits in passive avoidance behavior induced by the 5-HT_4 antagonists SDZ 205557 (10 mg kg^{-1} i.p.) and GR 125487 (10 mg kg^{-1} i.p.) was prevented, in the mouse passive avoidance test, by pretreatment with the 5-HT_4 agonists BIMU 1 (20 mg kg^{-1} i.p.) and BIMU 8 (30 mg kg^{-1} i.p.), injected 20 min before the training session. Both 5-HT_4 agonists enhanced the entrance latency up to a value comparable to that produced by control animals (fig. 2).

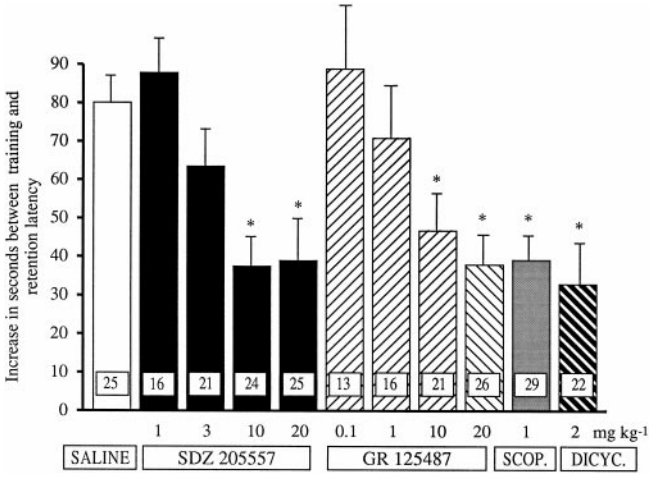

Fig. 1. Effect of SDZ 205557 and GR 125487 in comparison with scopolamine (scop.) and dicyclomine (dicyc.) in mouse passive avoidance test. All drugs were injected i.p. immediately after punishment. Inside the column is the number of mice. *P < .01 in comparison with saline-treated mice.

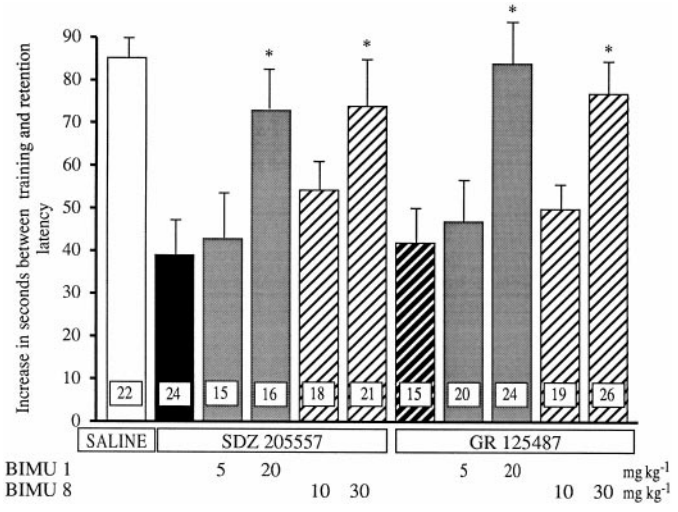

Fig. 2. Effect of BIMU 1 and BIMU 8 on amnesia induced by both SDZ 205557 (10 mg kg^{-1} i.p.) and GR 125487 (10 mg kg^{-1} i.p.) in mouse passive-avoidance test. BIMU 1 and BIMU 8 were administered 20 min before training session although SDZ 205557 and GR 125487 were injected immediately after punishment. All drugs were injected i.p. Inside the column is the number of mice. *P < .01 in comparison with mice treated with SDZ 205557 or GR 125487.

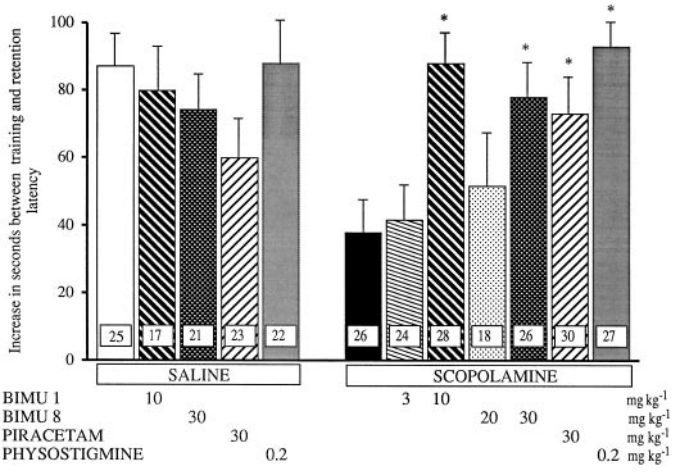

Fig. 3. Effect of BIMU 1 and BIMU 8 in comparison with both piracetam and physostigmine on amnesia induced by scopolamine (1 mg kg^{-1} i.p.) in the mouse passive avoidance test. BIMU 1, BIMU 8, piracetam and physostigmine were administered 20 min before training session although scopolamine was injected immediately after punishment. All drugs were injected i.p. Inside the column is the number of mice. *P < .01 in comparison with mice treated with scopolamine.

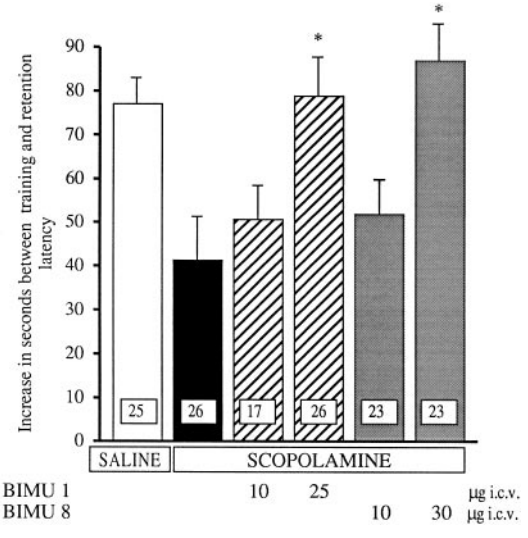

Fig. 4. Effect of BIMU 1 and BIMU 8 administered i.c.v. on amnesia induced by scopolamine (1 mg kg^{-1} i.p.) in the mouse passive avoidance test. BIMU 1 and BIMU 8 were administered 20 min before training session. Inside the column is the number of mice. *P < .01 in comparison with mice treated with scopolamine.

BIMU 1 and BIMU 8, at 5 and 10 mg kg^{-1} i.p. respectively, were completely ineffective (fig. 2).

BIMU 1 (10 mg kg^{-1} i.p.; 25 μg/mouse i.c.v.) and BIMU 8 (30 mg kg^{-1} i.p.; 30 μg/mouse i.c.v.) were also able to completely prevent scopolamine (1 mg kg^{-1} i.p.; figs. 3 and 4) and dicyclomine (2 mg kg^{-1} i.p.; fig. 5) induced deficits in passive avoidance behavior. The doses of 3 mg kg^{-1} i.p. and 10 μg i.c.v. BIMU 1 and the doses of 20 mg kg^{-1} i.p. and 10 μg i.c.v. BIMU 8 were unable to protect against scopolamine-induced (figs. 3 and 4) and dicyclomine-induced deficits in passive avoidance behavior (fig. 5).

Exposure to a hypoxic environment produced deficits in passive avoidance behavior that were completely prevented by pretreatment with the two 5-HT$_4$ agonists BIMU 1 and BIMU 8 at the doses of 10 and 30 mg kg^{-1} i.p. respectively (fig. 6).

The antiamnesic effect produced by i.p. injection of BIMU 1 and BIMU 8 was comparable to that produced by the well known nootropic drug piracetam (30 mg kg^{-1} i.p.) and the cholinesterase inhibitor physostigmine (0.2 mg kg^{-1} i.p.) as illustrated in figures 3, 5 and 6.

Doses of BIMU 1 and BIMU 8 higher than 10 and 30 mg kg^{-1} i.p., respectively, were not investigated because a complete prevention of scopolamine-, dicyclomine- and hypoxia-induced deficits in passive avoidance behavior used was already reached.

BIMU 1 and BIMU 8, when given alone, at the highest doses used, had no effect on mouse passive avoidance test in comparison with saline-treated mice (fig. 3). No statistically significant difference among the entrance latencies for each compound tested in the training session of the passive avoidance test was observed (data not shown). Table 1 reports the

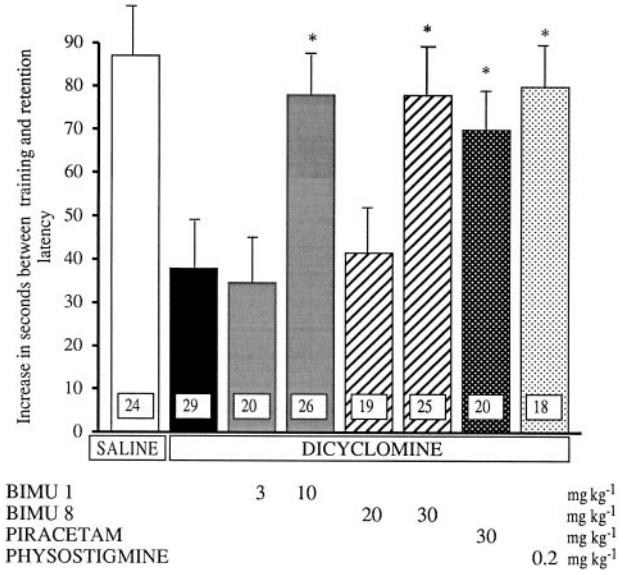

Fig. 5. Effect of BIMU 1 and BIMU 8 in comparison with both piracetam and physostigmine on amnesia induced by dicyclomine (2 mg kg^{-1} i.p.) in the mouse passive avoidance test. BIMU 1, BIMU 8, piracetam and physostigmine were administered 20 min before training session although dicyclomine was injected immediately after punishment. All drugs were injected i.p. Inside the column is the number of mice. *P < .01 in comparison with mice treated with dicyclomine.

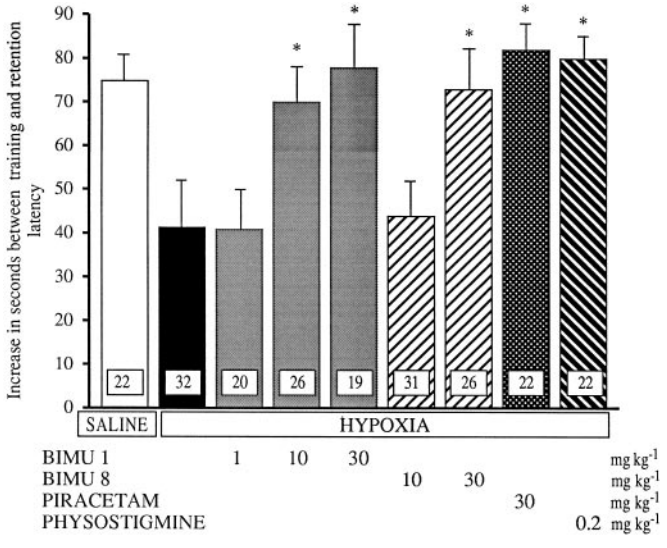

Fig. 6. Effect of BIMU 1 and BIMU 8 in comparison with both piracetam and physostigmine on amnesia induced by hypoxia (O_2 5% + N_2 95%) in the mouse passive avoidance test. BIMU 1, BIMU 8, piracetam and physostigmine were administered 20 min before training. Mice were submitted to hypoxia immediately before punishing. All drugs were injected i.p. Inside the column is the number of mice. *P < .01 in comparison with mice submitted to hypoxia.

entrance latency values in the training and retention sessions for BIMU 1, BIMU 8, SDZ 205557 and GR 125487 at the highest effective doses, taken as an example.

Lack of effect by the 5-HT_3 antagonist ondansetron. Ondansetron, up to the dose of 1 mg kg^{-1} i.p., was not able to prevent scopolamine (1 mg kg^{-1} i.p.), SDZ 205557 (10 mg kg^{-1} i.p.) and hypoxia-induced deficits in passive avoidance behavior (fig. 7).

Effect of 5-HT_4 agonists and antagonists on mouse rota rod, Animex and hole board tests. It should be noted

TABLE 1
Effect of BIMU 1, BIMU 8, SDZ 205557 and GR 125487 in the mouse passive avoidance test

Pretreatment (mg kg^{-1} i.p.)	Treatment (mg kg^{-1} i.p.)	Latency To Enter The Dark Compartment(s)	
		Training	Retention
Saline	Saline	18.4 ± 2.3	98.8 ± 7.5
BIMU 1 10	Saline	16.9 ± 2.1	95.9 ± 7.3
BIMU 8 30	Saline	18.1 ± 1.9	93.0 ± 8.5
Piracetam 30	Saline	19.2 ± 2.0	86.9 ± 8.2
Physostigmine 0.2	Saline	17.9 ± 2.2	99.7 ± 7.9
Saline	SDZ 205557 10	18.7 ± 3.1	55.8 ± 9.2[a]
Saline	GR 125487 20	20.3 ± 2.9	58.1 ± 8.1[a]
Saline	Scopolamine 1	19.7 ± 2.6	57.5 ± 8.3[a]
Saline	Dicyclomine 2	19.8 ± 1.5	53.1 ± 9.4[a]

Pretreatment and treatment drugs were administered, respectively, 20 min before and immediately after the training session. The number of mice ranged between 18 and 25.
[a] P < .01 *vs.* saline-treated mice.

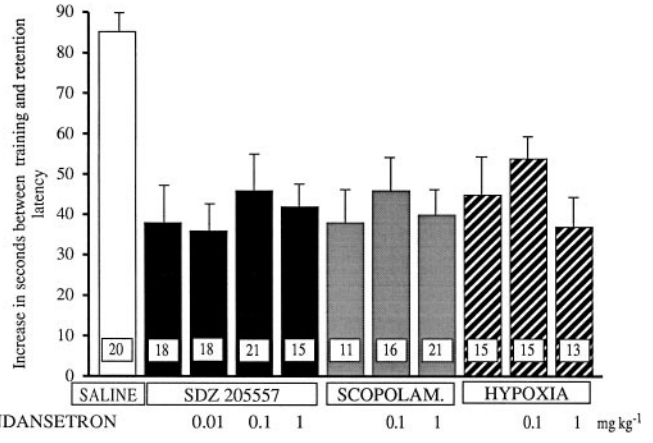

Fig. 7. Effect of ondansetron on amnesia induced by SDZ 205557 (10 mg kg^{-1} i.p.) scopolamine (1 mg kg^{-1} i.p.) and hypoxia (O_2 5% + N_2 95%) in mouse passive-avoidance test. Ondansetron was administered 20 min before training session although SDZ 205557 and scopolamine were injected immediately after punishment. Mice were submitted to hypoxia immediately before punishing. All drugs were injected i.p. Inside the column is the number of mice.

that the 5-HT_4 agonists and antagonists we investigated elicited their modulatory effect on cognitive processes without changing either gross behavior or motor coordination as revealed by the rota rod test (fig. 8). None of the drugs, administered at the highest active doses, increased the number of falls from the rotating rod in comparison with saline- and vehicle-treated mice (fig. 8). The number of falls in the rota rod test progressively decreased because mice learned how to balance on the rotating rod.

The spontaneous motility and inspection activity of mice was unmodified by administration of the above-mentioned 5-HT_4 modulators as revealed by the Animex (fig. 9) and hole board (fig. 10) tests in comparison with saline-treated mice. In the hole board test D-amphetamine (6 μg/mouse i.c.v.), used as a reference drug, increased both parameters evaluated. Because both i.p. and i.c.v. administration of BIMU 1 and BIMU 8 prevented deficits in passive avoidance behavior, the effect of the two 5-HT_4 agonists on mouse locomotor activity was evaluated after i.p. (Animex, fig. 9) and i.c.v. (hole board, fig. 10) injection.

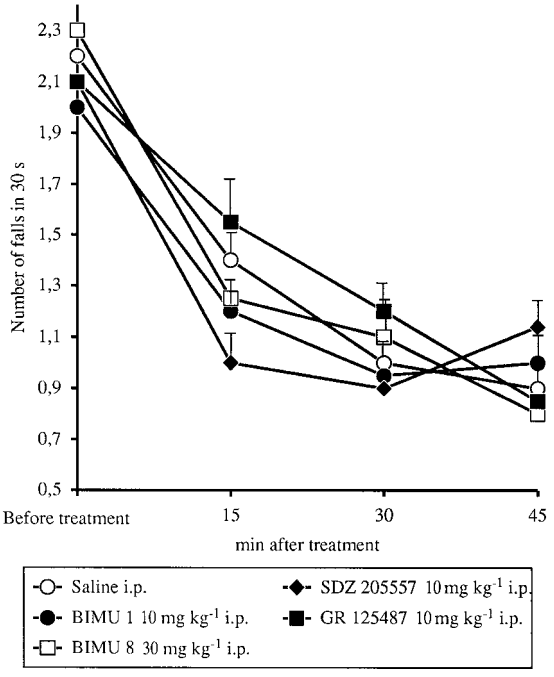

Fig. 8. Lack of effect of BIMU 1, BIMU 8, SDZ 205557 and GR 125487 on mouse rota rod test. *P < .01 in comparison with saline controls. Each point represents the mean of 10 mice.

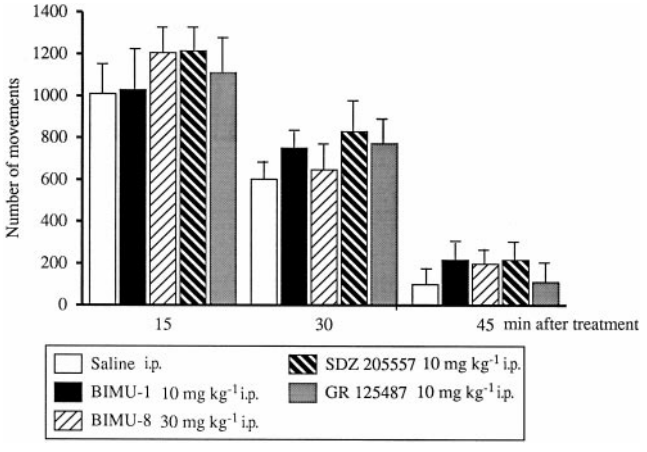

Fig. 9. Lack of effect by BIMU 1, BIMU 8, SDZ 205557 and GR 125487 in mouse Animex test. Each column represents the mean of 15 mice.

Discussion

5-HT$_4$ receptors appear to be involved in the regulation of cognitive processes in mice. Our results demonstrate that the administration of 5-HT$_4$ receptor antagonists (SDZ 205557, GR 125487) provoke amnesia in the mouse passive avoidance test of severity comparable to that induced by the amnesic drugs scopolamine and dicyclomine. The 5-HT$_4$ receptor agonists (BIMU 1, BIMU 8) are able to prevent the amnesia induced not only by 5-HT$_4$ receptor antagonists, but also by antimuscarinic drugs and exposure to a hypoxic environment. Previous data, demonstrating the amelioration in rats of social olfactory memory (Letty *et al.*, 1997) and associative memory (Marchetti-Gauthier *et al.*, 1997) produced by the 5-HT$_4$ agonist BIMU 1, confirmed our results.

BIMU 1 and BIMU 8 have been reported to be endowed also with 5-HT$_3$ receptor antagonistic activity (Turconi *et al.*, 1990, 1991). However, their procognitive effect seems unrelated to the blockade of 5-HT$_3$ receptors because ondansetron, a highly selective 5-HT$_3$ receptor antagonist (Turconi *et al.*, 1991), did not exert any effect on hypoxia-, scopolamine- and 5-HT$_4$ antagonists-induced amnesia. The lack of improvement of social olfactory memory in rats by ondansetron (Letty *et al.*, 1997) confirms the hypothesis that 5-HT$_3$ receptors are not involved in the regulation of memory processes.

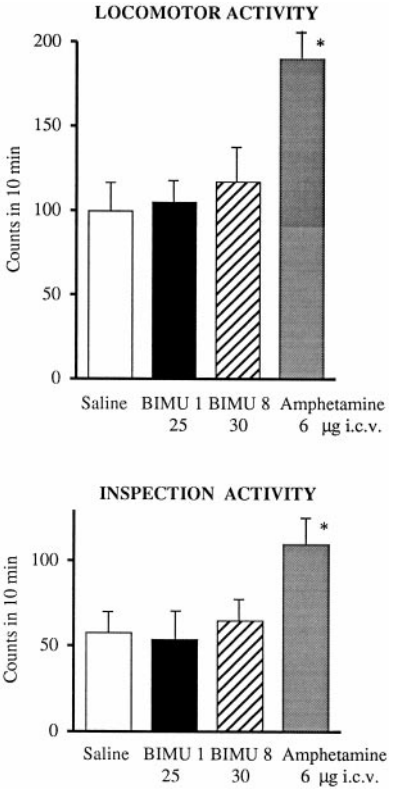

Fig. 10. Lack of effect of BIMU 1 and BIMU 8 administered i.c.v. in comparison with amphetamine in the mouse hole board test. Test was performed between 15 and 20 min after administration. Each column represents the mean of 10 mice. *P < .01 in comparison with saline controls.

5-HT$_4$ receptors have a restricted distribution in guinea pig and human brains, suggesting that they have specific functions and indicating that they play a role in neuronal excitability and neurotransmitter release. Rat interpeduncular nucleus, thalamus and hippocampus contain a high degree of 5-HT$_4$ receptors (Waeber *et al.*, 1993). At the hippocampal level the distribution is clearly laminar along the pyramidal cell layers CA1, CA2, CA3 and dentate gyrus (Waeber *et al.*, 1993). In the human brain 5-HT$_4$ receptors have been observed in the frontal cortex, superior colliculi, limbic structures and basal ganglia (Waeber *et al.*, 1993; Grossman *et al.*, 1993). A high density of 5-HT$_4$ receptors have been observed especially in the nigro-striatal pathway and hippocampus (Waeber *et al.*, 1993). The location of 5-HT$_4$ receptors in the hippocampus from several species is also consistent with a role for the receptor in cognitive processes (Bockaert *et al.*, 1994a; Letty *et al.*, 1997; Marchetti-Gautier *et al.*, 1997; Fontana *et al.*, 1997). 5-HT$_4$ receptor activation has been found to inhibit after-hyperpolarization in rat CA1 hippocampal neurones and such effects may contribute to the induction of long-term potentiation, an elementary biochemical and cellular process for learning and memory (Andrade

and Chaput, 1991). In mouse colliculi neurones, moreover, 5-HT_4 receptor agonism elevates intracellular adenylyl cyclase and, consequently, inhibits voltage-sensitive potassium channel opening time (Dumuis *et al.*, 1989; Andrade and Chaput, 1991; Bockaert *et al.*, 1994a, b). Prolonged closure of potassium channels, and thus neuronal hyperexcitability, was seen even after very short exposure to 5-HT (Bockaert *et al.*, 1994b). These mechanisms may be involved in the induction of hippocampal CA1 late stage long-term potentiation (Frey *et al.*, 1993), a potential mechanism for explicit forms of memory. Furthermore, a marked loss of 5-HT_4 receptors, labeled with [^{3}H]-GR 113808, in hippocampal and cortical regions in the brains of patients with Alzheimer's disease (Reynolds *et al.*, 1995) supports the hypothesis of a role for 5-HT_4 receptors in memory processes.

An *in vivo* study indicated that i.c.v. injection of zacopride and renzapride, two 5-HT_4 agonists, increased the energy of low frequency hippocampal theta rhythm and other frequency bands (Boddeke and Kalkman, 1990). These effects were blockaded by scopolamine, suggesting a cholinergic step in the effects of 5-HT_4 agonists. The involvement of the cholinergic system in the central effects of 5-HT_4 agonists was confirmed by rat microdialysis studies in which an increase in ACh extracellular levels was observed after BIMU 1 and BIMU 8 administration (Consolo *et al.*, 1994). Furthermore, Ghelardini *et al.* (1996) demonstrated that the antinociceptive effect of BIMU 1 and BIMU 8 is mediated via activation of cholinergic neurotransmission. Because it has long been known that the stimulation of the cholinergic system improves cognitive processes (Coyle, 1995) the facilitatory effect induced by the 5-HT_4 agonists BIMU 1 and BIMU 8 could be due, at least in part, to activation of the cholinergic system.

BIMU 1 and BIMU 8 exerted their antiamnesic effect by acting centrally. It was possible to reach the same intensity of analgesia by injecting directly into the cerebral ventricles doses (25–30 μg/mouse) of BIMU 1 and BIMU 8 which were much lower than those needed parenterally. This finding excludes the possibility that the antiamnesic action can depend on retrodiffusion of the two 5-HT_4 agonists from the cerebral ventricles to the periphery.

The 5-HT_4 receptor agonists BIMU 1 and BIMU 8 have been reported to be endowed with analgesic properties (Ghelardini *et al.*, 1996). In our experimental conditions these compounds were administered before receiving the punishing stimulus in correspondence with their maximum antinociceptive activity. It is, however, unlikely that their analgesic effect may have influenced the results obtained. Analgesic drugs, by reducing the perception of the punishing stimulus (electric shock), may produce a false amnesic effect. BIMU 1 and BIMU 8, even at the highest doses used, were always able to prevent amnesia indicating that the degree of antinociception produced was insufficient to reduce the perception of the electric shock applied.

The 5-HT_4 receptor agonists and antagonists, at the highest doses used, did not modify the animals' gross behavior. Nor did these compounds impair motor coordination as revealed by the rota rod test or modify locomotor and inspection activity as indicated by the hole board and Animex tests. We can, thus, suppose that the effects produced by 5-HT_4 receptor modulators were not imputable to compromised viability. Higher doses of all compounds were not investigated because the maximum amnesic (SDZ 205557, GR 125487) and antiamnesic (BIMU 1, BIMU 8) effect was already reached.

In conclusion, these results indicate the important role played by 5-HT_4 receptors in the regulation of memory processes. On these bases, the 5-HT_4 receptor agonists could be useful in the treatment of cognitive deficits although 5-HT_4 receptor antagonists may represent pharmacological tools for investigation of new potential antiamnesic drugs.

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