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Oral administration of the 5-HT_6 receptor antagonists SB-357134 and SB-399885 improves memory formation in an autoshaping learning task

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Abstract

In this work we aimed to re-examine the 5-HT_6 receptor role, by testing the selective antagonists SB-357134 (1–30 mg/kg p.o.) and SB-399885 (1–30 mg/kg p.o.) during memory consolidation of conditioned responses (CR%), in an autoshaping Pavlovian/instrumental learning task. Bioavailability, half-life and minimum effective dose to induce inappetence for SB-357134 were 65%, 3.4 h, and 30 mg/kg p.o., and for SB-399885 were 52%, 2.2 h, and 50 mg/kg p.o., respectively. Oral acute and chronic administration of either SB-357134 or SB-399885 improved memory consolidation compared to control groups. Acute administration of SB-357134, at 1, 3, 10 and 30 mg/kg, produced a CR% inverted-*U* curve, eliciting the latter dose a 7-fold increase *relative* to saline group. Acute injection of SB-399885 produced significant CR% increments, being 1 mg/kg the most effective dose. Repeated administration (7 days) of either SB-357134 (10 mg/kg) or SB-399885 (1 mg/kg) elicited the most significant CR% increments. Moreover, modeling the potential therapeutic benefits of 5-HT_6 receptor blockade, acute or repeated administration of SB-399885, at 10 mg/kg reversed memory deficits produced by scopolamine or dizocilpine, and SB-357134 (3 and 10 mg/kg) prevented amnesia and even improved performance. These data support the notion that endogenously 5-HT acting, via 5-HT_6 receptor, improves memory *consolidation*.

Keywords: Memory formation; Serotonin; Receptors; 5-HT_6 receptors; Autoshaping; Rats

1. Introduction

A growing body of evidence from snails to humans indicates that serotonin (5-hydroxytryptamine, 5-HT) system participates in memory formation. It remains unclear if this 5-HT influence is either tonic or phasic, and involves different 5-HT receptors (for reviews see Graves et al., 2003; Roth et al., 2003; Bockaert et al., 2004; Costall and Naylor, 2004; Lanfumey and Hamon, 2004; Leysen, 2004; Terry, 2004; Thomas and Hagan, 2004; Woolley et al., 2004) and/or multiple cell signaling pathways (Raymond et al., 2001). Actually, compromised serotonergic function may have an important contribution to cognitive decline related to aging, Alzheimer's disease (AD) and schizophrenia. Thus, serotonergic system became a potential target for treatment of memory dysfunctions (Meneses, 1999, 2003; Roth et al., 2003), and opens opportunities for the exploration of 5-HT agonists, antagonists, inverse agonists and agonists/antagonists (see e.g., Millan et al., 2004). In this regard, recent reviews provide further support to the notion that, 5-HT_1 to 5-HT_4 and 5-HT_6 and 5-HT_7 receptors may be useful in the treatment of cognitive dysfunctions (Graves et al., 2003; Roth et al., 2003; Bockaert et al., 2004; Costall and Naylor, 2004; Lanfumey and Hamon, 2004; Leysen, 2004; Terry, 2004; Thomas and Hagan, 2004; Woolley et al., 2004). Actually, these publications also allow highlighting the fact that investigation of 5-HT system in learning and memory has been greatly benefited from the identification, classification and cloning of multiple receptors and development of selective

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compounds (Hoyer et al., 1994, 2002). For instance, the discovery of novel 5-HT_6 and 5-HT_7 receptors represents an important area for the study of cognitive impairment associated to amnesia, AD, and schizophrenia (see e.g., Meneses, 2001b; Roth et al., 2003). Regarding cognition and 5-HT_6 receptors function, we have reported (Meneses, 2001a) that the 5-HT_6 receptor antagonist Ro 04-6790 administration improved memory formation in an autoshaping learning task. Amnesic effects of scopolamine or dizocilpine were also reversed by Ro 04-6790 (Meneses, 2001a). That this Ro 04-6790 facilitatory effect was specifically related to 5-HT_6 receptors, is supported by the fact that such effect was unaltered by the blockade of 5-HT_{1A}, 5-$HT_{2A/2C}$, 5-HT_3 or 5-HT_4 receptors (Meneses, 2001a,b). In addition, the amnesia induced by scopolamine, dizocilpine or the 5-HT agonist/antagonist mCPP was completely reversed or partially antagonized by Ro 04-6790 or unaffected, respectively. It should be *noted* that the muscarinic cholinergic antagonist scopolamine and the non-competitive NMDA glutamatergic antagonist dizocilpine *have been used* for modeling memory impairment (see e.g., Berger-Sweeney et al., 2004; Meneses, 2003; Santucci and Haroutunian, 2004). Inhibiting glutamatergic activity may be considered to have a general depressive effect on the nervous system. The direct contributions of the cholinergic system to memory function have been explored extensively, in particular the roles of muscarinic cholinergic receptors (e.g., blocked by scopolamine) in memory consolidation, and the facilitation of memory consolidation by muscarinic agonists (e.g., oxotremorine) (McGaugh et al., 2002, 2003). Available evidence indicates a growing interest regarding the notion that, 5-HT_6 receptors may improve normal memory or normalized dysfunctional memory (Rogers and Hagan, 2001; Stean et al., 2002; Foley et al., 2004; Woolley et al., 2003; for recent review see Woolley et al., 2004; however, see Russell and Dias, 2003). In this regard, using an autoshaping test Szczepanski et al. (2002) and Linder et al. (2003) have recently attempted to replicate our facilitatory effects of memory consolidation induced by 5-HT_6 receptor antagonist Ro 04-6790 in normal or scopolamine-amnesic rats (Meneses, 2001b). In an abstract form (kindly provided by R. Schreiber) Szczepanski et al. (2002) found that as training days passed, performance improved in control and treated groups, comparison between control and treated groups revealed that the 5-HT_6 receptor antagonist, Ro4368554 enhanced autoshaping learning (active dose: 3 mg/kg i.p.). According to these authors, it appears to enhance learning and memory processes, particularly in disease models (i.e., scopolamine-treated rats) and the procognitive effects of 5-HT_6 receptor antagonists may be modulated through cholinergic neurotransmission. In contrast, Linder et al. (2003) did not find evidence of improved acquisition or retention with Ro 04-6790 in the autoshaping task, even with repeated testing no effects were detected for Ro 04-6790, either in normal or scopolamine-induced deficits. Importantly, both Szczepanski et al. (2002) and Linder et al. (2003) groups contacted us, but it is not clear what the critical differences are mainly between Linder et al. group, and our previous findings (Meneses, 2001a). It is noteworthy that, using other behavioral tasks contradictory evidence about 5-HT system involvement in normal and impaired mnemonic processes in mammals has been reported (see Meneses, 1999; Schechter et al., 2002; Roth et al., 2003, for references), which could be attributable to methodological differences respect to timing and sites (systemic or central) of administration, behavioral test and drugs used (see Meneses, 1999, 2003). Certainly the reasons for the discrepancies between our work and Linder et al. (2003) are unclear, since both Szczepanski et al. (2002) and Linder et al. (2003) groups supposedly used identical protocols; however see discussion for some important methodological differences. In addition, since Ro 04-6790 poorly penetrates the brain (Russell and Dias, 2003); the present work was designed to further analyze the possible 5-HT_6 receptors involvement on memory formation. We decided herein to re-examine the role of $5HT_6$ receptor by using the two new and orally (dosage 1–30 mg/kg; per os, [p.o.] by mouth) active 5-HT_6 receptor antagonists *N*-(2,5-Dibromo-3-fluorophenyl)-4-methoxy-3-piperazin-1-ylbenzenesulfonamide (SB-357134) and *N*-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide (SB-399885) in the memory consolidation of an autoshaped response.

2. Methods

2.1. Animals

Male Wistar rats (12 weeks-old) were collectively housed in a temperature- and light-controlled room under a 12 : 12 h light / dark cycle (light on at 7:00 a.m.) with water and food provided ad libitum for a week. After that period, body weights were reduced to 85% by gradually reducing food intake (see below).

2.2. Autoshaping learning task

The local institutional committee for the use of animal subjects approved the present experimental protocol (Project No. 047/02). Autoshaping test has been previously described (Meneses and Hong, 1999; Meneses, 2001a, 2004; Meneses and Terron, 2001; Meneses et al., 2004; see Meneses, 2003; for review). The *n* per group was 8 animals and used once. *The autoshaping learning (Coulbourn Instruments, Lehigh Valley, PA) task apparatus included a standard attenuation system, and had the following inner dimensions: 25 cm width, 29 cm in length, and 25 cm in height.* Solid-state programming equipment was used for control and recording. An acrylic retractable lever was

mounted 4 cm above the floor and 10 cm from the right and left walls. The lever microswitch was adjusted to require a 10 g force for operation. A food magazine for rat pellets (Bio Serv, Frenchtown, NJ) was located 5 cm to the right of the lever and 3 cm above the floor. A house light was located in the right top corner and maintained turned on during session period.

2.2.1. Food-magazine training

Individually each rat was placed in an experimental chamber for a habituation period (≈15 min), having access to 50 food pellets (45 mg each) previously placed inside the food-magazine. Once the animal ate all food-pellets and presented 150 nose-pokes (as measured by a photocell) into the food-magazine, immediately afterwards the autoshaping program was initiated.

2.2.2. Autoshaping training

Autoshaping program consisted of discrete trials. A trial consisted in the presentation of an illuminated retractable lever for 8 s (conditioned stimulus, CS) followed by delivery of a 45 mg food pellet (unconditioned stimulus, US) with time intertrials (ITT) of 60 s; however, when the animal pressed the CS, the trial was then shortened, the lever was retracted, light was turned off, and a food pellet (US) was immediately delivered, and then the ITT began. The response during CS was regarded as a conditioned response (CR) and its increase or decrease was considered as an enhancement or impairment measure of learning, respectively. Since, the possibility and degree of engram manipulation are related both to the training amount and to the strength of post-training treatments (Meneses, 2003), and considering that 10 rather than, 5 or 20 trials, better detected the drug-induced changes on autoshaped response (*for references* see Meneses, 2003), hence the first session consisted of 10 (lasting ≈12 min) trials and the second of 20 trials (lasting ≈24 min). Following the first autoshaping training session, the testing session took place 24 h later and the results shown represent this latter autoshaping session. It should be *noted* that, notwithstanding sometimes neglected such as an important advance, the earlier recognition of the memory time-dependent nature and post-training drug administration (see McGaugh, 1966, 1989, 2003), allows excluding unspecific effects related to perception, motivation and motor activity, and thus studying selectively memory consolidation. Particularly important in this context is the food-intake aspect, since the bar pressing rates (i.e., CR) reported in the autoshaping task are very low (approximately 10%). This is consistent with the previous reports from us and Linder et al. (2003). Notably, in our case this minimal training produced learning. Of course, under these conditions a low floor effect means that the paradigm must be very sensitive to manipulation by non-specific effects such as change in activity level, food-intake or impulsivity.

2.3. Experimental protocol

2.3.1. Drug administration

For acute administration 30 min before the first autoshaping training session the animals received either methylcellulose vehicle, SB-357134 (1–30 mg/kg; p. o.) or SB-399885 (1–30 mg/kg; p.o.). Following the first autoshaping session, animals were placed in their home cages and the autoshaping session testing was performed 24 h later. For repeated or chronic administration, during 7 successive days previous to autoshaping training, animals received either methylcellulose vehicle, SB-357134 (1–30 mg/kg; p.o.) or SB-399885 (1–30 mg/kg; p.o.). Following the first autoshaping session, animals were placed in their home cages and the autoshaping session testing was performed 24 h later. Notably, using these acute and chronic administration schedules of drug administration, memory formation is affected. Finally, in the interaction experiments aiming to model an impaired memory SB-357134 or SB-399885 was coadministered with either acute scopolamine (0.17 mg/kg) or dizocilpine (0.1 mg/kg) (i.p.) administration. After 24 h, the session test was performed. The doses of scopolamine and dizocilpine were selected based on previous experiments (see Meneses, 1999, 2003). In addition, in order to address the question of food-intake of both 5-HT_6 receptor antagonists, naïve food-deprived rats were treated acutely, and observed 0.5 and 24 h following the vehicle, SB-357134 or SB-399885 administration.

2.4. Drugs

The drugs used were: SB-357134 and SB-399885 (both from GalxoSmithKline, Harlow-Essex, UK; Drs. W. Hirst and A. Chuang); dizocilpine HCl, scopolamine HCl purchased from Research Biochemical Inc., Wayland, MA. 5-HT_6 receptor antagonists were suspended in methylcellulose (at 25% of concentration) and given orally, while scopolamine or dizocilpine were dissolved in saline solution and given IP. The vehicle was administered p.o. in combination with scopolamine or dizocilpine. All drugs were administered in a volume of 1 ml/kg.

2.5. Statistical analysis

Responses in the presence of the CS (CR) were divided by the trials number during last session (i.e. 20 trails), and were expressed as a percentage. Only one CR was possible per trial. Multiple group comparisons were made using ANOVA followed by Tukey test (e.g., vehicle vs. SB-399885; or SB-39885 vs. antagonist plus scopolamine). To analyze food-intake results a two way ANOVA was performed, comparing food-deprived vehicle vs. treated groups, at 0.5 and 24 h following drug administration. In all statistical comparisons, $p < 0.05$ was used as criterion for significance. The n per group was 8 and 5 animals, for autoshaping and food-intake experiments, respectively, and were used only once.

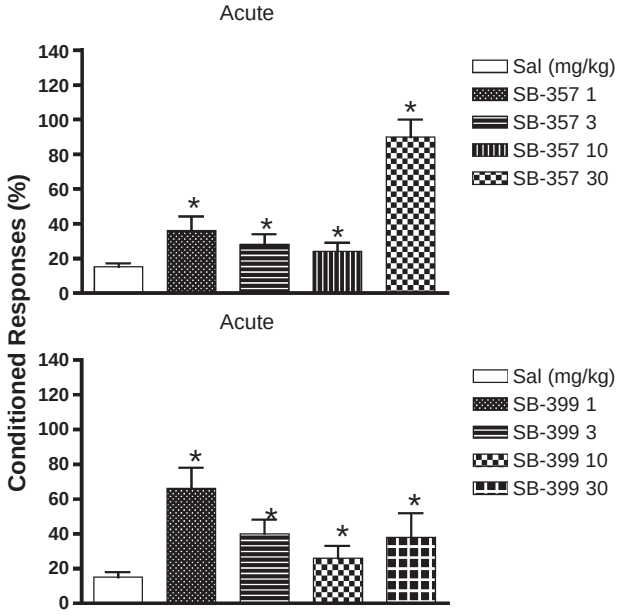

Fig. 1. Effects of acute administration (p.o.) of SB-357134 (upper) and SB-399885 (bottom) in the autoshaping task. Data of conditioned responses percentage (CR%) are plotted as mean±error standard (ES). All rats received injection 30 min before the autoshaping session and were tested 24 h later. Values represent the mean±(ES) error standard from 8 different animals and were analyzed by ANOVA, followed by Tukey test, $*p<0.05$ control saline vs. antagonist.

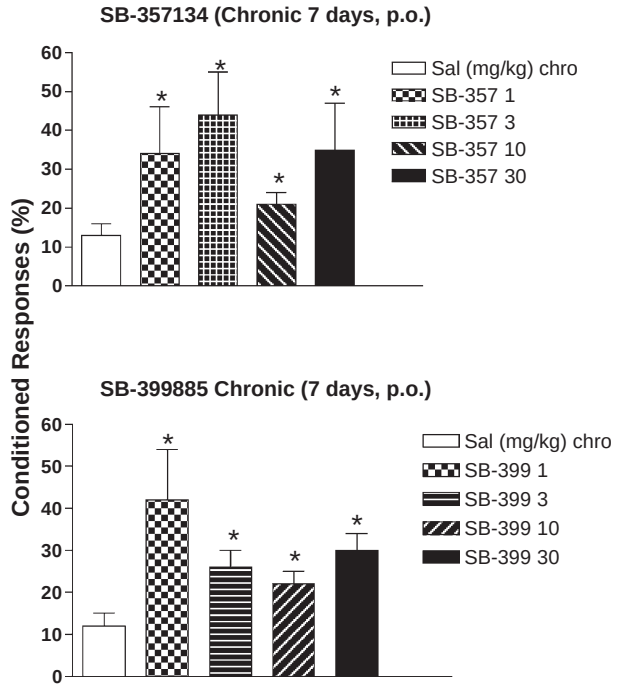

Fig. 2. Effects of chronic administration (p.o.) of SB-357134 (upper) and SB-399885 (bottom) in the autoshaping task. Data of conditioned responses percentage (CR%) are plotted as mean±error standard (ES). All rats received injection during 7 consecutive days and in the eighth they received the first training session and 24 h later the session test. Values represent the mean±(ES) error standard from 8 different animals and were analyzed by ANOVA, followed by Tukey test, $*p<0.05$ control saline vs. antagonist.

3. Results

3.1. 5-HT$_6$ receptors blockade

ANOVA revealed that compared with control saline groups (CR%=11±2), either acute chronic administration of SB-357134 [$F(4,39)=4.1$; $P<0.05$] (Fig. 1) or SB-399885 [$F(4,39)=3.1$; $P<0.05$] produced significant increments of CR%, and, post hoc Tukey test showed that these effects were produced at 1–30 mg/kg doses of either SB-357134 or SB-399885 (Fig. 1). Repeated administration of similar dosage of SB-357134 [$F(4,39)=5.3$; $P<0.05$] or SB-399885 [$F(4,39)=4.4$; $P<0.05$] also elicited significant increases of the CR% (Fig. 2).

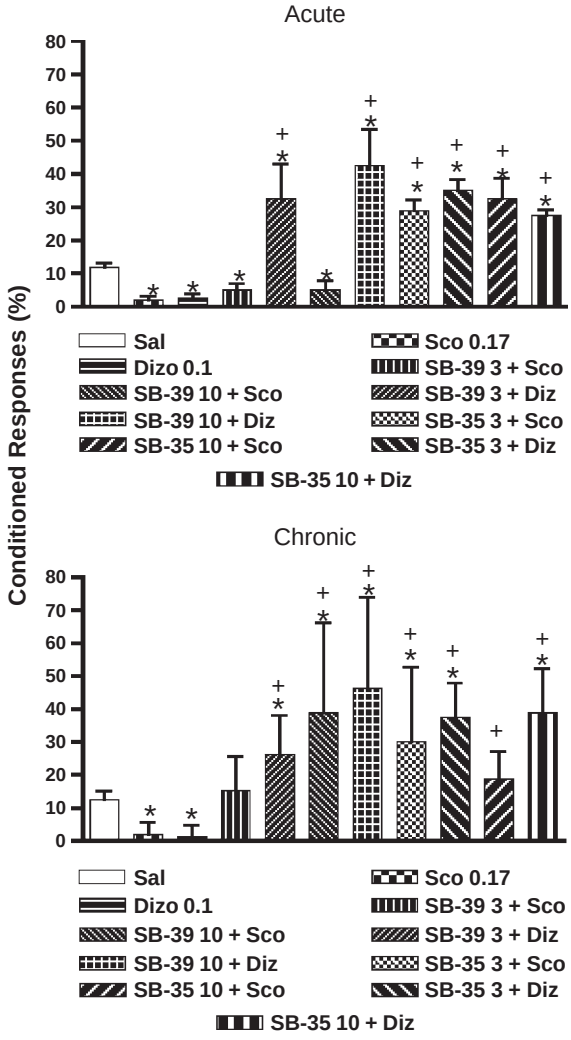

Fig. 3. Effects of acute (upper) or chronic (bottom) administration (p.o.) of SB-357134 and SB-399885 on the autoshaping task, in fasted animals treated with scopolamine or dizocilpine. Data are plotted as conditioned responses (CR%). All rats received saline, scopolamine or dizocilpine alone or the 5-HT$_6$ receptor antagonists plus scopolamine and dizocilpine. Values represent the mean±(ES) error standard from 8 different animals, Tukey test, $p<0.05$, *control vs. scopolamine or dizocilpine alone or +amnesic drugs plus 5-HT$_6$ antagonists. For the values of the SB-357134 or SB-399885 alone see text and Figs. 1 and 2.

3.2. 5-HT$_6$ receptors blockade and memory impairment

Acute post-training administration of scopolamine (0.17 mg/kg) or dizocilpine (0.1 mg/kg) (Fig. 3) significantly decreased percentage of CR (2±1 and 2±2, respectively), and these effects were antagonized by acute or repeated administration of SB-357134 [$F(10,87)$= 8.3; $P<0.0001$] or SB-399885 [$F(10,87)=7.8$; $P<0.0001$]. Post hoc Tukey test showed that, acute administration of SB-399885 (at 10, but not 3, mg/kg) completely and significantly reversed scopolamine- or dizocilpine-induced decrements of the CR%, and even increased performance (Fig. 3), producing 32±10 and 42±10 of CR%, respectively. Acute administration of SB-357134, at either 3 or 10 mg/kg, antagonized scopolamine (28±3 and 35±3 of CR%, respectively) and dizocilpine (32±6 and 27±1 of CR%, respectively) effects, and also increased the CR%. Moreover, repeated SB-399885 administration at 10, but not 3, mg/kg antagonized scopolamine-induced decrements, producing 27±6 of CR%; nevertheless, this same drug, at 3 and 10 mg/kg, completely and significantly reversed dizocilpine-induced decrements (39±19 and 47±35 of CR%), even produced improvement performance (Fig. 3). Except for the 3 mg/kg dose of SB-357134, which only normalized scopolamine or dizocilpine (30±18 and 20±6 of CR%, respectively) decrements on performance, all other doses antagonized scopolamine and dizocilpine (39±8 and 40±11 of CR%) impairment-effects and even provoked significantly improvements of CR% (Fig. 3).

3.3. Food-intake and 5-HT$_6$ receptors

ANOVA of food-intake results indicated that acute administration of 5-HT$_6$ antagonists produced significant differences (Table 1) between food-deprived vehicle vs. treated groups [$F(2,24)=6.2$, $P<0.001$], and timing of drug treatments [$F(1,1)=5.2$, $P<0.001$], indicating that both 5-HT$_6$ antagonists suppressed food-intake in food-deprived *animals* at 24 h following drugs.

Table 1
Food-intake in food-deprived vehicle and treated rats 0.5 and 24 h following administration of 5-HT$_6$ receptor antagonists

	Drug (mg/kg)	Acute administration	
		1	10
		Food-intake (g)	
	Saline		
Timing (h)	0.5	7±2	
	24	14±2	
	SB-357134		
Timing (h)	0.5	15±5*	12±4
	24	9±5	7±3*
	SB-399885		
Timing (h)	0.5	11±3	8±3
	24	5±2*	2±1*

*Values represent the means±error standard (ES) from 5 different animals and were analyzed by Multivariate repeated-measures ANOVA, followed by Tukey test, *$p<0.05$ control vehicle vs. antagonist.

4. Discussion

The most important results of the present work were observed following oral acute (−30 min before autoshaping training), or repeated (during 7 consecutive days) administration of either SB-357134 or SB-399885, which improved memory formation in the autoshaping learning task. It is certainly possible that the drugs are simply increasing the activity or appetite of the rats, particularly in the first session. Nevertheless, since, 1) posttraining administration of these drugs produced similar facilitatory effects, but different magnitude (Meneses, unpublished results); 2) 5-HT$_6$ receptor antagonists provoked significant reduction in food consumption (Table 1; for review see Woolley et al., 2004); and 3) the bioavailability values of these antagonists (see below) indicate the drug may not be active by the time of the second session. Considering the possibility that the 5-HT$_6$ antagonists did decrease appetite in the first training session and in the subsequent test session there was a "rebound" effect, such that drug-treated rats had an increased appetite. This possibility is excluded by the finding that the acute administration of either 5-HT$_6$ receptor antagonists suppressed food-intake in food-deprived at 24 h time. Hence drugs affected memory formation alone and not improved "motivation". Importantly, both 5-HT$_6$ receptor antagonists reversed the amnesia induced by scopolamine or dizocilpine. Notably, similar enhancements have been reported in non-appetively motivated tasks such as passive avoidance, water maze, and novel object discrimination tasks (see below). These data are consistent with previous evidence (Meneses, 2001a; Rogers and Hagan, 2001; Stean et al., 2002; Szczepanski et al., 2002; Foley et al., 2004; King et al., 2004) and provided further support to the notion that the serotonergic, glutamatergic, and cholinergic systems interact in cognitively impaired animals (Berger-Sweeney et al., 2004; Meneses, 2003; Santucci and Haroutunian, 2004).

4.1. The 5-HT$_6$ receptor antagonists SB-357134 or SB-399885 enhanced memory formation

Acute administration (−30 min) before training session of either SB357134 or SB-399885 improved memory formation. Effective doses of both antagonists, ranged from 1 to 30 mg/kg. Interestingly, SB-357134, produced *an inverted-U* curve of facilitated memory formation, the higher dose eliciting a 7-fold increase relative to control *saline* group (Fig. 1), a level of facilitated memory formation not previously reported in the autoshaping Pavlovian/instrumental task. The inverted-U dose–response curve is frequently seen in memory facilitation studies (Santucci and Haroutunian, 2004). Actually, it has been

reported with drugs affecting the acetylcholine, norepinephrine, dopamine and glucocorticoid receptors (see e.g., McGaugh, 2004). Significant facilitation of memory formation was produced by acute administration of SB-399885, at 1–30 mg/kg, but in this case 1 mg/kg was the most effective dose. On the other hand, repeated administration of SB-357134 (at 1–30 mg/kg) or SB-399885 (at 1–30 mg/kg) facilitated memory formation; in both cases low doses were more effective (Fig. 2). Certainly, the absence of a dose–response relation between cognitive facilitation and these 5-HT_6 receptor antagonists, suggests a complex interaction of efficacy and memory formation, for which we do not have a clear explanation yet. Hence, considering this complex pattern of efficacy, in the interaction experiments, we decided to test SB-357134 and SB-399885 at low and high dosage.

4.2. The 5-HT_6 receptor antagonists SB-357134 and SB-399885 reversed amnesia and even improved memory formation

The cholinergic deficit hypothesis has become central to the study of AD, including the notion that activation of muscarinic receptors is required during memory formation, and more recently glutamatergic integrity to neural plasticity and memory (Berger-Sweeney et al., 2004; Santucci and Haroutunian, 2004; Terry and Buccafusco, 2003). Thus, cholinergic muscarinic (e.g., scopolamine) or glutamatergic (e.g., dizocilpine) antagonists *have been used for modeling* mechanisms of dysfunctional memory and for developing new drugs acting on dementia. Actually, 5-HT, ACh and glutamatergic systems present a complex interaction in regions such as cortex, hippocampus, and amygdala, which likely depend on which specific receptors are involved and phase of memory formation (see Berger-Sweeney et al., 2004; Meneses, 1999, 2003; Santucci and Haroutunian, 2004). As noted earlier, acute or repeated administration of either SB-357134 or SB-399885 reversed the amnesic effects induced by scopolamine or dizocilpine; even unexpectedly some doses facilitated memory formation (Fig. 3). An anonymous referee had noticed another interpretation namely, that the doses of scopolamine and dizocilpine were inactive on the enhancing effect of both 5-HT_6 antagonists. Interestingly, this interpretation would implicate a protecting effect. For instance, acute administration of SB-399885 (at 10 mg/kg) not only reversed scopolamine-induced amnesic effect but improved memory formation. Similar effects were observed with (acute and repeated) administration of either SB-357134 or SB-399885 (at 3 or 10 mg/kg), regarding dizocilpine-induced amnesic effects. Thus, repeated administration of both 5-HT_6 receptor antagonists normalized memory impairments of scopolamine and dizocilpine and, once again even facilitated memory formation (Fig. 3). Certainly, we did not test enough number of doses to establish dose-dependent curves. Nevertheless in the present work, higher doses always were more effective than lower ones in amnesic animals, while the opposite was true, at least regarding SB-399885 in normal rats (Figs. 1 and 2). Notably together these findings are consistent with the notion that serotonergic tone, via 5-HT_6 receptor, is exerting an inhibitory action during memory formation (Meneses, 2003), which become more important under amnesic conditions. In this context, it is noteworthy that emergent evidence (Meneses, 2004; Meneses et al., 2002; Pérez-García and Meneses, in press) suggests a dominant role for some 5-HT receptors during memory formation which could be "unmasked" under either procognitive or amnesic conditions. In this connection, modeling dysfunctional memory with cholinergic or glutamatergic blockade, serotonergic function appeared to be modified, at least regarding 5-HT_6 (5-HT_{1A} or 5-HT_7) receptors (Meneses, 2004; Pérez-García and Meneses, in press). Of course, these considerations should be taken with caution since recently a novel and selective 5-HT_6 receptor agonist (WAY-466) was reported (Schechter et al., 2004), which produced robust elevations in cortical and hippocampal GABA levels and decreases in stimulated glutamate, but not basal, levels.

4.3. Related findings

Reproducibility and reliability among behavioral tasks and/or laboratories are major concerns (see Meneses, 1999, 2003; Meneses et al., 2004). Procognitive effects of 5-HT_6 receptor antagonists, in normal or amnesic animals, have been previously reported by a number of authors (Meneses, 2001a,b; Foley et al., 2004; Hirst et al., submitted for publication; King et al., 2004; Rogers and Hagan, 2001; Stean et al., 2002; Szczepanski et al., 2002; Woolley et al., 2004) in behavioral task such as passive avoidance, water maze, novel object discrimination, autoshaping, etc. (but see Linder et al., 2003). For instance, Szczepanski et al. (2002), reported in an abstract that, repeated administration (dosage 1–30 mg/kg) of the 5-HT_6 receptor antagonist Ro4368554, at 3 mg/kg, significantly facilitated autoshaped memory consolidation during second, third and fourth training sessions. Notably, during testing sessions, their control and treated animals showed accumulating improved performance in 20 trails per session. This evidence is consistent with the present and previous studies (see Meneses, 2001a,b, 2003). Szczepanski and co-workers concluded that Ro4368554 appears to enhance learning and memory, particularly in "disease models" (e.g. scopolamine-treated rats), Ro4368554 reversed the effects of scopolamine in Step-Down Passive Avoidance (active doses: 100 mg/kg p.o.; 10, 30 mg/kg; i.p.), Object Recognition (active doses: 3, 10 mg/kg i.p.), Social Recognition (active doses: 3, 10 mg/kg i.p.), but not in Radial Arm Maze (1–10 mg/kg; p.o.) or Step-Through Passive Avoidance (1–30 mg/kg; i.p.). In untreated rats, Ro4368554 enhanced autoshaped performance (active dose: 3 mg/kg i.p.), having not effect in the Morris Water Maze (1–10 mg/kg p. o.) in aged rats. In tests sensitive to antipsychotics, Ro04368554 was inactive in

Sidman Avoidance (3–30 mg/kg; i.p.), and failed to reverse the deficits induced by amphetamine, dizocilpine or neonatal lesions of the hippocampus in prepulse inhibition (1–10 mg/kg; i.p.). Thus, Ro4368554 appears to enhance learning and memory processes, particularly in disease models (i.e., scopolamine-treated rats), via modulation of cholinergic neurotransmission. The present findings provide further support to this conclusion. Moreover, Linder et al. (2003) in a full paper failed to replicate any of the positive results reported in autoshaping test. For example, they did not find evidence of improved acquisition or retention with Ro 04-6790 in the autoshaping task. They comment that we (Meneses, 2001a) reported autoshaped responses rates of 10% for the vehicle-treated control group on the test day, and in their experiments, vehicle-treated presented CR on 6–7% of the 20 trials, which is within the expected range of Meneses' experiments, but it is so low that there are potential floor effects which may reduce the sensitivity of this test (Linder et al., 2003).

Since the above points and considering that we have been contacted with questions about autoshaping, herein some details are discussed. It should be noticed that, conditioned responses result of associative learning to the anticipation of food reward, while being effective in preventing or eliminating autoshaped responses noncontigent control procedures, including pseudoconditioning and, of course, the lack of association. For instance, using a truly random Pavlovian control procedure of CS and US presentations (according Rescorla, 1967 seminal work), we found no evidence of all of the autoshaped responses (Meneses, unpublished data). Even though, Linder et al. increased the CS duration from 8 to 30 s, decreased ITT duration from 60 to 10 s and increased in the number of trials (from 20 to 50) in subsequent training sessions, these changes did not increase the probability of CR occurrence. Probably because long intertrial intervals facilitate CR acquisition (see Gibbon and Balsam, 1981). Spaced vs. massive training produces a more stable and durable learning (Meneses, 2003). Indeed, 5 or 10 trials detect better drugs effect (see Meneses, 1999, 2003; for reviews), which allows to give animals limited training, and thus excluding extensive training, which could make the results difficult to interpret. Certainly, as demonstrated by Tomie et al. (2003), wide variability inter-individuals could occur in the acquisition and maintaining of autoshaped responses, indicating that control rats might be unable to master learning autoshaping. Hence, it is worthy to know what determines the rate to acquire CR during Pavlovian autoshaping. In Pavlovian autoshaping acquisition speed of autoshaping response depends on critical variables, such as the ITT (I), the time during the trial (T) for which the CS is presented, and the fractional number of US per CS presentations (see Kakade and Dayan, 2002; for recent review). Actually, autoshaped response emerges more rapidly when trials are widely spaced in time (Gibbon and Balsam, 1981) or relatively shorter CS duration (Kakade and Dayan, 2002). For instance, the resulting median number of trials required for the CR acquisition depends on the *ratio* of I/T (not on I and T separately), being the number of reinforcements approximately inversely proportional to I/T. In consideration to the inter-animals variability to acquire the CR in Pavlovian autoshaping, usually research on acquisition rates are often measured by the number of trials until a certain behavioral criterion is met (e.g., autoshaped responses occurring on three out of four consecutive trials). In order to diminish the autoshaped response inter-animals variability, since 1986 we have used an autoshaping Pavlovian/instrumental learning task, consisting in a contingent delivery of US following autoshaped response, an acrylic and illuminable retractable lever as CS (Meneses, 2003) and a certain minimum number of head-pokes into the food-magazine during food-magazine training (see Meneses, 2003, for references). In this regard, probably major differences between our work and that of Linder et al. (2003) could be that they used a metallic retractable lever illuminated by a house light located at the top of the cage directly above the lever. Instead, we used an acrylic and illuminated retractable lever as CS, which improves CS salience. Importantly, under these conditions Pavlovian/instrumental autoshaping learning task is better conceptualized as an instance of systems processing styles stimulus–stimulus, stimulus–response and stimulus–reinforcer [S–Rf] learning (Meneses, 2003), which requires brain areas such as dentate gyrus, hippocampal CA1, basolateral amygdaloid nucleus and prefrontal cortex (Luna-Munguía et al., 2005; Manuel-Apolinar et al., 2005; Meneses et al., 2004).

4.4. Brain areas and memory formation

Although in the present study only systemic treatments were used, some considerations of sites of action are important. Thus, the present behavioral test is an appetitively-rewarded behavioral task, which involves diverse brain areas, including the basolateral amygdala (Meneses et al., 2004). Regarding memory facilitation, extensive studies (see e.g., McGaugh, 2004) have shown that the amygdala, and especially the basolateral amygdala is critically important in mediating posttraining drug effects on memory consolidation and memory formation itself (for review see Power et al., 2003). These authors have highlighted that memory can also be affected by post-training activation of muscarinic cholinergic receptors in the hippocampus, striatum and cortex. Evidence of increases in hippocampal and cortical ACh levels following learning experiences supports the view that endogenous cholinergic release is involved in long-term memory consolidation. The notion that muscarinic cholinergic receptor activation is involved in the storage of information in these brain regions is supported by the findings indicating that muscarinic cholinergic receptor drug treatments influence plasticity in the hippocampus and in sensory cortices (Power et al., 2003). Notably, other possible interactions in the cortex,

nucleus basalis, hippocampus/medial septum system could exist. For instance, in the context of instrumental acquisition, prefrontal cortex has been conceptualized to represent declaratively the contingencies between instrumental actions and their outcomes (see Cardinal and Everitt, 2004, for review). Conceptualizations whose practical implications, includes the fact that the present autoshaping task combines, both Pavlovian and instrumental conditioning, which offers the opportunity to study hippocampus-mediated declarative memory and striatum-mediated S–R "habit formation", in rats (Meneses, 2003) and mice (see Barrett and Vanover, in press). It detects little changes (increases or decreases) on diverse behavioral parameters not redundant (i.e., not measuring the same event), including the sign tracking (i.e., conditioned behavior directed toward the localized retractable and illuminated lever, CS), and the goal tracking (i.e., the place where the US is delivered) (Meneses, 2003). This latter is quite important, since it allows the study of bi-directional expression of an enhanced or impaired memory formation. As earlier mentioned, autoshaped response emerges rapidly when trials are spaced in time, with its acquisition rate relatively constant when inter-trial interval ratio to CS and US interval is constant; therefore, the difference on CR number between the first and second autoshaping sessions is modest *giving* animals a limited training. For instance, under our specific experimental conditions we found 8 to 12±2% of CR in saline-control groups while scopolamine-treated groups present 2–3 to 4±1% of CR (see Meneses, 2001a, 2004; Meneses and Terron, 2001; Manuel-Apolinar and Meneses, 2004). Some of these results have been consistent with data reported in genetic deletion of 5-HT_{1A} or 5-HT_{1B} receptors (see Meneses, 2003, for references) and expression of diverse 5-HT receptors during memory formation (Luna-Munguía et al., 2005; Manuel-Apolinar et al., 2005; Meneses et al., 2004). Limited training has an important implication, since the possibility and degree of engram manipulation are related both to the training amount and strength of post-training treatments. Indeed, e.g., even though Ro 04-6790 appears poorly brain penetrant (Russell and Dias, 2003), when Linder et al. (2003) treated a group with 3 mg/kg of Ro 04-6790 a higher non-significant number of CR (nearly 8±2) with respect to the other groups was observed. Interestingly, at the Ro 04-6790 same doses we found a significant beneficial peak effect in memory consolidation (Meneses, 2001a). The present data clearly demonstrated that selective, potent and brain penetrant 5-HT_6 agents improved formation. Actually, effective doses of acute injection of either SB-357134 or SB-399885 improved memory formation, ranged from 1 to 30 mg/kg. Notably, the bioavailability (BA), half-life ($T_{½}$) and minimum effective dose to induce inappetence (MED) for the 5-HT_6 receptor antagonists are: SB-357134, BA=65%, $T_{½}$=3.4 h, MED=30 mg/kg p.o.; and SB-399885, BA=52%, $T_{½}$=2.2 h, MED=50 mg/kg p.o. (personal communication provided by A.T Chaung, GlaxoSmithKline). A practical connection of the present data is the fact that, atypical antipsychotics have higher affinities for 5-$HT_{2A/2C}$, 5-HT_6 and 5-HT_7 receptors (Meneses, 2001b; Roth et al., 2003; Thomas and Hagan, 2004; Woolley et al., 2004). And although it is unclear whether the 5-HT_6 receptor C267T polymorphism plays a major role in susceptibility to the development of schizophrenia and/or cognitive impairment in schizophrenic patients; notably, compared to patients with the T/C 267 genotype, those with T/T 267 genotype showed less severe positive symptoms and general psychopathology, including cognitive dysfunctions (Lane et al., 2004).

5. Conclusions

In conclusion, acute and chronic oral administration of either SB-357134 or SB-399885 improved memory consolidation in naïve animals. Modeling the potential therapeutic benefits of 5-HT_6 receptor blockade, acute or repeated administration of SB-399885, at 10, but not 3, mg/kg reversed memory deficits produced by scopolamine or dizocilpine. SB-357134 (at 3 and 10 mg/kg) did not only prevent amnesia but even improved performance. These data confirm that: 1) endogenously 5-HT acting, via 5-HT_6 receptor, modulates memory formation, while blockade of these receptors improves it; and 2) 5-HT_6 receptor antagonists can reverse amnesia and even facilitate memory formation. Hence, potent 5-HT_6 receptor antagonists can be useful in the treatment of dysfunctional memory in aged-related decline and Alzheimer's disease. Particularly since 5-HT_6 receptors seem diminished in AD patients (see Garcia-Alloza et al., 2004).

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