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Abbreviations

AD, Alzheimer's disease; ANOVA, analysis of variance; ATP, adenosine triphosphate; CHO, Chinese hamster ovary; cAMP, cyclic adenosine monophosphate; CNS, central nervous system; HEK, human embryonic kidney; 8-OH-DPAT, 8-hydroxy-aminotetralin; 5-CT, 5-carboxytryptamine; 5-HT, 5-hydroxytryptamine, LSD, lysergic acid diethylamide; ns, non significant; SDZ

205,557, 4-amino-5-chloro-2-methoxy-benzoic acid-(diethylamino) ethyl ester hydrochloride; RID, ratio of investigation duration.

Section: Behavioural Pharmacology

Abstract

SL65.0155 (5-(8-amino-7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-3-[1-(2-phenyl ethyl)-4-piperidinyl]-1,3,4-oxadiazol-2(3H)-one monohydrochloride), is a novel benzodioxanoxadiazolone compound with high affinity for human 5-HT_4 receptors (Ki of 0.6 nM) and good selectivity (greater than 100-fold for all other receptors tested). In cells expressing the 5-$HT_{4(b)}$ and 5-$HT_{4(e)}$ splice variants SL65.0155 acted as a partial agonist, stimulating cAMP production with a maximal effect 40-50% of serotonin. In the rat esophagus preparation SL65.0155 acted as a 5-HT_4 antagonist with a pK_b of 8.81. However, SL65.0155 potently improved performance in several tests of learning and memory. In the object recognition task it improved retention at 24 h when administered i.p. or p.o. (0.001 - 0.1 mg/kg). This effect was antagonised by the 5-HT_4 antagonist SDZ 205,557, itself without effect, demonstrating that the promnesic effects of SL65.0155 are mediated by 5-HT_4 agonism. SL65.0155 also reversed the cognitive deficits of aged rats in the linear maze task and the scopolamine-induced deficit in the water maze task in mice. Furthermore, the combined administration of an inactive dose of SL65.0155 with the cholinesterase inhibitor rivastigmine resulted in a significant promnesic effect suggesting a synergistic interaction. SL65.0155 was devoid of unwanted cardiovascular, gastrointestinal or CNS effects up to doses more than 100-fold higher than those active in the cognitive tests. These results characterise SL65.0155 as a novel promnesic agent acting via 5-HT_4 receptors with an excellent preclinical profile. Its broad range of activity in cognitive tests and synergism with cholinesterase inhibitors suggest that SL65.0155 represents a promising new agent for the treatment of dementia.

Alzheimer's disease (AD) is a chronic neurodegenerative disorder characterized by progressive cognitive decline, debilitating behavioral impairment and ultimately death. Neurofibrillary tangles and amyloid plaques are the pathological hallmarks of this disease which is also characterized by a dramatic loss of cholinergic neurons in brain regions involved in higher cognitive functions. It is widely accepted that the loss of cholinergic markers is the principal neurochemical deficit in AD and that this deficit correlates well with loss of cognitive abilities (Bierer et al., 1995; Palmer, 1996). Despite this, cholinergic therapies such as inhibitors of acetylcholinesterase have had only partial success (e.g. Volger, 1991), possibly due to the multifactorial nature of the deficits and poorly understood etiology of AD. Thus, there is a major need for novel therapeutic approaches which can alleviate the symptoms of the disease.

In addition to the cholinergic deficit, loss of other neurotransmitter systems has been reported in AD, including 5-hydroxytryptamine (5-HT) (Meltzer et al., 1998; Palmer, 1996). This therefore provides another opportunity for therapeutic intervention, either alone or in combination with compounds acting on the cholinergic system (Cassel and Jeltsch, 1995; Richter-Levin and Segal, 1996; Steckler and Sahgal, 1995). The numerous subtypes of 5-HT receptor that have been described (Hoyer et al., 1994) allow a variety of ways in which serotonergic compounds might affect cognition.

Although there is evidence for the involvement of several 5-HT receptor subtypes in cognitive processes (e.g. Barnes et al., 1990; Carli et al., 1997), an increasingly large body of evidence supports a major role for the 5-HT_4 subtype in learning and memory. This receptor is a member of the G-protein-coupled 7-transmembrane domain receptor super-family and is positively coupled to adenylyl

cyclase. 5-HT_4 receptors are heterogeneously located in the central nervous system with high concentrations in numerous structures considered important for mnemonic processes, such as the hippocampus and regions of the cortex (Compan et al., 1996; Jakeman et al., 1994). In the hippocampus they have been localized to pyramidal neurons (Roychowdhury et al., 1994), where there is *in vitro* evidence that their activation results in the inhibition of voltage dependent K^+ channels via stimulation of a cAMP-dependent protein kinase (Ansanay et al., 1995; Torres et al., 1995). Experiments have shown that only a brief stimulation of the 5-HT_4 receptor is necessary for a slowly-reversible blockade of these K^+ channels which results in a long-lasting increase in neuronal excitability (Andrade and Nicoll, 1987; Ansanay et al., 1995). Interestingly, there is evidence for a loss of 5-HT_4 receptors in Alzheimer's disease in the cortex (-23%) and particularly hippocampus (-66%) (Reynolds et al., 1995), consistent with their distribution and localisation on pyramidal cells and with a potential role in cognitive processing.

In a recent study (Robert et al., 2001), activation of the human 5-HT_4 receptor was shown to stimulate the secretion of the non-amyloidogenic soluble form of the amyloid precursor protein (sAPPα). Given the neuroprotective and enhancing memory effects of sAPPα (Mattson et al., 1993; Schubert and Behl; 1993; Behl et at. 1994) 5-HT_4 receptor agonists may have a therapeutic potential for the treatment of Alzheimer's disease. Further support for a therapeutic potential of 5-HT_4 receptor agonists in learning and memory disorders comes from a range of *in vivo* studies showing that they can improve performance in animal models of learning and memory. Most of these studies have used the relatively non-selective compounds BIMU-1 and BIMU-8 (Eglen et al., 1995), which have shown activity in an olfactory discrimination task (Marchetti-Gauthier et al., 1997), a social memory

task (Letty et al., 1997), in acquisition of an operant auto-shaping task when administered pre-conditioning (Meneses and Hong, 1997) and in reversing scopolamine-induced amnesia in a mouse passive avoidance task (Galeotti et al., 1998). A specific role for 5-HT_4 receptors in some of these studies is supported by the antagonism of these effects by selective 5-HT_4 antagonists (Galeotti et al., 1998; Letty et al., 1997; Marchetti-Gauthier et al., 1997). More selective compounds, such as RS17017 and RS 67333, have also been shown to enhance cognition in a delayed-match-to-sample task in aged primates (Terry, Jr. et al., 1998), and reversal of an atropine-induced deficit in the Morris water maze in rats (Fontana et al., 1997).

A potential drawback of 5-HT_4 agonists as therapeutic agents is their effects mediated in the periphery by this receptor subtype. These include increased gastric motility, cardiac inotropy and tachycardia, and release of adrenal hormones (Hegde and Eglen, 1996), all of which would be undesirable in a clinical setting. We anticipated that a compound with less efficacy at the 5-HT_4 receptor would retain the promnesic activity demonstrated by agonist compounds while avoiding the peripheral effects associated with this receptor. This report describes the properties of a selective 5-HT_4 partial agonist compound, SL65.0155 (5-(8-amino-7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-3-[1-(2-phenylethyl)-4-piperidinyl]-1,3,4-oxadiazol-2(3H)-one monohydrochloride) (Figure 1).

-- Figure 1 about here --

Methods

Radioligand binding studies

All radioligand binding studies were carried out either by CEREP (Celle L'Evescault, France) or MDS Pharma Service (Taipei, Republic of China). Unless otherwise indicated in the results, all studies were initially carried out at 10^{-5} M. If there was greater than 75% displacement this concentration, Ki values were determined. Over 100 binding sites were examined, including all other 5-HT receptors and all major classes of neurotransmitter receptor, uptake systems, ion channels and enzymes. In the case of the 5-HT receptor subtypes, all experiments were carried out using recombinant human receptors cloned into various cell types as indicated in table 1. Also shown in table 1 are the ligands used and their concentration.

Affinity of SL65.0155 for different splice variants of the 5-HT_4 receptor

Membranes were prepared from either CHO cells stably transfected with human isoforms of the 5-HT_4 receptor (isoforms c, d and e) or from transiently transfected COS-7 cells (isoform b) and the ability of SL65.0155 to displace [^{3}H]GR113808 (Amersham, Arlington Heights, Ilinois, USA) was examined as previously described (Blondel et al., 1998). Briefly, radioligand binding studies were performed in 500 µl of HEPES buffer (50 mM, pH 7.4) to which was added 50 µl of membrane preparation (100 - 200 µg), 20 µl of buffer solution containing the competing agent (8 concentrations of SL65.0155 were used or 10 µM of GR113808 to determine non-specific binding) and 20 µl of buffer containing [^{3}H]GR113808 to give a final concentration of 50% of the Kd value. The reaction was stopped by rapid vacuum filtration through Whatman GF/B filter paper (pre-soaked in a solution of

polyethylenimine (0.1 %) to reduce binding to filters) using a Brandel 48R cell harvester. Filters were subsequently washed with ice-cold buffer (50 µM Tris-HCl, pH 7.4) and placed overnight in 4 ml of Ready-Safe scintillation cocktail (Beckman, Fullerton, CA, USA). Radioactivity was measured using a Beckman model LS 6500C liquid scintillation counter. Each experiment was carried two or three times in triplicate. Binding data were analysed by computer assisted non-linear regression analysis (Prism; GraphPad software, SanDiego, CA, USA).

Functional effects at the 5-HT$_4$ receptor

Stimulation of cyclic adenosine monophosphate (cAMP) production in CHO cells expressing splice variants of the human 5-HT$_4$ receptor

The 5-HT$_{4(a)}$ variant of the human 5-HT$_4$ receptor was transiently expressed in COS-7 cells and changes in cAMP formation in response to compounds was evaluated as described previously using [^{3}H]-adenine to label the ATP pool (Claeysen et al., 1997). The 5-HT$_{4(b)}$ and 5-HT$_{4(e)}$ variants of the human 5-HT$_4$ receptor were expressed in CHO cells (transiently and stably, respectively) and changes in cAMP formation in response to compounds was evaluated as described previously using a radioimmunological kit (ERIA kit 79830 from Pasteur Diagnostics) (Blondel et al., 1998). At least six different concentrations were used and each concentration evaluated in 2 or 3 separate experiments. Non-linear curve fitting was used to determine the maximal effect and the EC$_{50}$ values.

Rat esophagus

The 5-HT$_4$ agonist activity of SL65.0155 was assessed by measuring the relaxation of the muscularis mucosae of rat distal esophagus pre-contracted with

carbachol compared to that induced by 5-HT. The thoracic esophagus was isolated from male Sprague-Dawley rats (350 - 450 g) and placed in Krebs solution (NaCl 118 mM; KCl 4.7 mM; $MgSO_4$ 1.64 mM; KH_2PO_4 1.18 mM; glucose 11.5 mM; $NaHCO_3$ 24.88 mM; $CaCl_2$ 2.52 mM). The outer striated muscle coat was cut longitudinally and peeled away to expose the inner muscularis mucosae. Tissues were mounted in 20 ml organ baths containing Krebs solution maintained at 32°C and gassed with 95% O_2/5% CO_2, pH 7.4. Contractions were recorded isometrically with an initial resting tension of 0.5 g applied to the preparation. After 30 min, carbachol (0.5 µM) was added to the bath and the response to 5-HT (1 µM) was determined. After washing out the 5-HT and a 20 min re-equilibration period, cumulative concentration effect curves were constructed for SL65.0155 (0.1 - 1 µM) in carbachol-precontracted tissue to determine its agonist effect. Responses to the addition of SL65.0155 were expressed as a percentage of the effect of 5-HT at 1 µM. At the highest concentration of SL65.0155 a cumulative concentration-effect curve to 5-HT was constructed and compared to a pre-established 5-HT control curve to determine the pKb as a measure of the antagonist properties of SL65.0155.

Guinea-pig ileum

Following sacrifice, the distal segment of the ileum was rapidly removed from male Dunkin-Hartley guinea-pigs weighing approximately 350 g. The ileum segment was cleaned and then cut into longitudinal segments approximately 1 cm in length. These segments were then placed in an organ bath containing oxygenated (95% O_2, 5% CO_2) physiological solution (118 nM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 11 mM glucose) at pH 7.4 and a temperature of 37°C and connected to an isotonic strain guage (Ugo Basile model

7006) to measure smooth muscle contraction. Experiments were carried out in the presence of pyrilamine (1 µM), methysergide (1 µM) and ondansetron (10 µM). After installation in the organ bath the tissue was placed under a 1g tension and allowed to stabilise for 60 min, after which the tension was readjusted to 1 g. Agonist dose-response curves were obtained by administering different concentrations of SL65.0155 or 5-HT to the bath at 40 min intervals. Each concentration was left in contact with the tissue until the maximal effect had been obtained or for a maximum of 5 min. The tissue was washed between drug administrations. Antagonist effects of SL65.0155 were evaluated by exposing the tissue to one of three concentrations (10, 30 or 100 nM) 60 min prior to the determination of a concentration-response curve to 5-HT (6 determinations per concentration of SL65.0155).

Evaluation of cognition-enhancing activity

Visual recognition memory in young rats

The effects of SL65.0155 on visual recognition memory were assessed using an object recognition task similar to that described by Ennaceur and Delacour (1988). Male Sprague-Dawley rats (180 - 230 g at testing) were housed in pairs with free access to food and water in a temperature-controlled animal room (21±1°C) on a 12h light/dark cycle (dark period from 0700 - 1900). The apparatus consisted of a uniformly lit hardboard enclosure (65 x 45 x 45 cm) observed from an adjacent room via a video monitoring system. Each experiment consisted of 3 sessions. During the first session the animals were allowed 2 min to become familiar with the experimental environment. Time spent active (animal moving around with or without sniffing and exploration) was measured. Twenty-four hours later, the animals were again placed in the enclosure in the presence of two identical objects for the amount

of time necessary to spend 20 s exploring these two objects to a limit of 3 min (exploration was defined as the animal having its head within 2 cm of the object while looking at it, sniffing or touching it). Any animal not exploring the objects for 20 s within the 3 min period was eliminated. After an interval of either 1 min or 24 h, the rats were again placed in the enclosure with a previously presented familiar object and a novel object for a period of 3 min. Time spent exploring the familiar and novel objects was recorded. Animals exploring the objects for less than 10 s were eliminated from the study. The objects used were a metal triangle (7 x 3 x 8 cm) and a plastic pyramid (9 x 3x 7 cm). Two different sets of objects were used in order to enable them to be wiped between one rat and the next, in order to prevent the possibility of olfactory recognition. SL65.0155 was studied at doses of 0.00001 - 0.1 mg/kg p.o. (doses expressed as salt) with tacrine (0.5, 1 and 2 mg/kg p.o.) as a reference. In the study examining the interaction of SL65.0155 with the 5-HT_4 receptor antagonist SDZ 205,557 doses of 0.1 mg/kg p.o. and 1 mg/kg i.p. were used respectively. In a study to determine the interaction between SL65.0155 and the cholinesterase inhibitor rivastigmine, doses of 0.0001 mg/kg po and 0.03 mg/kg ip were used respectively. Drugs were administered either 30 min (i.p.) or 60 min (p.o.) before each of the three experimental sessions. Data were analysed by analysis of variance for comparisons between treatments and between familiar and novel objects for each treatment.

Acquisition of a linear maze task in aged rats.

The effects of SL65.0155 on acquisition of a linear maze task were investigated in aged rats (21-23 months old at the start of experiments). These experiments used a total of 63 male Wistar rats housed 5 animals per cage (42 x

42 x 18 cm), in a temperature-controlled room (21 ∀ 1°C) with a 12h/12h dark/light cycle (lights on at 7 a.m.). Water was freely available but animals were kept on a food deprivation schedule of 30 min access to food per day. The linear maze consisted of a start box, six choice units (50 x 40 x 35 cm) with 10 cm wide openings between each of them and a goal box (25 x 25 x 35 cm). All units and boxes were made of beige PVC and start and goal boxes were separated from other units by sliding doors. The task required rats to learn a sequence of six successive left-right choices to reach the goal box and obtain food reinforcement. Vertical barriers, made of clear plexiglas, could be changed from one side to the other in each unit, to form blind alleys. Illumination was provided by six 60W-lamps, 2.5 m above each choice point, in order to avoid shadows in the apparatus. Six different maze configurations were used and randomized between animals: LRRLLR, LRLLRR, RRLLRL, RLRRLL, RLLRRL, LLRRLR (where R = right and L = left). The reinforcement (Kellogg's Chocopops) was placed in a white porcelain receptacle in the goal-box. Prior to training there was a habituation period (4 or 5 days) which allowed the animals to become familiar with the environmental context and to associate reinforcement and goal box. No barriers were present during this stage. The learning period consisted of 2 trials per day (one in the morning and one in the afternoon) for 10 days. For each trial, the rat was placed in the start-box, the door was immediately opened and the rat was allowed to reach the goal-box and obtain the reinforcement (maximum 15 min). The number of errors (defined as an entry into a blind alley) made by rats was recorded and summed for each pair of trials per day. After an error, rats could return towards the start-box thus allowing them to re-enter a blind alley more than once during the same trial. Statistical analyses were performed according to a multi-stage procedure. Data was analyzed using one- or

two-factor ANOVA for repeated measures, as appropriate, followed by additional ANOVA or t-test comparisons if significant ($p<0.05$). SL65.0155 (0.1 and 1.0 mg/kg i.p.) or rivastigmine (0.1 and 0.25 mg/kg i.p.) were dissolved in saline (0.9% NaCl w/v) and injected in a volume of 2 ml/kg, 30 min before each acquisition or reversal trial. Doses are expressed as salt.

Reversal of scopolamine-induced deficits in the water maze in mice

The water maze task evaluates spatial memory acquisition by testing the ability of animals to find an invisible platform located in a circular arena (1.5 m diameter filled with water at 23°C) using distal environmental cues. The platform (10 cm diameter transparent perspex) is made invisible by placing it 0.5 cm below the surface of the water. Male B6D2F_1 mice were placed in the water maze at one of four start points located around the perimeter and were required to swim in the maze until they found the platform. If they did not find the platform in 60 s they were placed on the platform for 10 s. Mice were subjected to 4 trials per day (each trial separated by 5 min, during which the mouse was replaced in the home cage) for 4 days. The fourth trial of the final day was a retention test in which the platform was removed and mice allowed to swim in the maze for 30 s. Possible confounding effects of motivational or visual deficits were evaluated with a training session using a visible platform (an opaque platform 0.5 cm above the surface of the water) carried out four days after the retention test. Four treatment groups were studied, each consisting of 9 mice: controls, scopolamine HBr (0.3 mg/kg i.p. in 0.9% NaCl) and two groups receiving scopolamine and either 0.1 or 0.3 mg/kg i.p. of SL65.0155 dissolved in a solution of 1% tween 80 and 0.01% dimeticone. All mice received two injections of drug or vehicle 20 min prior to the first trial each day of testing. The

trajectory taken by each animal was monitored by an image analysis system (Videotrack, Viewpoint, Lyon, France) which measured latency and distance taken to the platform for all acquisition and visible platform trials. During the retention trial the additional parameters of time spent in each quadrant and annulus crossing (ie number of occasions the mouse swam over the former platform location and corresponding positions in other quadrants) were recorded. Statistical analysis consisted of an initial ANOVA comparison of control and scopolamine-treated groups (treatment and session as factors) followed by a similar comparison of the scopolamine-treated group with those receiving scopolamine and SL65.0155.

Improvement of social olfactory recognition in rats.

The effects of SL65.0155 on social olfactory recognition were assessed using the procedure previously described (Pério et al., 1989). Juvenile Wistar rats (3 weeks old, 50 - 100 g, were housed 7/8 per cage) and 10 adult Wistar rats (400 - 500 g, housed individually (home cage dimensions 30 x 40 x 18 cm) were maintained under conditions of constant temperature (22 $\pm$ 2 °C) and on an inverted light-dark cycle (light on from 7.00 PM to 7.00 AM). Food and water were freely available. Experiments were conducted in the animal room, under red illumination during the dark phase. The juveniles were isolated for 30 min before the start of each experiment. In the spontaneous forgetting procedure, the juveniles were exposed to the tested conspecific, in the conspecific's home cage, on two successive presentations of 5 min duration separated by a 120 min interval during which the juveniles were returned to their isolation box. The experiments were monitored via a camera by an observer in an adjacent laboratory. Duration of

investigatory behaviour (nosing, sniffing, grooming, close following of the juvenile) was recorded by striking precoded keys on a computer system.

SL65.0155 was studied at doses of 0.001 – 0.03 mg/kg i.p. (doses expressed as base) and administered immediately after the first presentation of the juvenile. Each of the 10 adult rats were tested under each of the 5 treatment conditions on 5 consecutive days, with the treatments administered in a pseudo-randomised order. The time spent in social investigation was expressed as the ratio of individual investigation during the second exposure to that of the first exposure (ratio of investigation duration, RID). Individual RIDs were submitted to a Kruskal-Wallis tests for repeated measures (RS1 software) with subsequent post-hoc comparisons using the Wilcoxon test.

Side effect liability

Effect on general behavior in rats (Irwin observation test)

Potential behavioral and neurological effects of SL65.0155 and its effects on body temperature and weight were studied in male Sprague Dawley rats, (150 - 200 g at testing, n = 5/group). SL65.0155 (0.1 - 10 mg/kg expressed as the salt) or vehicle (1% tween 80, 0.01% dimethicone) were administered orally in a volume of 5 ml/kg. Each animal was observed continuously for autonomic and neurological changes over a 30-min period post-dose and then at times 1, 2, 4, 6 and 24 h after treatment. Observation of animals treated with SL65.0155 was carried out in parallel with that of control rats (receiving vehicle). Body temperature was measured using a rectal probe and animals were weighed before treatment and after 24 h.

Arterial blood pressure and heart rate in conscious normotensive rats

Male Sprague-Dawley rats (288 - 324 g at testing; n = 5/group) were anesthetized using an intraperitoneal injection of ketamine (116 mg/kg). A catheter filled with anticoagulant (povidone) solution was inserted into the femoral artery, passed under the skin and exteriorized between the scapula. The day after the implantation, the catheter was connected to a blood pressure transducer (Statham P23XL model). After a stable baseline was obtained, SL65.0155 was administered orally, dissolved in water at the doses of 0.1, 1 and 10 mg/kg, expressed as the salt, in a volume of 5 ml/kg. The effect of SL65.0155 on arterial blood pressure and heart rate was monitored continuously for 4 h. Haemodynamic parameters (systolic and diastolic arterial blood pressure and heart rate) were recorded every 15 min during the first two h, then every 30 min. The results are expressed as the difference from baseline values.

Haemodynamic profile in anesthetized dogs

Male beagle dogs, weighing 10 - 16 kg at testing (n = 5 per treatment group) were fasted for 24 h before use. On the day of testing anesthesia was induced with pentobarbital (30 mg/kg, i.v.) and then maintained with an i.v. infusion of pentobarbital (0.1 mg/kg/min) into the cephalic vein. Animals were placed under artificial ventilation (25 cycles/min, 200 ml/kg/min). Arterial blood pressure and pulmonary artery pressure were measured from catheters inserted into the right femoral aorta and a jugular vein, respectively. Left ventricular pressure was measured by means of a cannula inserted via the left carotid artery. Doppler probes (Transonic Systems T206) were positioned around the right carotid artery, the left circumflex coronary artery, the aorta and the left renal artery to monitor arterial flow

rates. Three electrodes were placed in order to measure the electrocardiogram. The haemodynamic parameters were measured before drug administration and at various times between 1 and 120 min after the intravenous administration of SL65.0155 at doses of 0.01, 0.1 and 0.5 mg/kg, expressed as the salt, or its vehicle (saline). In addition, arterial blood samples were taken at 15, 30, 60 and 120 min post-treatment for analysis of blood gases and electrolytes.

Intestinal transit in rats

The effects of SL65.0155 were studied in male Sprague-Dawley rats, weighing 150 - 190 g (n=15 per treatment group). Rats were fasted for 24 h prior to oral administration of either vehicle (1% w/v tween 80 in sterile water) or SL65.0155 (0.3, 3 or 30 mg/kg po, expressed as base) on the test day. Thirty minutes later, the rats received a charcoal meal (12.5 ml/kg of 10% w/v activated charcoal suspended in 0.25% w/v methyl cellulose and 0.5% w/v tween 80). A further 30 min later, the animals were sacrificed by an intra-cardiac injection of a lethal dose of pentobarbital. The small intestine was removed and the length of the intestine from the pylorus to the ileo-caecal junction was measured and also the length of the part colored by the charcoal. Intestinal transit was expressed as the percentage of intestine colored by charcoal.

Compounds

SL65.0155 HCl, rivastigmine HCl, tacrine HCl and SDZ 205,557 (4-amino-5-chloro-2-methoxy-benzoic acid-(diethylamino) ethyl ester hydrochloride) were all synthesised by the Sanofi-Synthélabo Medicinal Chemistry Group. Scopolamine HBr, carbachol, noradrenaline and serotonin were purchased from Sigma (St

Quentin Fallavier, France). The vehicle and volume of injection used is indicated for each study. All doses refer to salt except where stated.

Animals

Unless otherwise stated, all animals were obtained from Charles River Laboratories (two sources were used: Iffa-Credo, L'Arbresle, France and Charles River, St. Aubin les Elbeuf, France). Rats arrived at our animal quarters at least one week before use and were housed in groups of 2-5 per cage (see individual experimental methods for details). The beagle dogs used for the cardiovascular studies were obtained from Marshall Farms (North Rose, NY, USA) and acclimatised to our animal quarters where they were individually housed for at least 10 days prior to use. All animals were kept under standard conditions of humidity and temperature with lights on between 0700 and 1900. Food and water were freely available except in the case of the aged Wistar rats which were placed on a restricted diet of 30 min access to food per day.

The protocols used in these experiments have been approved by the Comité Expérimentation Animale (Animal Care and Use Committee) of Sanofi-Synthélabo Recherche. Furthermore, our animal facilities and animal care and use programmes are in accordance with the principles laid down in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and its appendix.

Results

Radioligand binding studies

SL65.0155 demonstrated high affinity for the human 5-HT_4 receptor (Table 1). In addition it was found to be extremely selective for this subtype, with more than a 1000-fold difference in affinities for the 5-HT_4 receptor compared to all other 5-HT receptors (Table 1). These studies used the 5-$HT_{4(e)}$ splice variant of the human 5-HT_4 receptor.

-- Table 1 about here --

In a separate series of studies, the affinity of SL65.0155 was evaluated at several of the splice variants of the human 5-HT_4 receptor. SL65.0155 displaced binding to the (b), (c), (d) and (e) isoforms of the 5-HT_4 receptor with affinities of 4.7±0.6; 6.1±2.5; 7.0±1.0 and 16.0±3.0, respectively (all values are Ki in nM). Although these values are slightly higher than that of the initial study, this experiment demonstrates that SL65.0155 does not discriminate between isoforms of the 5-HT_4 receptor.

In contrast to its high affinity for 5-HT_4 receptors, SL65.0155 was largely devoid of activity (Ki > 1 µM) at most of the other 100 binding sites studied (CEREP and MDS Pharma service binding profiles, data not shown).

The only receptors/binding sites for which SL65.0155 had significant affinity were the rat sigma receptor (Ki = 94 nM), the human noradrenergic α_{1D} receptor (Ki = 111 nM), the dopamine $D_{4.4}$ receptor (Ki = 129 nM) and the histamine H1 receptor (Ki = 171 nM).

Functional studies at the 5-HT$_4$ receptor

Stimulation of cAMP production in CHO cells expressing splice variants of the human 5-HT$_4$ receptor

In cells expressing the 5-HT$_{4(a)}$ variant of the 5-HT$_4$ receptor, none of the concentrations of SL65.0155 increased cAMP production and actually reduced basal conversion of ATP to cAMP with a maximal reduction of 32% at 100 nM. This inverse agonist effect was confirmed by the ability of SL65.0155 to antagonise the effect of 5-HT (100 nM) with a Kd of 2.6 nM.

In contrast to its antagonist activity against the 5-HT$_{4(a)}$ variant, SL65.0155 behaved as a partial agonist in cell lines expressing either the 5-HT$_{4(b)}$ or 5-HT$_{4(e)}$ isoform of the human 5-HT$_4$ receptor, stimulating cAMP production to a lesser extent than 5-HT (table 2).

-- Table 2 about here --

Rat esophagus

SL65.0155 was without agonist activity in this preparation, but antagonised the effect of 5-HT with a pK$_b$ of 8.81.

Guinea pig ileum

SL65.0155 was without contractile effects on the guinea-pig ileum preparation. However, pre-exposure to SL65.0155 resulted in a rightward shift of the concentration-response curve to 5-HT with no depression of the maximum response, consistent with competitive antagonism, and allowing the calculation of a pA$_2$ value of 8.29.

Cognitive effects

Object recognition task

This test exploits the tendency of rats to preferentially explore novel elements of their environment. Thus, when a rat is presented with both a novel and a recently presented familiar object, it will spend significantly more time exploring the novel object. If this discrimination task is carried out 24 h after the presentation of the familiar object however, the rat will no longer differentially explore the two objects, as seen in the 24 h control group (Figure 2A). Treatment with either tacrine or SL65.0155 prior to each of the three sessions reinstated recognition of the previously exposed object as demonstrated by significantly more exploration of the novel one (Figure 2). Although the magnitude of the effect was similar for both compounds, SL65.0155 was considerably more potent (minimal effective dose = 0.001 mg/kg p.o. or i.p.) and had a much wider range of active doses than did tacrine (doses of tacrine higher than 2 mg/kg could not be tested due to the appearance of cholinergic side-effects). SL65.0155 showed similar potency and activity via both the i.p. and p.o. routes of administration.

-- Figure 2 about here --

In order to investigate the mechanism of action of SL65.0155 in this task we evaluated its interaction with the 5-HT_4 receptor antagonist SDZ 205,557. SDZ 205,557 at a dose of 1 mg/kg i.p. was without effect in this task (Figure 3), whereas SL65.0155 again showed marked activity at 0.01 mg/kg po. Co-treatment with SDZ 205,557 completely abolished the ability of SL65.0155 to facilitate discrimination of the novel and familiar objects.

-- Figure 3 about here --

The possible interaction between SL65.0155 and the cholinesterase inhibitor rivastigmine was examined using doses of each compound (0.0001 mg/kg p.o. and 0.03 mg/kg i.p., respectively) that had no significant effect on object discrimination 24h after the training trial. However, when these two doses were combined, animals demonstrated a significant discrimination between familiar and novel objects suggesting an improvement in retention (Figure 4).

-- Figure 4 about here --

Linear maze task

Aged rats show a marked deficit in the acquisition of this task compared to young rats (2-3 months) as shown in the inset to Figure 5. Not only do they show much slower learning of the task, requiring many more trials to achieve asymptotic levels of performance, but they also continue to make more errors per trial after stable performance has been reached. Pretreatment of aged rats prior to each trial with either SL65.0155 (0.01 and 0.1 mg/kg i.p.) or rivastigmine (0.1 and 0.25 mg/kg i.p.) improved task performance as measured by both these parameters: both the initial rate of learning and the asymptotic level of performance were significantly better than aged animals receiving vehicle (Figure 5).

-- Figure 5 about here --

Reversal of scopolamine-induced deficits in the water maze in mice

Scopolamine pretreatment (0.3 mg/kg i.p.) induced a significant acquisition deficit in the water maze task as demonstrated by the increased latency to find the platform compared to vehicle-treated mice (control vs. scopolamine treated groups: $F(1,16) = 54.6$, $p<0.0001$; Figure 6). This effect was particularly marked on the second and third day of training and overall latencies collapsed across trials was also significantly increased. Similar differences were observed for distance ($F(1,16) = 66.4$, $p<0.0001$, not shown) and scopolamine-treated mice swam slightly, but significantly, faster during the third and fourth sessions.

-- Figure 6 about here --

Co-administration of SL65.0155 at both 0.1 and 0.3 mg/kg i.p. significantly attenuated the scopolamine-induced deficit, as demonstrated by the significant ANOVA comparison (latency: $F(2,24) = 8.14$, $p<0.01$; distance: $F(2,24) = 4.32$, $p<0.05$), with the higher dose having a slightly greater effect as indicated by the post-hoc comparisons shown in Figure 6. Examination of Figure 6 shows that the mice treated with SL65.0155 learned the task faster than the scopolamine-treated group, an interpretation supported by the significant session x treatment interaction ($F(6,72) = 2.74$, $p<0.05$). In contrast to its effects on acquisition of the water maze task, scopolamine had no effect on retention (as measured by relative exploration of maze quadrants during the probe trial, data not shown), showing that although scopolamine had retarded acquisition, the animals were still using a spatial strategy to locate the platform. Finally, none of the groups differed in their ability to swim to the visible platform, all groups having average latencies less than 10 s.

Improvement of social olfactory recognition in rats.

The promnesic effects of SL65.0155 are presented in Figure 7. Increasing doses of SL65.0155 reduced the amount of time spent investigating the juvenile during the second presentation as indicated by the significantly decreased RID values (Kruskal-Wallis test for repeated measures: Chi-square value = 17.26 (4 degrees of freedom), $p<0.01$, $n=10$). Post-hoc Wilcoxon comparisons between groups indicated that RIDs were significantly decreased compared to control values at 0.01 and 0.03 mg/kg.

-- Figure 7 about here --

Side effect liability

Irwin test

No deleterious effects of SL65.0155 were observed up to the highest dose tested (10 mg/kg p.o.) on any of the parameters measured. This represents a dose more than 1,000-fold greater than that found to be active in the object recognition task.

Cardiovascular effects in conscious normotensive rats

Neither heart rate nor blood pressure were modified by SL65.0155 at any of the tested doses (0.1 - 10 mg/kg p.o.) in comparison with rats receiving vehicle.

Haemodynamic profile in anesthetized dogs

No changes in arterial blood pressure, pulmonary artery pressure, left ventricular pressure (and derived parameters), blood flows or heart rate were observed at any of the tested doses of SL65.0155 (0.01 - 0.5 mg/kg i.v.).

Effect of SL65.0155 on gastric motility

SL65.0155 had no significant effects on gastrointestinal motility in rats. Control animals had 85±2 % of their intestine coloured by charcoal compared to 88±2, 87±2 and 90±1 for the 0.3, 3 and 30 mg/kg treated animals respectively.

Discussion

The present results characterise SL65.0155 as a potent and selective ligand for the 5-HT_4 receptor which, in common with several other 5-HT_4 compounds, shows robust cognition enhancing activity across a range of tasks in rodents. This activity was comparable in magnitude to that seen with acetylcholinesterase inhibitors, currently the only class of compound available for the symptomatic treatment of Alzheimer's disease, but was maintained over a much broader dose-range. In functional tests, SL65.0155 behaved as a competitive antagonist when evaluated in the rat esophagus and guinea-pig ileum preparations but demonstrated a broader range of functional interaction with the 5-HT_4 receptor when studied against individual 5-HT_4 receptor splice variants. This activity ranged from inverse agonism at the 5-$HT_{4(a)}$ isoform to partial agonist activity (43 - 48% of the maximal activity of 5-HT) at the 5-$HT_{4(b)}$ and 5-$HT_{4(e)}$ isoforms (see below for further discussion). Thus, unlike other 5-HT_4 compounds which have demonstrated cognition enhancing activity, SL65.0155 demonstrated much less intrinsic activity. Compounds such as BIMU-1, BIMU-8 and RS67333 all show moderate to marked efficacy in comparable *in vitro* preparations ranging from 50 to 90% of that obtained with 5-HT (Eglen et al., 1995; Mialet et al., 2000a), increasing the likelihood that they will show side-effects. Pre-clinical studies with 5-HT_4 compounds and clinical

experience with compounds possessing 5-HT_4 agonist activity (such as cisapride) suggest a propensity to induce tachycardia, increased gastrointestinal activity and urinary incontinence. SL65.0155 clearly lacks cardiovascular and gastrointestinal effects, consistent with its antagonist activity in isolated peripheral tissues. The data presented here therefore demonstrate not only that SL65.0155 is an effective cognition enhancer with a novel mechanism of action but also suggest that it will have a better clinical side-effect profile than currently available treatments for memory deficits.

Given that the literature supports a role for *agonist* effects at the 5-HT_4 receptor in mediating pro-cognitive effects (see Introduction) and that the effect of SL65.0155 in the object-recognition task was antagonised by the selective 5-HT_4 receptor antagonist SDZ 205,557, it seems reasonable to conclude that SL65.0155 is also acting as an agonist to improve learning and memory performance. The absence of promnesic effects of SDZ 205,557 also supports this suggestion. The partial agonist effect demonstrated in some of the *in vitro* functional tests is therefore sufficient to produce a significant promnesic activity. Given the clear absence of any of the peripheral effects normally associated with 5-HT_4 agonists it is tempting to speculate further that SL65.0155 might have greater efficacy at central 5-HT_4 receptors (ie acting as an agonist or partial agonist) than at peripheral 5-HT_4 receptors, where it appears to act as an antagonist or at least lacks functional agonist activity.

Data obtained in the studies evaluating SL65.0155 at individual splice variants of the 5-HT_4 receptor provide evidence that such a functional dissociation between differently located 5-HT_4 receptors is a possibility. In humans, at least eight splice variants of the 5-HT_4 receptor have been identified to date (labelled a - h), all

differing in their C-terminal region (except the h isoform) and resulting in receptors with different degrees of intrinsic coupling to their second messenger systems (Blondel et al., 1998; Claeysen et al., 1999). Corresponding isoforms have been reported in rats and mice, where the isoforms originally referred to as 5-HT_{4S} and 5-HT_{4L} correspond to human 5-$HT_{4(a)}$ and to 5-$HT_{4(b)}$, respectively (Claeysen et al., 1996; 1997). Although the data currently available suggests a heterogeneous distribution of mRNA for the different isoforms in brain regions and peripheral tissues, none are purely central or peripheral, apart from the 5-$HT_{4(d)}$ which is limited to the intestine, and no brain region expresses only a single isoform (Bender et al., 2000; Blondel et al., 1998; Claeysen et al., 1999; Gerald et al., 1995; Medhurst et al., 2001; Ullmer et al., 1996).

Although there is increasing evidence for pharmacological differences between isoforms, it is interesting that this is manifested not so much in terms of affinity, as few compounds appear to discriminate between the isoforms in binding studies , but in terms of compound efficacy in stimulating coupling to adenylate cyclase (Blondel et al., 1998; Bender et al., 2000; Mialet et al., 2000a; 2000b; Pindon et al., 2002). The data presented here supports this: SL65.0155 did not differ markedly in its affinity for different isoforms of the human 5-HT_4 receptor (when measured under similar conditions) but showed marked variation in intrinsic activity. This ranged from inverse agonism at the 5-$HT_{4(a)}$ variant to partial agonist activity at the 5-$HT_{4(b)}$ and 5-$HT_{4(e)}$ variant. Although these experiments evaluating the functional interaction of SL65.0155 with splice variants of the 5-HT_4 receptor were not carried out under identical conditions they nevertheless demonstrate that under certain circumstances SL65.0155 is capable of interacting with 5-HT_4 receptors as either a partial agonist or an antagonist.

It thus seems likely that SL65.0155 could have sufficient agonist activity at one of the 5-HT_4 receptor splice variants to elicit an improvement in cognition, but insufficient agonist activity at any isoform to elicit unwanted side-effects, particularly in peripheral tissues. Whether this functional selectivity reflects a difference in the way SL65.0155 interacts with the various 5-HT_4 receptor isoforms or a difference in the local environment around central and peripheral 5-HT_4 receptors that affects efficiency of coupling remains to be determined. Whatever the mechanism, the data presented in this paper suggest that SL65.0155 can be described as a functionally selective partial agonist for those 5-HT_4 receptor isoforms mediating improved cognition.

The improvements in learning and memory induced by SL65.0155 were observed in several tests assessing different areas of cognitive performance and against a variety of deficits. The object recognition task assesses recognition memory in young, nominally unimpaired, rats but even under these conditions SL65.0155 improved performance at 24 h when control animals no longer discriminated between objects, i.e. they could no longer recognise one as familiar and one as novel. Reversal of a recognition deficit was also observed in the social recognition test, where SL65.0155 improved the ability of an adult rat to remember a juvenile rat presented 2 h earlier. In the linear maze, aged animals demonstrate a marked deficit in their ability to remember a sequence of left right turns. Such a deficit might reflect an inability to remember sequences or lists, but might also include an element of disorientation: both features of cognitive decline in age and Alzheimer's disease. It is also important to note that in this experiment rats received twenty administrations of SL65.0155 over 10 days and that the cognitive improvement was maintained over this period. Finally, SL65.0155 was also effective

against the cognitive-deficit induced by the muscarinic acetylcholine receptor antagonist scopolamine in the Morris water maze task for spatial memory. Scopolamine induces a cognitive deficit in man and has been used as a means of mimicking the hypo-cholinergic state of AD. These studies therefore suggest that SL65.0155 has a broad potential for treating a variety of cognitive deficits across a number of disease states.

One area of potential difficulty for the therapeutic application of 5-HT_4 compounds as cognition enhancing agents is that of side-effect liability (Eglen et al., 1995). In particular, compounds with agonist activity are likely to have both unwanted gastric and cardiovascular effects. In keeping with its antagonist profile as measured in the rat esophagus and at the 5-$HT_{4(a)}$ isoform, SL65.0155 was devoid of 5-HT_4 agonist-like actions in two studies evaluating a wide range of cardiovascular parameters in rats and in dogs. Similarly, it had no gastrointestinal prokinetic activity as measured by intestinal transit times in the rat. This absence of agonist-like activity at doses over 1000-fold higher than those active in cognitive tests demonstrates a wide safety margin and also reinforces the suggestion that SL65.0155 has some degree of functional selectivity for CNS 5-HT_4 receptors. It is also important to note that despite the moderately high affinity of SL65.0155 for α1 adrenoceptors, there were none of the unwanted effects (particularly cardiovascular) normally associated with antagonists at this receptor and in the rat vas deferens it only weakly antagonised (IC_{50}>1 µM) the effect of noradrenaline.

Despite the recent advances in our understanding of Alzheimer's disease there is still a clear need for symptomatic treatments that act via different mechanisms to those currently available. Such compounds not only offer the prospect of improved efficacy and reduced side-effect liability but may also be useful

as add-on therapies for patients already receiving cholinesterase inhibitors in order to enhance the modest improvements in cognitive function reported with this class of compounds. This is particularly supported by our demonstration that SL65.0155 can act synergistically with rivastigmine. A similar finding was recently reported with the 5-HT_4 agonist RS67333 and a galanthamine analogue in a place and object recognition task (Lamirault and Simon, 2001).

In conclusion, the data presented here demonstrates that SL65.0155 has potent and robust cognitive enhancing activity which is mediated by an agonist activity at 5-HT_4 receptors. This is supported by the partial agonist activity of SL65.0155 at the (b) and (e) isoforms of 5-HT_4 receptors *in vitro* and by the ability of a 5-HT_4 antagonist to reverse the cognition-enhancing activity of SL65.0155. In contrast, SL65.0155 acts as an antagonist at 5-HT_4 receptors in peripheral tissues *in vitro* and at the cloned (a) isoform of 5-HT_4 receptors. This functional selectivity is almost certainly responsible for the absence of gastrointestinal and cardiovascular effects and suggests that SL65.0155 will have a low propensity for side effects in the clinic. We propose that SL65.0155 represents a promising new agent for the treatment of patients suffering from memory deficits or dementia.

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Figure Legends

Figure 1. The structure of SL65.0155

Figure 2. Effect of SL65.0155 on object recognition performance in the young rat. Introducing a 24 h delay between test and recall sessions induced a marked decrease in discrimination between familiar and novel objects (panel A). This deficit was reversed by either i.p. (panel B) or p.o. (panel C) administration of SL65.0155. Oral tacrine was also effective in improving object recognition (panel D). The black and grey columns show exploration time for the familiar and novel object respectively. * $p<0.05$; ** $p<0.01$ paired t-test comparison between familiar and novel object exploration following a significant ANOVA.

Figure 3. Reversal of the promnesic effect of SL65.0155 (0.1 mg/kg p.o.) in the object recognition task by the 5-HT_4 antagonist SDZ 205,557 (1 mg/kg i.p.). ** $p<0.01$ paired t-test comparison between familiar and novel object exploration following a significant treatment by object interaction by ANOVA.

Figure 4. Synergism between SL65.0155 (0.0001 mg/kg p.o.) and rivastigmine (0.03 mg/kg ip) in the object recognition test. ** $p<0.01$ paired t-test comparison between familiar and novel object exploration following a significant treatment by object interaction by ANOVA.

Figure 5. Effect of SL65.0155 and rivastigmine on linear maze performance in the aged rat. The insert in the right-hand panel shows the deficit in aged rats compared to young animals. Statistical analysis of the SL65.0155 data (following a significant ANOVA result for factor treatment) revealed that both doses significantly improved performance: 0.01 mg/kg $P<0.05$, 0.1 mg/kg $p<0.01$. A similar analysis of the rivastigmine data revealed that the group receiving 0.1 mg/kg differed from the controls ($p<0.05$) but that the higher dose, 0.25 mg/kg just failed to reach significance ($p=0.058$).

Figure 6. Reversal of the scopolamine-induced deficit of water-maze performance in mice by SL65.0155. The value for each session is the mean of four trials per animal. The insert shows the data collapsed over all four acquisition trials. ## $p<0.01$ compared to vehicle treated controls; * $p<0.05$, ** $p<0.01$ compared to scopolamine alone, Dunnett's test following significant ANOVA.

Figure 7. Promnesic effect of SL65.0155 in the social olfactory recognition test in rats. SL65.0155 was administered immediately after the first presentation of the juvenile rat. The results are expressed as the ratio of the time spent investigating the juvenile during the second presentation compared to the first (ratio of investigation duration, RID) and are the means of 10 rats, each tested under each of treatment conditions. The time spent investigating the juvenile during the first period did not differ significantly between groups (the values for the five treatment conditions were: 98.3 s, 103.1 s, 86.1 s, 103.7 s and 110.5 s, Kruskall-Wallace comparison Chi-squared = 3.94, ns). * $p<0.05$, ** $p<0.01$ compared to control group (black histogram), Wilcoxon test).

Table 1. Affinity of SL65.0155 for human recombinant 5-HT receptor subtypes. All binding studies were carried out by Cerep except for 5-HT_{1D}, 5-HT_3, and the 5-HT transporter which were carried out by MDS Panlabs (see www.mdsps.com and www.cerep.com for further experimental details).The cell type used for expression is indicated: CHO = chinese hamster ovary, HEK = human embryonic kidney. All ligands were tritiated except RTI-55 which was iodinated. GR125743: N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl) benzamide; GR65630: 3-(5-methyl-1H-imidazol-4-yl)-1-(1-methyl-1H-indol-3-yl)-1-propanone; GR113808: [1-[2-methylsulphonylamino ethyl]-4-piperidinyl]methyl 1-methyl-1H-indole-3-carboxylate; RTI-55: 3 beta-(4-iodophenyl) tropane-2 beta-carboxylic acid methyl ester.

Receptor	**Cell type**	**Ligand and concentration (nM)**		**Affinity of SL65.0155 (Ki, nM)**
5-HT_{1A}	CHO	8-OH-DPAT	0.3	1020
5-HT_{1B}	CHO	GR125743	0.5	5500
5-HT_{1D}	CHO-K1	5-CT	0.5	7990
5-HT_{2A}	CHO	ketanserin	2	1320
5-HT_{2B}	CHO-K1	LSD	1.2	1560
5-HT_{2C}	CHO	mesulergine	0.7	4780
5-HT_3	HEK-293	GR65630	0.69	>10000
5-$HT_{4(e)}$	CHO	GR113808	0.2	0.6
5-HT_{5A}	HEK-293	LSD	1	>10000
5-HT_6	HEK-293	LSD	2	2580
5-HT_7	CHO	LSD	4	3260
transporter	HEK-293	RTI-55	0.15	905

Table 2. Stimulation of cAMP production in CHO cells expressing either the 5-$HT_{4(b)}$ or 5-$HT_{4(e)}$ splice-variant of the human 5-HT_4 receptor

	5-$HT_{4(b)}$		**5-$HT_{4(e)}$**	
	EC_{50} (nM)	maximal effect	EC_{50} (nM)	maximal effect
5-HT	28	1.00	0.8	1.00
SL65.0155	244	0.48	29	0.43

Figure 1

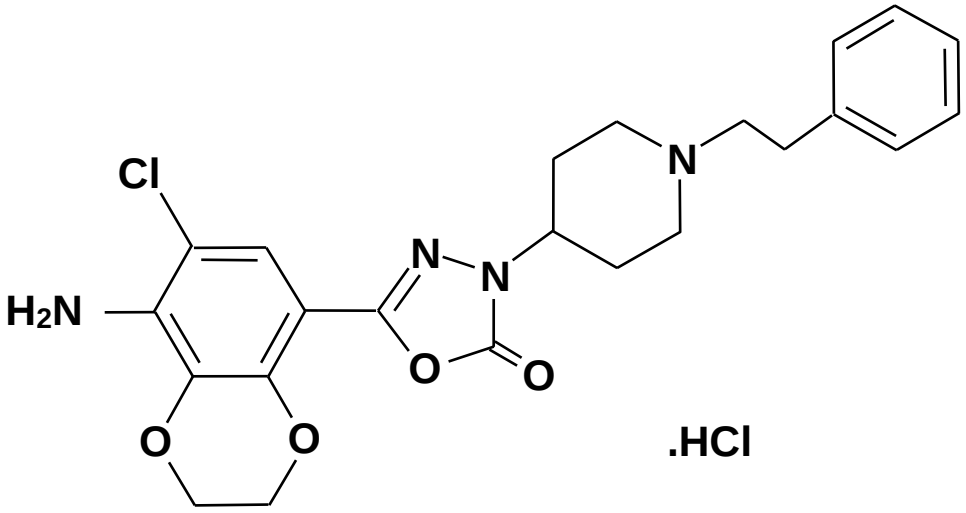

Figure 2

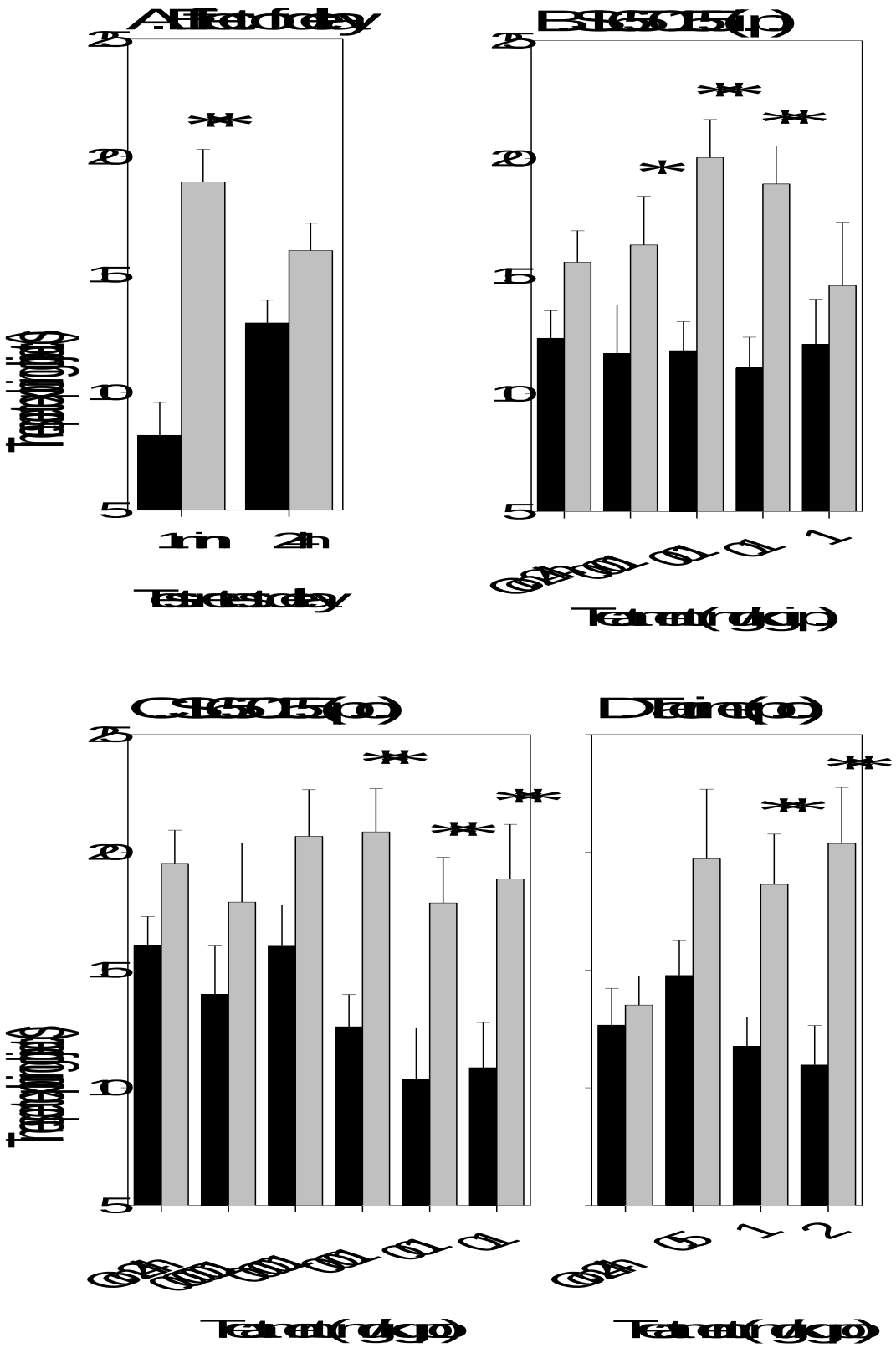

Figure 3

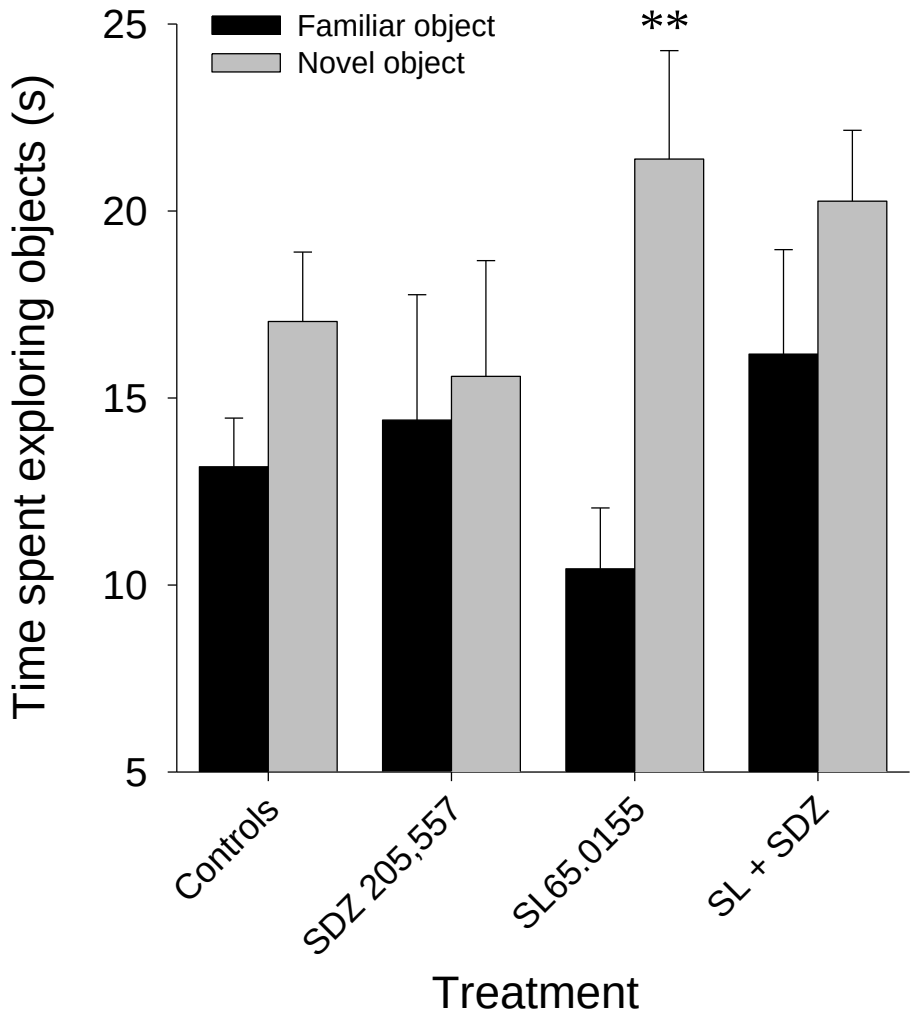
Familiar object
Novel object
**
Time spent exploring objects (s)
25
20
15
10
5
Controls
SDZ 205,557
SL65.0155
SL + SDZ
Treatment

Figure 4

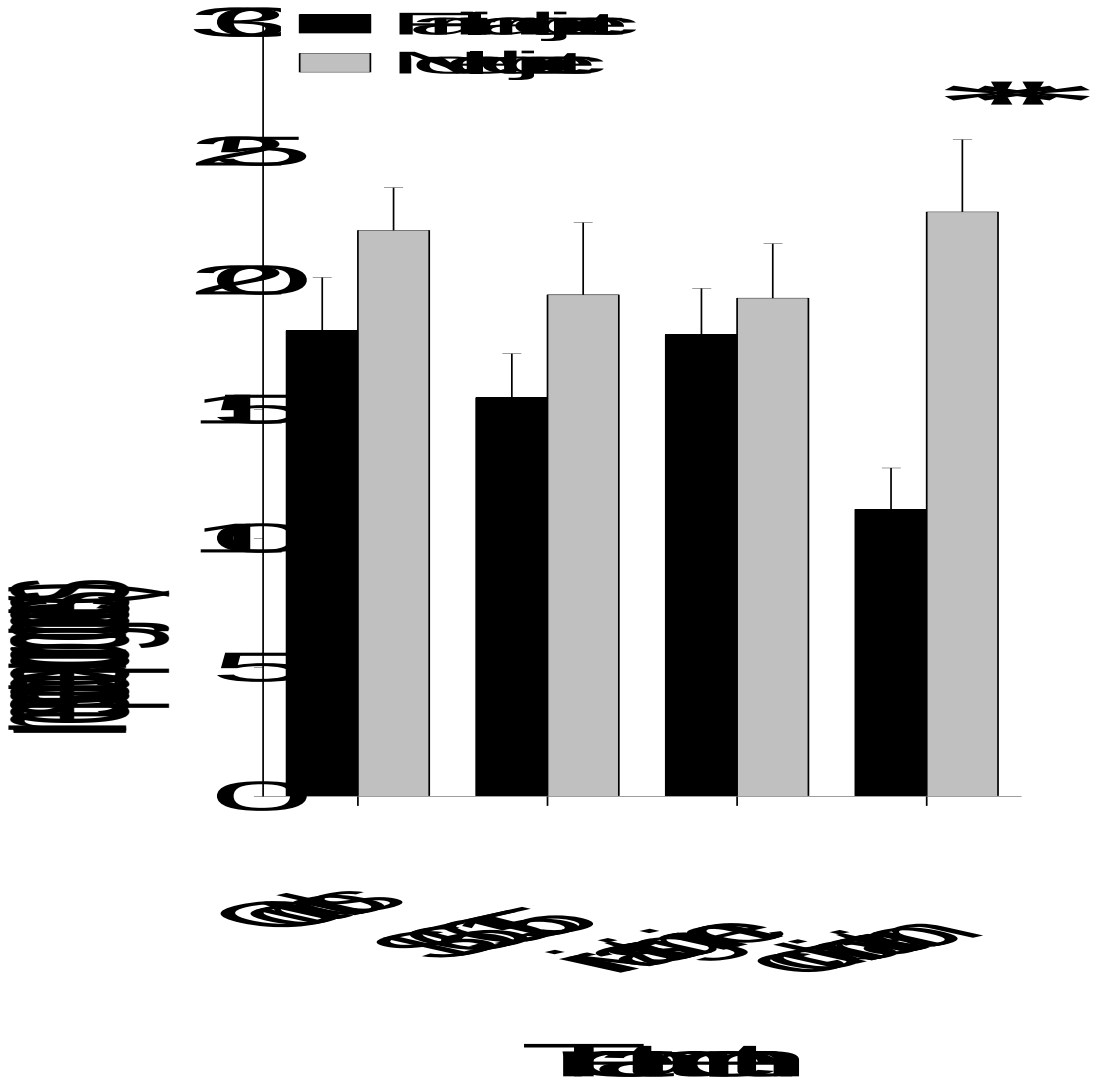

Figure 5

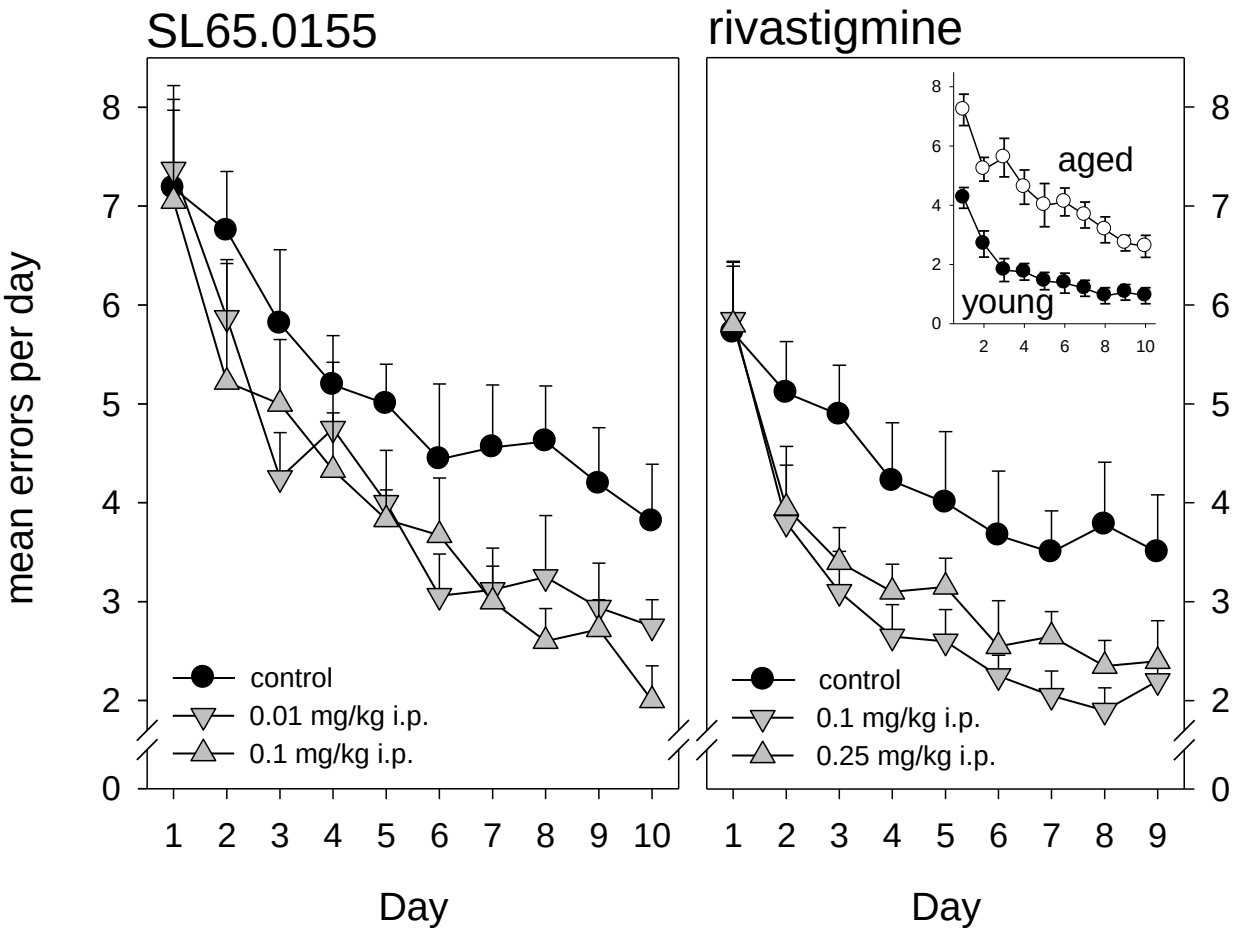
SL65.0155
rivastigmine
mean errors per day
Day
Day
control
0.01 mg/kg i.p.
0.1 mg/kg i.p.
control
0.1 mg/kg i.p.
0.25 mg/kg i.p.
aged
young

Figure 6

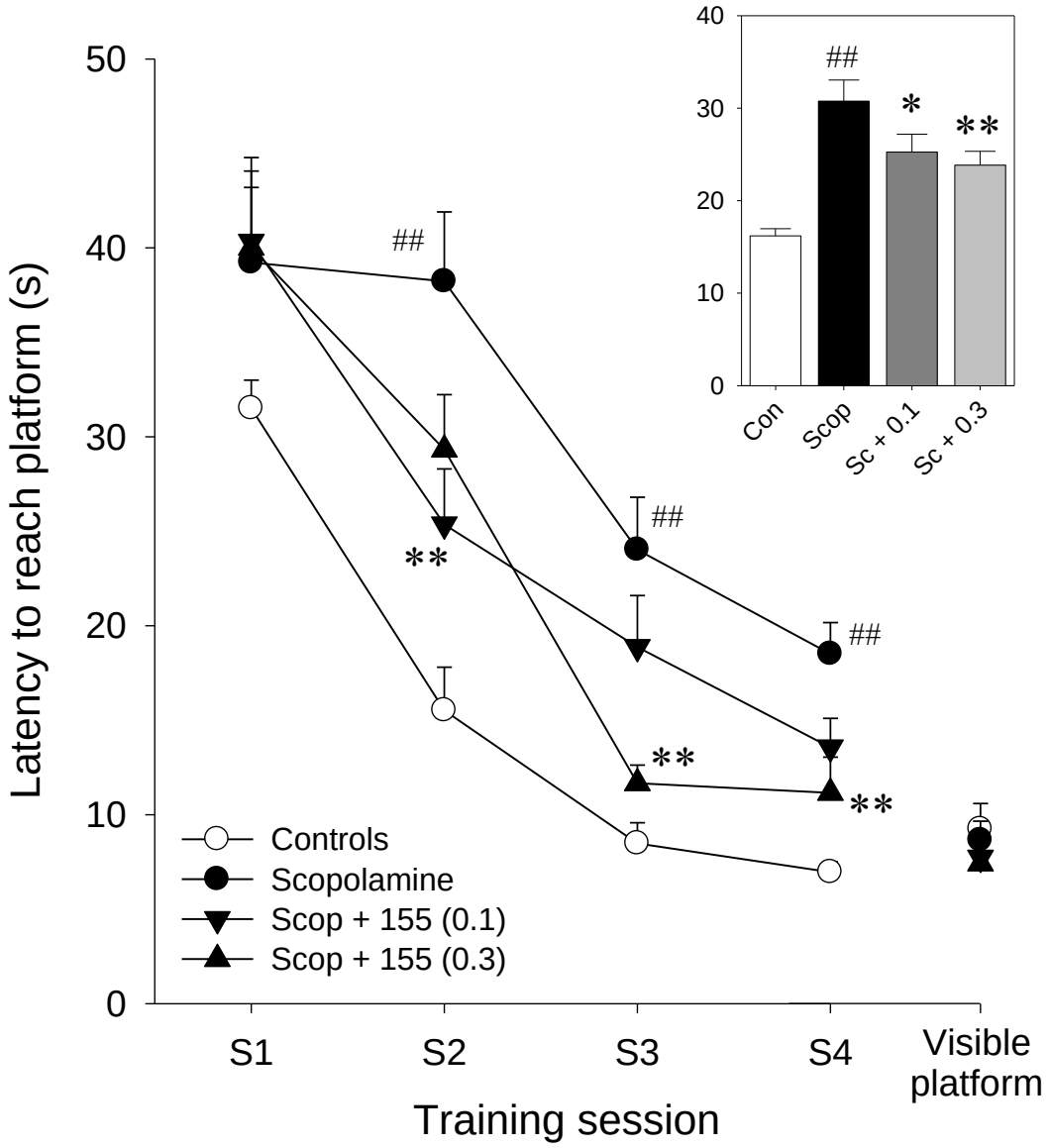
Latency to reach platform (s)
50
40
30
20
10
0
S1
S2
S3
S4
Visible platform
Training session
Controls
Scopolamine
Scop + 155 (0.1)
Scop + 155 (0.3)
##
**
40
30
20
10
0
Con
Scop
Sc + 0.1
Sc + 0.3
*

Figure 7