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SCH 57790, a selective muscarinic M_2 receptor antagonist, releases acetylcholine and produces cognitive enhancement in laboratory animals

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Abstract

The present studies were designed to assess whether the novel muscarinic M_2 receptor antagonist 4-cyclohexyl-α-[4[[4-methoxyphenyl]sulphinyl]-phenyl]-1-piperazineacetonitrile (SCH 57790) could increase acetylcholine release in the central nervous system (CNS) and enhance cognitive performance in rodents and nonhuman primates. In vivo microdialysis studies show that SCH 57790 (0.1–10 mg/kg, p.o.) produced dose-related increases in acetylcholine release from rat hippocampus, cortex, and striatum. SCH 57790 (0.003–1.0 mg/kg) increased retention times in young rat passive avoidance responding when given either before or after training. Also, SCH 57790 reversed scopolamine-induced deficits in mice in a passive avoidance task. In a working memory operant task in squirrel monkeys, administration of SCH 57790 (0.01–0.03 mg/kg) improved performance under a schedule of fixed-ratio discrimination with titrating delay. The effects observed with SCH 57790 in behavioral studies were qualitatively similar to the effects produced by the clinically used cholinesterase inhibitor donepezil, suggesting that blockade of muscarinic M_2 receptors is a viable approach to enhancing cognitive performance.

Keywords: Muscarinic M_2 receptor antagonist; Acetylcholine; Cognitive enhancer; (Rodent); (Primate)

1. Introduction

Augmentation of cholinergic neuronal systems within the central nervous system (CNS) has been shown to improve cognitive performance in rats (Baratti et al., 1979; Smith et al., 1996), and monkeys (Aigner and Mishkin, 1986; Rupniak et al., 1989, 1997). In addition, increasing acetylcholine neurotransmission by blockade of cholinesterase enzymes with drugs such as physostigmine, tacrine, donepezil, and rivastigmine has been associated with modest improvements in memory function in both healthy volunteers (Davis and Mohs, 1982) and patients with Alzheimer's disease (Christie et al., 1981; Farlow et al., 1992; Rogers and Friedhoff, 1998; Rösler et al., 1999). However, in Alzheimer's patients, the use of this class of drug has been restricted due to both mechanism- and nonmechanism-based side-effects that limit patient tolerability (Beerman, 1993; Watkins et al., 1994). The difficulty with compliance, as well as questionable efficacy in some patients, has resulted in a continued search for better pharmacotherapies for the treatment of the cognitive decline associated with Alzheimer's disease and other dementias.

Subtypes of acetylcholine muscarinic receptors have been characterized and designated muscarinic M_1–M_5 (Wess, 1996). Results from behavioral studies suggest that alterations in memory and learning are mediated primarily via receptors of the muscarinic M_1 subtype (Hunter and Roberts, 1988; Messer et al., 1990). Muscarinic M_1 receptors are located postsynaptically and activation of these receptors with direct and indirect acting agonists has been reported to lessen the cognitive decline associated with early Alzheimer's disease (Christie et al., 1981; Farlow et al., 1992; Soncrant et al., 1993). In the CNS, muscarinic M_2 receptors appear to be predominately located on presy-

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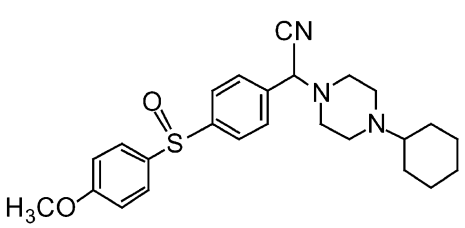
naptic terminals of acetylcholine containing neurons (Levey et al., 1995). Moreover, pharmacological and electrophysiological studies suggest these M_2 receptors serve as autoreceptors to regulate acetylcholine release, such that stimulation results in decreased cholinergic transmission (Consolo et al., 1987; Egan and North, 1986; McKinney et al., 1993; Pohorecki et al., 1988; Stillman et al., 1996). Thus, an alternative mechanism by which cholinergic neurotransmission could be augmented is to facilitate its release from cholinergic presynaptic nerve terminals by blocking muscarinic M_2 receptors with selective antagonists, thereby inhibiting autoreceptor feedback and increasing acetylcholine release. Indeed, Lapchak et al. (1989), Auld et al. (2000), and Richard et al. (1991) have shown that blockade of muscarinic M_2 receptors produce increases in measured acetylcholine release both in vitro and in vivo. Additionally, Billard et al. (1995) showed using in vivo microdialysis techniques that a range of muscarinic receptor antagonists facilitated acetylcholine release from rat brain in a manner that correlated with the affinity of the antagonists for muscarinic M_2 receptors.

The present studies were designed to assess the effects of a high-affinity muscarinic M_2 receptor antagonist 4-cyclohexyl-α-[4[[4-methoxyphenyl]sulphinyl]-phenyl]-1-piperazineacetonitrile (SCH 55790) (Kozlowski et al., 2000) in both rodents and a nonhuman primate species. SCH 57790 binds to cloned human muscarinic M_2 receptors expressed in Chinese hamster ovary cells with high, nanomolar affinity and 40–50-fold selectivity over the muscarinic M_1 receptor. In pharmacological studies in vitro, it acts as a competitive antagonist at the muscarinic M_2 site (Lachowicz et al., 1999) (Fig. 1). In the present study, using in vivo microdialysis, we have investigated the ability of SCH 57790 to release acetylcholine in discrete regions of the CNS of conscious rats, and associated this release with cognitive performance in rats and mice in a variety of behavioral tests. Additionally, we have extended previous studies utilizing muscarinic M_2 antagonists in vivo by assessing the effects of SCH 57790 in a cognitive task in squirrel monkeys (Moerschbaecher et al., 1984; Pakarinen and Moerschbaecher, 1993).



SCH 57790

Fig. 1. SCH 57790, 4-cyclohexyl-α-[4[[4-methoxyphenyl]sulphinyl]-phenyl]-1-piperazineacetonitrile.

2. Materials and methods

2.1. Animals and housing

Male Sprague–Dawley rats [30–50 g (passive avoidance responding) or 250–350 g (microdialysis, Irwin, cardiovascular)], and male Balbc mice (20–30 g) were obtained from Charles River laboratories. Mice and adult rats were housed five per cage, young rats were housed eight to ten per cage. Rats and mice were acclimated to a 12:12 h light/dark cycle (lights on at 6:00 a.m.), at a room temperature of 22–23 °C with 50% humidity for several days prior to the day of experimentation. Rats and mice were given standard rodent chow, with water available ad libitum.

Adult male squirrel monkeys (*Saimiri sciureus*) were maintained at approximately 85–90% of their free feeding weight with a diet consisting of banana-flavored food pellets (F0021, Bio-Serv) earned during the experimental session, Purina monkey chow, fruits and vegetables. Between sessions, monkeys were housed in individual home cages maintained on 12:12 h light/dark cycle (lights on at 6:00 a.m.), where they had unlimited access to water. All studies were carried out in accordance with the NIH Guide to the Care and Use of Laboratory Animals and the Animal Welfare Act, and guidelines established by Schering-Plough Research Institute and its Animal Care and Use Committee, at Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities.

2.2. Microdialysis

Male Sprague–Dawley rats (250–350 g) were anesthetized with ketamine/xylazine, and implanted with a stainless-steel guide cannula into the frontal cortex, hippocampus, and striatum using the following stereotaxic coordinates taken from bregma; cortex–probe angled 28° from vertical, A/P +1.2 mm, L ±1.3 mm, D/V −2.5 mm; hippocampus, probe angled 18° from vertical, A/P −3.8 mm, L −2.5 mm, D/V −2.5 mm; striatum A/P +1.0 mm, L ±4 mm, D/V −4 mm (Paxinos and Watson, 1997). Following at least 3 days recovery, a perfusable microdialysis probe (CMA/12, 3 × 0.5 mm, CMA, Acton, MA) was inserted through the guide cannula and into brain area of interest. Following insertion of the probe, rats were placed into a large Plexiglas chamber where the probe was perfused for 90 min to allow for equilibration (2 μl/min) with Ringer's buffer (NaCl 147 mM; KCl 3.0 mM; $CaCl_2$ 1.2 mM; $MgCl_2$ 1.0 mM) containing 1 μM neostigmine bromide (RBI, Natick, MA) at pH 7.4. Following the period of equilibration, 20-μl fractions were collected every 10 (cortex, striatum) and 30 min (hippocampus) for up to 3 h using a refrigerated collector (CMA/170, or CMA/200). The initial three or four fractions were used to calculate the baseline level of

acetylcholine. Drug or vehicle was administered between the 3rd to 5th sample times, and the effect of drug on the levels of acetylcholine was measured. Following completion of the microdialysis experiments, rats were sacrificed and probe placement was verified histologically.

The amount of acetylcholine in the collected samples was measured using high-pressure liquid chromatography (Waters, Millford, MA) with electrochemical detection. Briefly, acetylcholine was separated on an analytical ion exchange column (BAS, West Lafayette, IN), and then converted to hydrogen peroxide inside a column filled with a polymeric matrix to which acetylcholinesterase and choline oxidase enzymes had been covalently linked. The hydrogen peroxide formed was detected electrochemically by oxidation on a platinum electrode at +500 mV vs. an Ag/AgCl reference electrode. The amount of acetylcholine released was not corrected for recovery, and the effects of vehicle or drug administration were determined by expressing the amount of acetylcholine in each fraction as a percentage of the level of acetylcholine in the baseline samples.

2.3. Young rat passive avoidance responding

The rat passive avoidance responding apparatus consisted of black and clear Plexiglas chambers (20 × 20 × 24 cm) connected by a doorway (6 × 9 cm). The floor of the dark chamber was made up of stainless steel rods 5 mm in diameter spaced 1.7 cm apart, which were connected to a shock generator (Coulbourn Institute, Allentown, PA). Passive avoidance responding experiments were carried out using a procedure modified from Jarvic and Essman (1960). Briefly, 18–22 day old rats (Smith et al., 1996) were given a single training and test session. Training sessions consisted of placing rats into the light chamber facing away from the opening to the dark chamber. When rats entered the dark chamber, a guillotine door was closed and rats received a scrambled foot shock (0.5 mA) for 3 s. Rats were immediately returned to their home cages. Rats were tested for retention 24 h later. At this time, rats were placed into the light chamber and the time for each animal to move from the light chamber into the dark chamber was recorded (step-through latency). Sessions ended either when rats moved into the dark chamber or when 180 s had elapsed. Drugs were administered either before the training session (before training procedure), or immediately after rats had experienced the scrambled foot shock (after training procedure). Drugs were considered to have memory enhancing effects if they produced a statistically significant increase in step-through latency during the testing session.

2.4. Mouse passive avoidance responding

The mouse passive avoidance responding apparatus consisted of black and clear Plexiglas chambers (42 × 16 × 20 cm) connected by a doorway (2.5 × 2.5 cm). The floor of the dark chamber was made up of 3 mm diameter stainless-steel rods spaced 0.5 cm apart, which were connected to a shock generator (Coulbourn Institute). A similar experimental protocol was used for Balbc mice as for young rats. Before training, the nonselective muscarinic antagonist scopolamine (3.0 mg/kg, s.c.) was administered 10 min before various doses of SCH 57790 (0.003–0.1 mg/kg, i.p.). Following a further 10-min pretreatment, mice were placed into the light chamber of the passive avoidance responding apparatus facing away from the door to the dark chamber. When mice entered the dark chamber, a door was closed behind them and a 1-mA scrambled foot shock was delivered to the grid floor for 1 s. Following delivery of the foot shock, mice were removed from the apparatus and returned to their home cage. Retention testing took place 24 h later. Mice were placed into the light chamber and the time for each animal to move from the light chamber into the dark chamber was recorded. Sessions ended either when mice moved into the dark chamber or when 180 s had elapsed.

2.5. Fixed-ratio discrimination with titrating delay

During experimental sessions, monkeys sat in a Plexiglas primate chair (Plas-Labs, Lansing MI), located inside a ventilated, sound attenuated chamber. The chair was positioned in front of an operant response panel (Coulbourn Institute) equipped with three response levers arranged horizontally 3.5 cm apart within easy reach of the monkey. The center "response" lever was bracketed by two "choice" levers. Three stimulus lights were positioned 2.5 cm above each lever. Food pellets, to serve as reinforcers, could be delivered from a pellet dispenser into an aperture measuring 3 × 3 cm located 3 cm above the center response lever. Depression of the levers with a force of approximately 0.25 N resulted in an audible click and was counted as a response. A shielded 3 W house light was located at the top and center of the operant panel. The presentation of the schedule was controlled by a PC with SKED-11 software and interface.

Monkeys were trained to respond under a schedule of fixed-ratio discrimination with titrating delay. Under this schedule, an amber stimulus light was illuminated above the center response lever, and the monkey was required to complete one of two fixed-ratios (8 or 16) on this lever. Completion of the ratio turned off the stimulus light above the center lever and turned on amber stimulus lights above the left and right choice levers. If the ratio completed was fixed-ratio 8, a response on the left lever was designated as correct and resulted in the delivery of a food pellet and illumination of the house light for 2 s. If the ratio completed was fixed-ratio 16, a response on the right lever was designated correct and produced delivery of the food pellet reinforcer and house light illumination. Incorrect responses produced a brief 2-s time-out during which all lights were

extinguished and responding had no programmed consequences. Fixed-ratios were presented in a pseudorandom order determined by the controlling computer program, such that approximately half were fixed-ratio 8 and half were fixed-ratio 16 in any one session.

During drug testing, monkeys completed one of two fixed-ratios on the response lever, which extinguished the stimulus light above the center lever and illuminated the stimulus lights above the two choice levers. As above, a correct choice was reinforced with a food pellet and illumination of the house light for 2 s. An incorrect response resulted in all lights being extinguished for 2 s during which responding had no programmed consequences. Trials were presented in blocks of five and after completion of four of five correct choices, a 0.1-s delay was imposed between completion of the fixed-ratios on the center lever and illumination of the stimulus lights above the choice levers. If performance was correct in four of five trials at this delay, the time between completion of the fixed-ratios on the center lever and stimulus light illumination above the choice levers was increased by a further 0.3 s, for a total delay of 0.4 s. Thus, the delay was increased by 0.3-s intervals if the monkey completed four of five correct trials in a block of five. If two or more incorrect choices were made in a block of five trials, the delay was lowered by 0.3 s to the preceding delay. Thus, using this schedule, the delay between completion of the fixed-ratios on the response lever and illumination of the stimulus lights above the choice levers was titrated by 0.3-s intervals up or down as the session progressed in a manner that was dependent on the performance of individual monkeys. Test sessions were of 30-min duration, and were carried out 5 days/week. Once performance had stabilized under the schedule of fixed-ratio discrimination with titrating delay, vehicle or doses of drug were given intramuscularly or orally, 10 and 60 min before testing. Behavior was assessed as the mean delay at which monkeys could perform accurately under both the vehicle or the drug conditions. During these studies, a cross-over design was utilized such that half of the group of monkeys received drug and half received vehicle on any particular treatment day. Then, following at least 1-week washout, treatments were crossed over.

2.6. Observational studies

Behavioral, neurological, and autonomic changes following administration of drug were measured using a modified version of the method of Irwin (1964). Vehicle (0.4% methylcellulose) and doses of SCH 57790 (0.1–100 mg/kg, s.c.) were administered to male Sprague–Dawley rats. One hour following injection, a semi-quantitative scoring scale was used to assess any changes in a battery of behavioral, neurological and autonomic measures. Each treatment group consisted of six animals, with each group receiving a single dose of either vehicle or SCH 57790. In two separate groups of rats, the behavioral, neurological and autonomic changes following administration of scopolamine (0.1–100 mg/kg, s.c.) and donepezil (0.3–30 mg/kg s.c.) were assessed.

2.7. Heart rate studies

Rats were anesthetized with isofluorane following an overnight fast. The caudal artery was cannulated with PE_{50} tubing (Clay Adams, Parsippany, NJ) to allow monitoring of arterial blood pressure. The arterial line was connected to a Spectramed blood pressure transducer and the arterial waveform signal was digitized at a rate of 500 Hz by a Gould P3 Plus physiology platform (Gould Instrument Systems, Valley View, PA). Heart rate was derived from the pulsatile pressure waveform. The analog signal was displayed continuously on a direct writing chart recorder. Following catheter implantation, the rats were placed into plastic restrainers where they rapidly regained consciousness. Rats were allowed to acclimate for 60 min prior to drug administration. Hemodynamic variables were recorded prior to drug administration (basal values were determined as an average over 5 min) and as 1 min averages at 15, 30 and 45 min post dosing. Additional recordings were obtained at 30-min intervals for 4 h post dosing.

2.8. Data analysis

In microdialysis experiments, percent changes in measured acetylcholine release were analyzed for individual rats and expressed as the mean $\pm$ 1 S.E.M. for three to six rats at each treatment. Differences between vehicle and drug treated groups were analyzed using analyses of variance with Duncan's multiple range statistic. In rat and mouse passive avoidance responding and rat heart rate studies, comparisons between vehicle-treated and drug-treated animals were carried out using one analysis of variance followed, where appropriate, by post hoc Dunnett's *t*-tests. In passive avoidance responding studies, a P value of less than 0.05 was employed to denote statistical significance. In heart rate studies, P less than 0.01 was used. This allowed a 60% power to detect differences in drug and vehicle responses that were within $\pm$2 S.D.'s of one another. In monkey fixed-ratio discrimination experiments, mean delay for individual monkeys were expressed a percentage of the mean delay of the last nondrugged session before the treatment day, and averaged for the group of monkeys. The effects of vehicle or drug administration were analyzed with paired *t*-tests. In Irwin studies of behavioral, neurological and autonomic function, measures that were normally present (e.g., spontaneous activity, alertness, pupil size) were assigned a "normal" score of 0, or scores of $\pm$1, $\pm$2 and $\pm$3 indicating slight, moderate, and marked increases (+) or decreases (−) from "normality". When a measure occurred that is not normally present (e.g., convulsions, twitches), the magni-

tude of the effect was graded on a 1–3 scale. All animals were retained for 24 h following the last injection to determine whether any delayed deaths occurred. When observed, an effect was considered significant if a score of 2 or greater was recorded in 50% or more of the treated animals.

2.9. Drugs

SCH 57790 was synthesized within the chemical research division of Schering-Plough Research Institute. Donepezil (Aricept®) was generously donated by Eisai. Scopolamine was purchased from RBI. Drugs were dissolved in 0.4% methylcellulose and doses are expressed as free base in milligrams per kilogram (mg/kg). Drugs were administered via intraperitoneal (i.p.), subcutaneous (s.c.) or oral (p.o.) routes as indicated in figure legends.

3. Results

3.1. Microdialysis

Under the present experimental conditions, analysis of baseline samples revealed that average acetylcholine levels were 1.12 ± 0.16 pmol from hippocampus, 0.77 ± 0.05 pmol from frontal cortex, and 4.18 ± 0.27 pmol from striatum. Administration of SCH 57790 (0.1–10 mg/kg, p.o.) produced dose-related increases in measured acetylcholine release from the hippocampus of conscious rats (Fig. 2A). The levels of acetylcholine measured peaked 30–60 min after injection, and reached a maximum of approximately 400% of baseline levels following administration of the 10 mg/kg dose. After this time, the levels steadily declined. Also, administration of SCH 57790 (1.0–10.0 mg/kg, p.o.) produced a dose-related increase in acetylcholine release from cortex of conscious rats. The levels of acetylcholine measured peaked approximately 30–40 min following drug administration and reached a maximum of approximately 500% of control levels (Fig. 2B). Additionally, both SCH 57790 (1.0–10 mg/kg, p.o.) and nonselective muscarinic antagonist scopolamine (0.1–10 mg/kg, i.p.) produced dose-related increases in acetylcholine release from striatum (Table 1). In striatum, SCH 57790 (10 mg/kg, p.o.) increased acetylcholine release to approximately 230% of baseline. Similarly, scopolamine increased acetylcholine release from striatum such that levels were approximately 330% of baseline following administration of 10 mg/kg, i.p.

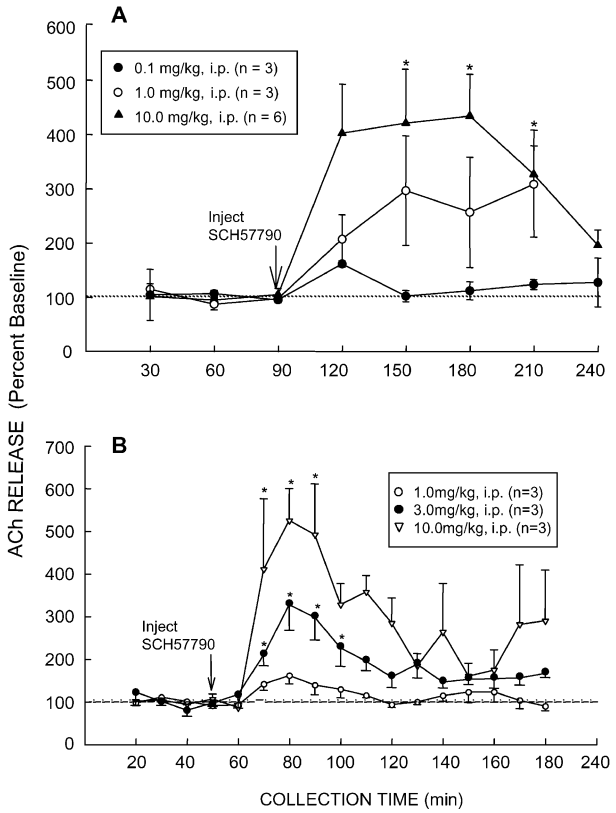


Fig. 2. Effect of SCH 57790 on acetylcholine release from rat hippocampus (A) and frontal cortex (B) following oral administration. Abscissae: time (min). Ordinates: increase in measured acetylcholine as percent of predrug baseline. Each data point is mean ± S.E.M. of three to six rats at each dose. Significant differences from predrug control are indicated by $^{*}p < 0.05$ (analysis of variance with Duncan's multiple range statistic).

3.2. Young rat passive avoidance responding

SCH 57790 produced increases in the mean step-through latency times in young rats whether given before or after the training session. In the before-training paradigm, SCH 57790 (0.003–3.0 mg/kg, p.o.) produced statistically significant increases in step-through latency following doses of 0.01 and 0.1 mg/kg [$F(7, 387) = 3.31$, $P = 0.0019$]. Under these conditions, step-through latencies increased from approximately 50 s following vehicle injections up to 86 and 92 s for the groups following injections of 0.01 and 0.1 mg/kg, respectively (Fig. 3A). When SCH 57790 was given immediately following the training session, significant elevations [$F(7, 244) = 6.29$, $P < 0.001$] in step-through latency were observed following doses of 0.3–1.0 μg/kg, p.o., which reached a maximum of approximately 100 s following an injection of 0.03 μg/kg SCH 57790 compared to a mean step-through latency of 23 s after vehicle injection. (Fig. 3B). These effects were qualitatively similar to those observed following administration of the clinically used cholinesterase inhibitor donepezil. Administration of doses of donepezil (0.01–1.0 mg/kg) before the training session resulted in significant increases in step-through latency observed 24 h later. In this regard, donepezil increased mean step-through latencies from approximately 87 s following injections of vehicle up to 168 s following a dose 1.0 mg/kg, p.o. (Fig. 3 insert).

Table 1
Effect of methylcellulose vehicle (p.o.), SCH 57790 (1.0–10.0 mg/kg, p.o.) and scopolamine (Scop) (0.1–10.0 mg/kg, i.p.) on ACh release from striatum of conscious rats

	Dose (mg/kg)	Acetylcholine release (percent baseline)								
		20 min	40 min	60 min	80 min	100 min	120 min	140 min	160 min	180 min
Vehicle		101 ± 4	100 ± 2	117 ± 4	115 ± 6	100 ± 5	109 ± 6	111 ± 10	125 ± 14	107 ± 6
SCH 57790	1.0	98 ± 3	100 ± 2	96 ± 6	121 ± 8*	124 ± 7*	125 ± 6*	116 ± 9*	113 ± 4	118 ± 5*
	3.0	93 ± 3	99 ± 4	98 ± 5	147 ± 16*	142 ± 9*	136 ± 9*	137 ± 11*	146 ± 8*	132 ± 14*
	10.0	96 ± 9	104 ± 5	103 ± 7	230 ± 23*	157 ± 20*	156 ± 20*	152 ± 12*	154 ± 12*	141 ± 9*
Scop	0.1	95 ± 2	101 ± 2	100 ± 6	112 ± 17	113 ± 19	111 ± 22	112 ± 13	101 ± 19	95 ± 16
	1.0	95 ± 4	101 ± 7	134 ± 23*	232 ± 17*	176 ± 23*	152 ± 28*	130 ± 12*	117 ± 27	94 ± 31
	10.0	103 ± 36	166 ± 34*	294 ± 11*	216 ± 16*	187 ± 14*	167 ± 19*	151 ± 12*	132 ± 9*	97 ± 5*

Data presented are in 20 min bins for brevity, and are means ± 1 S.E.M. of percent increase above predrug baseline for three rats at each dose.
* $p < 0.05$ indicates significant increase in ACh release above predrug baseline.

3.3. Mouse passive avoidance responding

During retention testing, mice that received injections of scopolamine had significantly shorter step-through latencies than mice that had received vehicle injections. Step-through latencies were decreased from approximately 150 s following saline injection to around 55 s following injections of scopolamine (3.0 mg/kg, s.c.) [$F(5, 54) = 5.52$, $P < 0.001$]. The scopolamine-induced passive avoidance responding deficit observed was dose-dependently reversed by increasing doses of the M_2 antagonist SCH 57790 (0.003–0.03 mg/kg, i.p.), such that following injection of 0.03 mg/kg SCH 57790, step-through latencies were not statistically different ($P > 0.05$) from saline treated animals and averaged 122 s for the group (Fig. 4). Doses of SCH 57790 above 0.03 mg/kg did not produce further reversal of the scopolamine-induced deficit in passive avoidance responding.

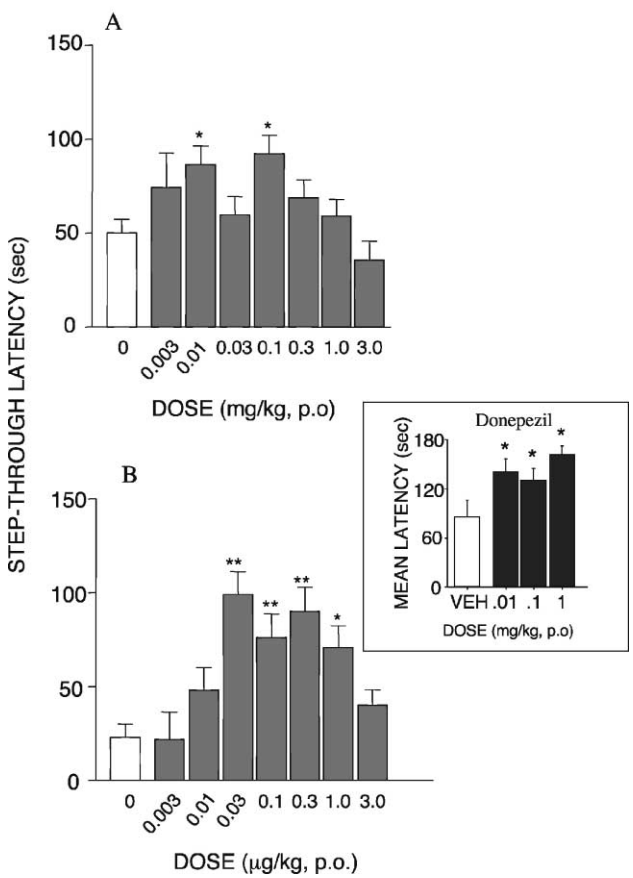


Fig. 3. Effect of SCH 57790 in a young rat passive avoidance responding assay. SCH 57790 (0.01–10 mg/kg, p.o.) was given 60 min before the training session (A), and SCH 57790 (0.03–30.0 μg/kg, p.o.) was given immediately following the training session (B). Insert indicates effects of Aricept (0.01–1.0 (mg/kg, p.o.) given before the training session. Abscissae: drug dose. Ordinates: mean step-through latency (s). Each bar indicates mean ± 1 S.E.M. step-through latency of 36 rats at each dose. Significant differences from vehicle are indicated by * $p < 0.05$ and ** $p < 0.01$ (one-way ANOVA followed by post hoc Dunnett's *t*-test).

3.4. Fixed-ratio discrimination in primates

Under control conditions, squirrel monkeys performed the fixed-ratio discrimination with titrating delay task in a

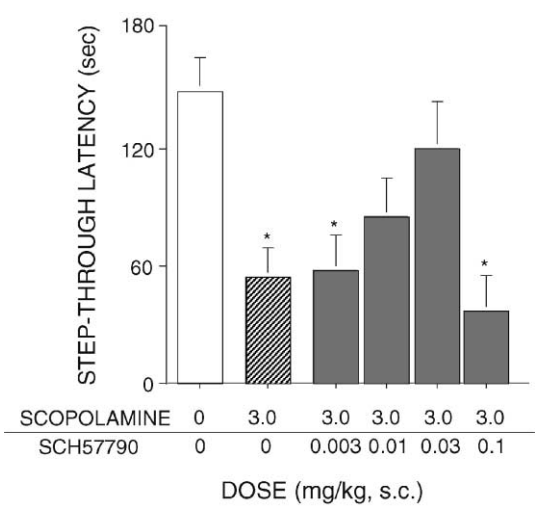


Fig. 4. Effect of SCH 57790 (0.003–0.1 mg/kg, s.c.) on passive avoidance responding deficit induced by scopolamine (3.0 mg/kg, s.c.) in male CD-1 mice. Abscissae: drug dose. Ordinates: mean step-through latency (s). Each bar indicates mean ± 1 S.E.M. step-through latency of 10 mice at each dose combination. Significant differences from vehicle are indicated by * $p < 0.05$ (one-way ANOVA followed by post hoc Dunnett's *t*-test).

manner that maintained stable baseline responding for the duration of the current studies. The mean delays between completion of the response requirements on the center lever, and the responses on the choice levers that monkeys could tolerate before inaccurate performance, varied between individuals and ranged from 0.7 up to 1.5 s for individual monkeys (data not shown).

In the present study, oral administration of 0.4% methylcellulose vehicle produced little or no change in the mean delays that monkeys could tolerate when compared to predrug control days. SCH 57790 (0.01–0.03 mg/kg, p.o.) produced a dose-related percent increases in mean delay that monkeys could tolerate and still maintain accurate performance This increase reached statistical significance ($P < 0.05$) following administration of 0.03 mg/kg and extrapolated to approximately 155% of predrug control performance (Fig. 5A). This percent increase in mean delay was qualitatively similar to the percent increases observed following administration of the cholinesterase inhibitor donepezil (Fig. 5B). In this regard, donepezil (0.3–1.0 mg/kg) produced significant increases ($P < 0.05$) in mean delay that reached a maximum of approximately 175% of predrug control levels.

In contrast, the nonselective muscarinic antagonist scopolamine (0.001–0.01 mg/kg, i.m.) produced a dose-related decrease in the accuracy of performance in this operant task such that percent mean delays decreased to approximately 36% of control values following a dose of 0.01 mg/kg (Fig. 5 insert). Additionally, increasing doses of scopolamine disrupted ongoing operant performance as measured by overall rates of responding (data not shown).

3.5. Observational studies

The behavioral, neurological and autonomic changes induced by SCH 57790 was assessed following administration of doses ranging from 0.1 up to and including 100 mg/kg. Little or no changes were observed in any measures following doses up to 3.0 mg/kg. Doses above 10.0 mg/kg appeared to produce a general increase in alertness/reactivity, with an increase in vocalization also observed. Additionally, doses of SCH 57790 above 10.0 mg/kg produced a dose-related dilation of the pupils. No tremors, convulsions or lethalities were observed in any rat at any dose of SCH 57790 (Table 2). Administration of scopolamine produced similar responses to that of SCH 57790. Doses above 10 mg/kg increased alertness and activity, and no tremors, convulsions or lethalities were observed in any rat at any dose of scopolamine up to 100 mg/kg. Following administration of 3.0 mg/kg donepezil, rats appeared to show an overall decrease in activity and alertness. Also, tremors were observed in all rats following the 3.0 mg/kg dose of donepezil. Administration of higher doses (10–30 mg/kg) produced seizures and lethality.

Table 2
Minimum effective doses (MEDs) of SCH 57790, scopolamine and donepezil to produce significant behavioral, autonomic and neurological effects in male Sprague–Dawley rats

Drug	MED (mg/kg, s.c.)					
	PAR	Arousal	Sedation	Tremors	Seizure	Leathality
SCH 57790	0.01	10.0	> 100	> 100	> 100	> 100
Scopolamine	NA	10.0	> 100	> 100	> 100	> 100
Donepezil	0.01	–[a]	3.0	3.0	10	30

NA—Not active.
PAR—passive avoidance responding.
[a] No effect measured.

3.6. Heart rate studies

SCH 57790 (1.0–10 mg/kg, s.c.) produced transient, dose-related increases in heart rate in male Sprague–Daw-

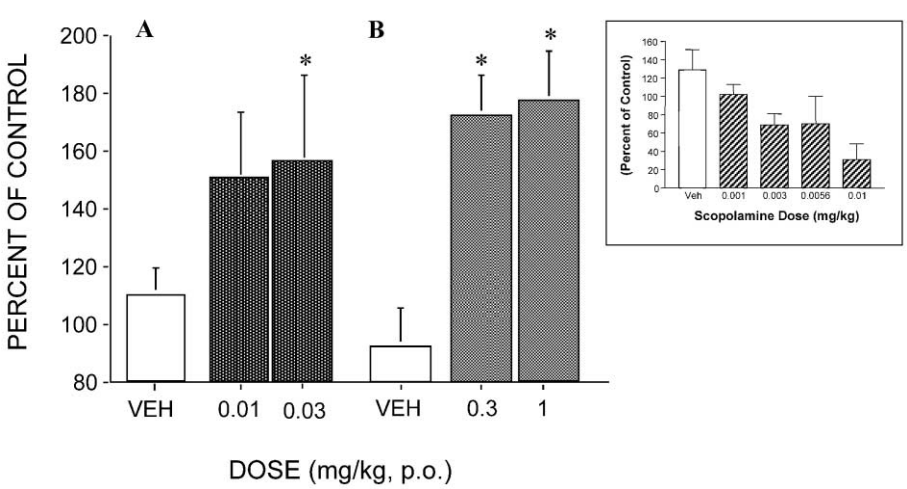


Fig. 5. Effect of (A) SCH 57790 (0.01–0.03 mg/kg p.o.) and (B) donepezil (0.3–1.0 mg/kg, p.o.) on mean delay performance in squirrel monkeys working under a schedule of fixed-ratio discrimination with titrating delay. Insert indicates effects of scopolamine (0.001–0.01 mg/kg, i.m.). Abscissae: drug dose. Ordinates: mean delay as percent of predrug control level. Data represented is mean ± 1 S.E.M. of mean titrating delay of five to seven squirrel monkeys at each dose. Significant differences from vehicle are indicated by * $p < 0.05$ (paired t-test).

Table 3
Effects of SCH 57790 (1.0–10.0 mg/kg, s.c.) and scopolamine (0.3–3.0 mg/kg, s.c.) on mean blood pressure and heart rate in conscious Sprague–Dawley rats

	MBP (mm Hg)							Heart rate (beats/min)						
	Vehicle	SCH 57790			Scopolamine			Vehicle	SCH 57790			Scopolamine		
	0 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg	0 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg
Basal	100±2	97±2	98±2	97±2	98±4	100±4	101±2	372±12	371±10	363±12	387±14	362±8	369±22	366±12
Δ at (h)														
0.25	+2±2	+2±2	+2±3	−3±1	+10±4	+10±5	+4±4	+7±13	+79±13	+102±12*	+114±27*	+143±12*	+144±26*	+134±17*
0.5	+2±3	−7±2	0±1	−5±4	+5±4	+5±5	+4±4	+7±15	+21±9	+60±15	+77±26	+98±13*	+111±30*	+103±11*
0.75	−1±2	−4±3	+2±2	−6±1	+6±2	+6±3	+2±3	−27±8	+12±11	+68±8*	+38±19*	+60±13*	+87±21*	+71±16*
1.0	+5±1	0±3	+2±2	−3±3	+3±3	+4±6	+5±3	−5±10	+12±15	+58±15	+49±16	+36±11	+55±23	+79±15*
1.5	+1±2	−2±2	+4±2	−2±2	+1±4	+8±4	+2±4	−16±8	+4±10	+58±9*	+41±17*	−3±1	+31±13	+46±18*
2.0	+5±2	+1±1	−2±2	+1±3	+4±5	+5±3	+3±3	+2±12	+16±10	+23±9	+50±18	−5±6	−11±9	+30±25
2.5	+5±2	+2±2	+1±3	−2±3	+3±6	+9±4	+6±3	+2±10	+20±17	+53±32	+21±13	−8±7	−14±13	+15±26
3.0	+3±1	+3±3	+2±2	+2±1	+2±5	+7±4	+4±3	+7±10	+17±11	+32±15	+36±17	−19±21	+16±11	+7±20
3.5	+1±2	+2±3	+2±3	+2±4	+9±3	+11±5	+7±4	−10±5	+3±9	+36±27	+38±25	−5±12	−12±30	−6±24
4.0	+7±2	+1±2	+1±1	+2±3	+4±6	+11±4	+7±4	+27±5	−3±9	+31±18	+55±27	−19±16	−21±10	−19±24

Values represent means ± SEM for five rats per treatment. An asterisk denotes a significant ($P < 0.01$, ANOVA and Dunnett's *t*-test) difference from the corresponding response in vehicle-treated animals.

ley rats. Heart rate in vehicle treated rats remained relatively stable throughout the course of the experiment. SCH 57790 (1.0 mg/kg, s.c.) did not produce a statistically significant change in heart rate. SCH 57790, 3.0 and 10 mg/kg, s.c., produced a statistically significant ($P < 0.01$) dose-related elevation in heart rate during the initial 60 min of the study (Table 3). The nonselective muscarinic antagonist scopolamine also elevated heart rate at doses of 0.3–3.0 mg/kg, s.c. Heart rate was elevated following the 0.3 mg/kg, s.c. scopolamine dose by approximately 140 beats/min. Increasing doses of scopolamine produced no further increases the heart rate, with scopolamine at 1.0 and 3.0 mg/kg, s.c. elevating heart rate by approximately 140 and 134 beats/min, respectively. Following scopolamine administration, heart rate was elevated for 45 min following the 0.3 and 1.0 mg/kg doses, and 90 min following the 3.0 mg/kg dose. At the doses tested, SCH 57790 or scopolamine did not alter mean blood pressure.

4. Discussion

SCH 57790 is a high-affinity, competitive antagonist at muscarinic M_2 receptors with approximately 50-fold selectivity for muscarinic M_2 over muscarinic M_1 sites (Lachowicz et al., 1999), and further has no significant affinity at over 20 other receptor and ion channel types (Lachowicz, personal communication). In the present study, SCH 57790 produced dose-related increases in acetylcholine release in rats, improved passive avoidance responding in rodents, and improved working memory performance in squirrel monkeys at doses that did not produce overt side-effects.

Peripheral administration of SCH 57790 produced a dose-related increase in measured ACh release from the hippocampus, cortex, and striatum of conscious rats. These data are similar to the findings of Quirion et al. (1995) and Vannucchi et al. (1997) who showed that administration of the relatively nonselective muscarinic M_2 receptor antagonists 5,11-dihydro-8-chloro-11-[[4-[3-[(2,2-dimethyl-1-oxopentyl)ethylamino]propyl]-1-piperidinyl]acetyl]-6*H*-pyrido[2,3-b][1,4]benzodiazepin-6-one (BIBN-99) and 5,11-dihydro-11-[2-[2-[(*N*,*N*-dipropylaminomethyl)piperidin-1-yl]ethylamino]-carbonyl]-6*H*-pyrido[2,3-b][1,4]benzodiazepin-6-one (AF-DX 384), respectively, increased acetylcholine release from hippocampus and cortex of aged rats. In separate experiments, the nonselective muscarinic receptor antagonist scopolamine also augmented acetylcholine release, suggesting that the in vivo microdialysis assay is unable to differentiate nonselective and selective muscarinic antagonists simply by measuring acetylcholine release. However, previous work from this laboratory (Billard et al., 1995) showed that the ability of muscarinic receptor antagonists such as scopolamine, pirenzapine, 11-[[2-[(diethylamino)methyl]-1-piperidinyl]

acetyl]-5, 11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepine-6-one (AF-DX 116) and himbacine to increase acetylcholine release correlated with their affinity for muscarinic M_2 sites, and not with affinity at other muscarinic receptors. These data further supports a role for blockade of muscarinic M_2 sites in the facilitation of release of acetylcholine within the CNS.

In behavioral studies, administration of SCH 57790 improved memory retention in a young rat passive avoidance test, an assay that may possess construct validity for the cholinergic deficits associated with certain dementias. Young rats (18–22 days old) display a naturally occurring deficit in memory retention as measured by passive avoidance responding, and it has been shown that these cognitive deficits appear to be specific to the cholinergic system. In particular, cholinomimetics such as muscarinic and nicotinic agonists or cholinesterase inhibitors reverse this deficit, whereas agents from other pharmacological classes do not (Smith et al., 1996). The effects of muscarinic M_2 receptor blockade were independent of whether SCH 57790 was given before or after training. These data suggest that SCH 57790 can improve both memory acquisition and consolidation in this assay, and the effects observed were comparable to the effects produced by the cholinesterase inhibitor donepezil. These data generated with SCH 57790 extend previous behavioral studies in which blockade of muscarinic M_2 receptors with relatively nonselective antagonists such as AF-DX 116 (3-fold M_2 vs. M_1), AF-DX 384 (2-fold M_2 vs. M_1) and BIBN 99 (20 fold M_2 vs. M_1) was shown to improve cognitive function in mice as measured by passive avoidance responding (Baratti et al., 1993), reverse memory deficits associated with traumatic brain injury (Pike and Hamm, 1995), and improve spatial memory performance in water-maze in both normal adult and aged rats (Quirion et al., 1995; Packard et al., 1990; Vannucchi et al., 1997). These data add further to the extensive literature describing the effects of cholinomimetics in improving cognitive performance in behavioral tasks (for reviews, see Sarter et al., 1992; Levin, 1996; Brioni et al., 1997; Buccafuco and Terry, 2000).

Of interest are the potency differences with SCH 57790 observed in the young rat passive avoidance assay and microdialysis experiments. Increases in cognitive performance in passive avoidance responding were observed at doses 300–1000 times lower than doses that produce significant increases in measured acetylcholine release. The reason for these discrepancies is not immediately clear, but one possible explanation may be that the increases in acetylcholine release required to enhance cognitive performance are small, and below the detection capability of the chromatography apparatus used in the present experiments. It is only when the higher doses of SCH 57790 are used that sufficient acetylcholine is released into the extrasynaptic spaces to allow detection using the techniques described herein. However, the results observed in microdialysis and behavioral experiments suggest that the increased acetylcholine release observed following blockade of muscarinic M_2 receptors with SCH 57790 is functionally relevant and produces improved cognitive performance. Data from this and other laboratories have shown that while scopolamine produces a similar increase in acetylcholine release, presumably via blockade of muscarinic M_2 sites, its overriding behavioral action is to produce cognitive impairment via its post synaptic muscarinic M_1 receptor blocking actions. This effect was clearly demonstrated in the present studies in mouse passive avoidance responding and the primate response discrimination task. Administration of scopolamine to mice significantly reduced 24-h memory retention, and this retention deficit was reversed by doses of SCH 57790. These data suggest that SCH 57790 can compete with scopolamine for occupancy of presynaptic muscarinic M_2 sites and enhance acetylcholine release to a degree great enough to displace scopolamine from postsynaptic muscarinic M_1 sites.

In an operant procedure in squirrel monkeys, oral administration of SCH 57790 was shown to produce a significant improvement in working memory performance as measured by percentage increases in the delays that monkeys could tolerate and still maintain accurate performance in a response discrimination task. To our knowledge, this provides the first evidence that muscarinic M_2 receptor blockade can improve cognitive performance in a nonhuman primate species. The fixed-ratio discrimination with titrating delay schedule has the advantage that the task is made steadily more difficult (by titrating up the delays) as the experimental session progresses. This allows determination of a characteristic baseline performance for each monkey, which can then be used to investigate potential cognitive enhancers without the need to first disrupt performance with drugs or lesions. Under these conditions, it is apparent that selective blockade of muscarinic M_2 receptors, but not nonspecific muscarinic receptor blockade, produces improvements in cognitive performance. Previous studies in monkeys have shown that cholinesterase inhibitors such as tacrine and donepezil are able to reverse scopolamine-induced impairments of working memory (Rupniak et al., 1997, 1990). Also, Rupniak et al. (1997) demonstrated that low doses of donepezil were able to improve the performance of rhesus monkeys in a visual recognition task in the absence of a scopolamine induced deficit and, indeed, in the present studies, donepezil produced robust increases in working memory performance under our response discrimination schedule. Together, these data show that muscarinic M_2 receptor blockade or cholinesterase inhibition can produce comparable improvements in cognitive performance in squirrel monkeys in the absence of an imposed cholinergic deficit. It has been suggested that data generated in a nonhuman primate possess a higher degree of predictive validity compared to data generated in lower species (Buccafuco and Terry, 2000). As such, the improvement in cognitive performance

observed following administration of SCH 57790 in squirrel monkeys further supports muscarinic M_2 receptor blockade as a viable approach to improve cognition.

In observational studies, when groups of rats were administered high doses of drug, SCH 57790 did not produce overt behavioral, neurological or autonomic changes even up to 100 mg/kg. This contrasts with the cholinesterase inhibitor donepezil that, in our hands, produced decreases in alertness, sprawled body position, tremors, seizures and lethality at doses above 10 mg/kg. Given the mechanism- and nonmechanism-based side effects that can limit the use of cholinesterase inhibitors and direct acting muscarinic M_1 agonists clinically, the relatively innocuous nature of muscarinic M_2 receptor blockade observed following high doses of SCH 57790, suggests that this mechanism may have an advantage in its safety profile. Indeed comparison of the effects of SCH 57790 with scopolamine or donepezil indicate that high doses of the M_2 receptor antagonist produce effects similar to those of the nonselective muscarinic antagonist and not the cholinesterase inhibitor.

In addition to presynaptic localization on cholinergic neurons in the CNS, muscarinic M_2 receptors are also located in peripheral tissues including the heart (Levey, 1993). Muscarinic M_2 receptors are located postsynaptically in cardiac tissue where they serve to slow heart rate following stimulation by acetylcholine that is released from the parasympathetic nervous system. As predicted, blockade of these muscarinic M_2 sites with SCH 57790 or scopolamine increased the heart rate of Sprague–Dawley rats. The increase observed following administration of SCH 57790 occurred at doses of 3.0 and 10 mg/kg. These doses are at least 300–1000 times higher than the doses of SCH 57790 that improved cognitive performance in the young rat passive avoidance responding assay (0.01 mg/kg).

Even though there is substantial loss of cholinergic neurons in Alzheimer's disease (Francis et al., 1999), postmortem studies have shown that some function remains, even in the very late stages of the disease (Quirion et al., 1995). This residual neuronal activity suggests the potential for efficacy with cholinomimetics, such as the muscarinic M_2 receptor antagonist described in this manuscript, during the progression of Alzheimer's disease. In addition, evidence is mounting that muscarinic M_1 receptor activation by muscarinic M_1 agonists may have neuroprotective actions. Specifically, muscarinic M_1 stimulation may decrease the formation of the characteristic pathological markers of Alzheimer's disease, namely amyloid plaques and neurofibrillary tangles. Work by Eckols et al. (1995), and DeLapp et al. (1998) in vitro, and Nitsch et al. (2000) in patients with Alzheimer's disease suggests that muscarinic M_1 receptor stimulation may reduce deposition of amyloid protein by increasing the production of the soluble Aβ protein. Also, Sadot et al. (1996) showed that muscarinic stimulation may decrease tau-protein phosphorylation, thereby decreasing the production of neurofibrillary tangles. Whether indirect muscarinic M_1 receptor stimulation with acetylcholine released from presynaptic terminals via muscarinic M_2 receptor blockade also has these neuroprotective actions will require further investigation.

In summary, the present study demonstrated that the selective muscarinic M_2 receptor antagonist SCH 57790 produced robust increases in acetylcholine release in the CNS, and through this neurochemical mechanism produced consistent improvements in cognitive performance in both rodents and a nonhuman primate in a variety of tests. These improvements were qualitatively similar to those produced by the clinically used cholinesterase inhibitor donepezil. Further, in studies designed to assess behavioral, neurological and autonomic functions following high doses of drugs, SCH 57790 did not produce the same effects as a cholinesterase inhibitor. SCH 57790 did increase heart rate, but only at doses well above those required to increase cognitive performance. These studies demonstrate that blockade of muscarinic M_2 receptors with SCH 57790 is a viable mechanism by which to produce improved cognition in laboratory animals.

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