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Effects of nicotine on memory impairment induced by blockade of muscarinic, nicotinic and dopamine D2 receptors in rats

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Abstract

Scopolamine dose-dependently inhibits passive avoidance latency and decreases spontaneous alternation in the Y-maze, suggesting effects on long-term and short-term memory, respectively. Chlorisondamine (10 mg/kg), a compound which produces a long-lasting central nicotinic receptor blockade, did not affect short-term and long-term memory performance. In normal rats, nicotine at the doses of 0.3, 1.0, and 3.0 mg/kg administered once had a facilitating effect on short-term memory; a higher dose (3.0 mg/kg) did not show a more pronounced effect than a lower one (0.3 mg/kg). Nicotine, by activating the nicotinic acetylcholine receptors, attenuated the impairment of short-term memory induced by muscarinic or dopamine D2 receptor blockade. On long-term memory, a single dose of nicotine (0.3, 1.0, 3.0 mg/kg) did not affect memory performance, but improved it after chronic (10 consecutive days, 0.3 mg/kg) administration. The antiamnesic effect of nicotine administered once was observed in scopolamine-, scopolamine + chlorisondamine- or sulpiride-treated rats. These results suggest that the antiamnesic effect of nicotine can result from an action at nicotinic receptors subtypes not blocked by chlorisondamine or at nonnicotinic receptors.

Keywords: Chlorisondamine; Nicotine; Scopolamine; Sulpiride; Memory

1. Introduction

Although studies regarding the implication of the cholinergic system in learning and memory began long ago, the statement that in Alzheimer's disease patients, this system is impaired (James and Nordberg, 1995) has led to an intensification of research concerning the cholinergic structures involved (receptors, neurotransmitters) and their interaction with other nervous structures (for review, see Dutar et al., 1995; Van der Zee and Luiten, 1999). Muscarinic and nicotinic acetylcholine receptors mediate the action of acetylcholine. Clinical investigation of Alzheimer's disease patients does not indicate changes in number and function of muscarinic acetylcholine receptors. However, a significant decrease in nicotinic acetylcholine receptors density has been reported for the cerebral cortex and hippocampus, two major areas which are involved in learning and memory processes (James and Nordberg, 1995).

Chlorisondamine blocks central nicotinic receptors for 4–5 weeks via an unknown mechanism from the central and peripheral nervous system (Clarke and Kumar, 1983; Decker et al., 1994; Marenco et al., 2000). Because of the long-lasting blocking effect on nicotinic acetylcholine receptors, we used chlorisondamine as an experimental tool to study the involvement of nicotinic acetylcholine receptors in memory processes. Our data have shown that blockade of nicotinic acetylcholine receptors by chlorisondamine does not affect either short-term or long-term memory performance in rats. Concerning the influence of nicotine, a specific nicotinic acetylcholine receptor stimulus, experimental studies in animals and humans have yielded contradictory results. While some researchers (Decker et al., 1995; Hefco et al., 2000; Levin and Rezvani, 2000; Levin and Simon, 1998; Nitta et al., 1994) reported an improving effect of nicotine on memory, others (Dunnet and Martel, 1990; Heisham et al., 1994; Spilich et al., 1992) did not observe any effect or, on the contrary, reported negative effects.

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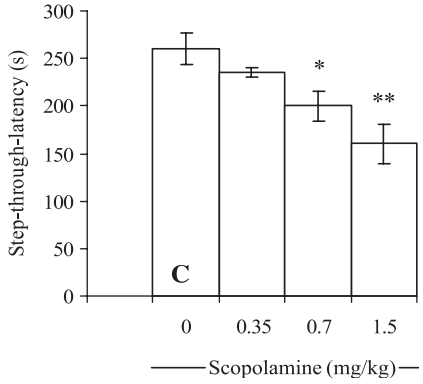

Fig. 1. Effects of scopolamine on step-through latency in the retention trial of the passive avoidance task in normal rats. Values are means ± S.E.M. ($n = 8$ per group). *$P < 0.05$, **$P < 0.01$ vs. respective control (C) group.

The precise mechanism responsible for the enhancement of learning and memory induced by nicotine has not yet been elucidated. Nicotine induces the release of a variety of neurotransmitters including acetylcholine, dopamine, γ-aminobutyric acid (GABA), norepinephrine, serotonin and glutamate (Levin and Simon, 1998; Yin and French, 2000). In the present study, we examined the effects of dose and duration of nicotine treatment on memory in normal rats, and in rats with muscarinic, nicotinic and dopamine D2 receptor blockade.

2. Materials and methods

The experiments were carried out on male Wistar rats weighing 200–250 g at the start of the experiment. They were fed and allowed to drink water ad libitum. Rats were treated in accordance with institutional guidelines.

2.1. Step-through passive avoidance task

In brief, a step-through type passive avoidance apparatus consisting of two compartments (25 × 15 × 15 cm high), one illuminated and one dark, both equipped with a grid floor, was used. The two compartments were separated by a guillotine door. In the acquisition trials, each rat was placed in the illuminated compartment; when the animal entered the dark compartment, the door was closed and an inescapable foot shock (0.3 mA, 5 s) was delivered through the grid floor. The rat was removed after receiving the foot shock and was placed back into the light compartment. The door was again opened 30 s later to start the next trial. The training continued until the rat stayed in the light compartment for a 120-s period on a single trial. After 24 h, each rat was placed in the light compartment and the step-through latency was recorded until 300 s had elapsed (retention trial). The step-through latency in the retention trial was used as the index of retention of the training experience (Yamada et al., 1996). Longer retention latencies were interpreted as indicating better retention of the training experience.

2.2. Y-maze task

Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) × 100 (Yamada et al., 1996). Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory.

2.3. Drug administration

All drugs were dissolved in sterile saline and injected intraperitoneally in a volume of 1 ml/kg b.w. (−)-Nicotine

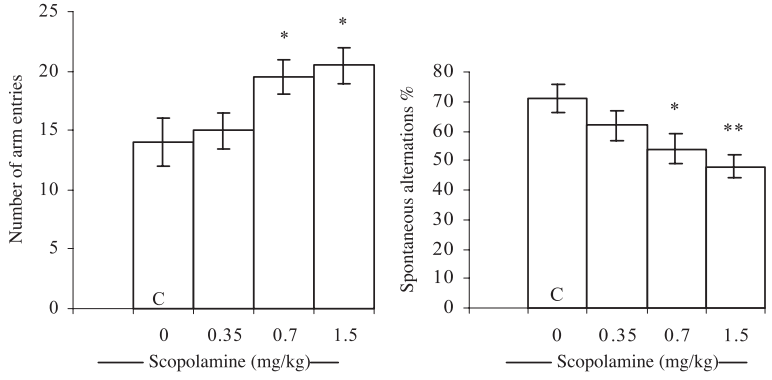

Fig. 2. Effects of scopolamine on number of arm entries and spontaneous alternation percent in normal rats. Values indicate means ± S.E.M. ($n = 8$ per group). *$P < 0.05$, **$P < 0.01$ vs. control (C) group.

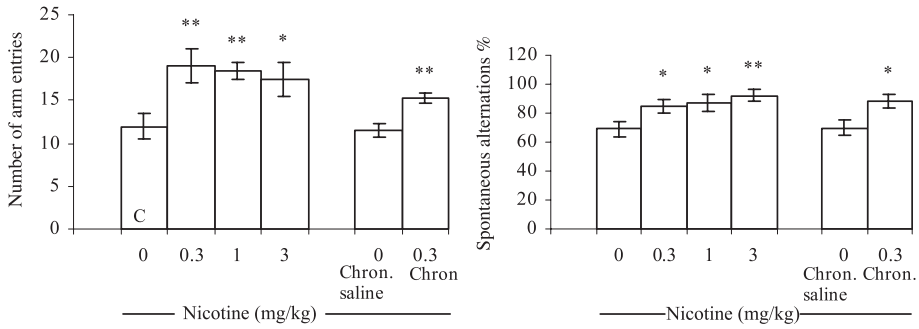

Fig. 3. Effects of a single or chronic (chron) dose of nicotine on the number of arm entries and spontaneous alternation percent in Y-maze test in normal rats. The data are means ± S.E.M. ($n = 8$ per group). *$P < 0.05$, **$P < 0.02$ vs. control group.

(free base; 0.3 mg, 1.0 mg and 3.0 mg/kg i.p., Sigma), scopolamine hydrobromide (0.35, 0.7 or 1.5 mg/kg i.p., Sigma) and sulpiride (10 mg/kg i.p., Sigma) were administered individually or in the combinations indicated in Section 3, immediately after the acquisition trial for the passive avoidance task to avoid a possible impairment effect per se on acquisition.

For the Y-maze task, the same doses of nicotine or scopolamine were administered 30 min before the experiments, and sulpiride (10 mg/kg i.p.) was injected 5 min before the administration of nicotine. Chlorisondamine (10 mg/kg i.p., Ciba Giegy) was injected 24 h before the experiments. Functional blockade of nicotinic acetylcholine receptors was confirmed in each animal after behavioral testing by determining if chlorisondamine protected against nicotine-induced prostration, a centrally mediated effect of nicotine (Abood et al., 1981).

Controls animals received an equal volume of sterile saline (1.0 ml/kg).

2.4. Statistical analysis

The results are expressed as means ± S.E.M. The results were analyzed using Student's *t*-test. Values of $P < 0.05$ were regarded as significant.

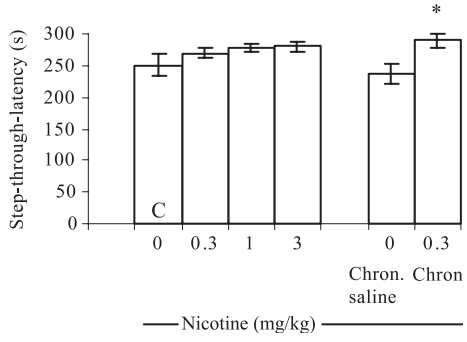

Fig. 4. Effects of a single or chronic (chron) dose of nicotine on performance of the passive avoidance task in normal rats. Values indicate means ± S.E.M. ($n = 8$ per group). *$P < 0.05$ vs. control group.

3. Results

3.1. The role of muscarinic acetylcholine receptors in learning and memory

Administration of scopolamine—a specific antagonist of muscarinic acetylcholine receptors—at the doses of 0.35, 0.7 and 1.5 mg/kg b.w. i.p., immediately after the acquisition period resulted in a dose-dependent impairment of memory retention in the passive avoidance test 24 h after scopolamine administration. Scopolamine, 0.35 mg/kg, induced a nonsignificant decrease in step-through latency (Fig. 1). Based on this result, in the next experiments we used scopolamine at the dose of 0.7 mg/kg.

Scopolamine also dose-dependently decreased short-term memory as evidenced by a significant decrease of the spontaneous alternation percentage in the Y-maze test (Fig. 2). This effect could not be attributed to decreased motor activity, which increased significantly as can be deduced from the number of arm entries (Fig. 2).

3.2. The role of nicotinic acetylcholine receptors in learning and memory

Stimulation of nicotinic receptors in normal rats with nicotine administered once at the doses of 0.3, 1.0 and 3.0 mg/kg i.p. improved short-term memory as evidenced by a

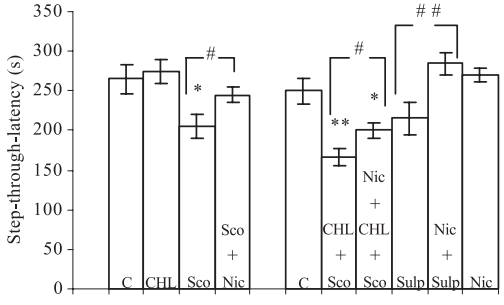

Fig. 5. Effects of nicotine (0.3 mg/kg) treatment on step-through latency in normal and chlorisondamine (CHL) (10 mg/kg), scopolamine (Sco) (0.7 mg/kg) or sulpiride (Sulp) (10 mg/kg)-treated rats in the passive avoidance test. The data are means ± S.E.M. ($n = 8$ per group). *$P < 0.05$, **$P < 0.02$ vs. control group. #$P < 0.05$, ##$P < 0.02$ vs. respective vehicle group.

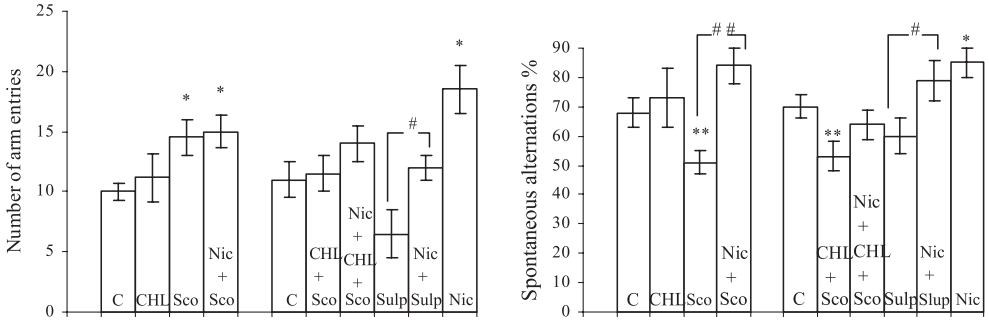

Fig. 6. Effects of nicotine (Nic) (0.3 mg/kg) treatment on number of arm entries and spontaneous alternation percent in normal and chlorisondamine (CHL) (10 mg/kg), scopolamine (Sco) (0.7 mg/kg) or sulpiride (Sulp) (10 mg/kg)-treated rats. Values are means ± S.E.M. ($n = 8$ per group). *$P < 0.05$, **$P < 0.02$ vs. control (saline) group. $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ vs. respective vehicle group.

significant increase in spontaneous alternation percentage. All doses were effective but the effects were not significantly different from each other (Fig. 3).

Regarding long-term memory, an acute dose of nicotine did not improve memory performance in normal rats, although a significant memory improvement was observed after chronic (10 consecutive days) nicotine administration (0.3 mg/kg) (Fig. 4). On the contrary, a single dose of nicotine (0.3 mg/kg) significantly attenuated the reduction in long-term memory performance in rats with muscarinic, muscarinic and nicotinic and dopamine D2 receptors blocked by scopolamine, scopolamine + chlorisondamine and sulpiride, respectively (Fig. 5). Regarding short-term memory, the beneficial effect of nicotine could not be evidenced only in rats with both muscarinic and nicotinic receptors blocked; nicotine attenuated the reduction in alternation performance induced by scopolamine or sulpiride alone (Fig. 6). Sulpiride itself did not show any significant effect on the performance in the Y-maze or in the passive avoidance task. The number of arm entries increased significantly after scopolamine, nicotine, or scopolamine and nicotine treatment (Fig. 6).

The blockade of nicotinic acetylcholine receptors by chlorisondamine—a long-lasting specific nicotinic acetylcholine receptor antagonist—does not affect performance in the passive avoidance (Fig. 5) or Y-maze (Fig. 6) tasks.

4. Discussions

Our results showed that muscarinic acetylcholine receptor blockade with scopolamine induced a significant decrease in both long-term memory (explored through the passive avoidance test) and short-term memory (evidenced in a Y-maze task). The inhibitory effect of scopolamine was dose-dependent. These data confirmed those from the literature concerning the facilitating role of muscarinic acetylcholine receptors in memory processes (Dutar et al., 1995).

About the role of nicotinic acetylcholine receptors explored with chlorisondamine, a long-lasting nicotinic acetylcholine receptor antagonist, we were unable to demonstrate an effect on short-term memory tested by means of the Y-maze or on long-term memory tested with the passive avoidance test. Functional blockade of nicotinic acetylcholine receptors was confirmed in each animal by determining if chlorisondamine protected against nicotine-induced prostration. Thus, our failure to observe effects of chlorisondamine on the performance in these memory tasks could not be attributed to inadequate blockade of nicotinic receptors. These results are in contrast to the impairment of memory with mecamylamine, an acute nicotinic receptors antagonist (Decker and Majchrzak, 1992; Riekkinen et al., 1990). The differences in the effects of nicotinic receptor blockade produced by mecamylamine and by chlorisondamine may perhaps be due to their different duration of action. Chronic nicotinic receptor blockade may be accompanied by compensatory mechanisms not engaged in rats receiving an acute nicotinic antagonist such as mecamylamine. Alternatively, different effects of these two nicotinic acetylcholine receptor antagonists on the performance of memory tasks may be related to selective actions of these compounds at nicotinic receptor subtypes or at nonnicotinic receptors.

About the role of nicotinic acetylcholine receptors explored with nicotine, a specific agonist of nicotinic acetylcholine receptors, some researchers have observed an ameliorating effect of nicotine on memory impairment (Decker et al., 1995; Levin and Simon, 1998; Levin and Rezvani, 2000; Nitta et al., 1994), while others did not observe any effect or on the contrary, have reported a negative effect (Dunnet and Martel, 1990; Heisham et al., 1994; Spilich et al., 1992). The equivocal results cited above may be due to differences in dosage, duration of drug treatment, animal strains or different tests used for memory evaluation.

Our present data show that nicotine administered once has a facilitating effect on short-term memory of normal rats; a higher dose of nicotine (3.0 mg/kg) did not show a more pronounced facilitator effect than did a lower dose (0.3 mg/kg).

Because acetylcholine release evoked by nicotine activates both muscarinic and nicotinic acetylcholine receptors,

we also investigated the effect of nicotine on cognitive functions in rats with muscarinic acetylcholine receptors blocked by scopolamine. Our data show that a single dose of nicotine (0.3 mg/kg) improved short-term memory in scopolamine-treated rats.

The dopamine system could also be involved in the nicotine and scopolamine effects on memory, because the dopamine neurons from the ventral tegmental area have both nicotinic and muscarinic acetylcholine receptors. Systemic or in vitro administration of nicotine excites dopamine neurons in the ventral tegmental area (White, 1996; Yin and French, 2000). Dopamine can exert its effect through five types of dopamine receptors, D1–D5. In the present study, using a selective antagonist for dopamine D2 receptors such as sulpiride, it was established that nicotine improved short-term memory in the sulpiride-treated rats. In our study, the beneficial effect of nicotine on short-term memory was not observed only in the (scopolamine + chlorisondamine)-treated rats. This suggests that nicotine exerts its action on short-term memory mainly through the nicotinic acetylcholine receptors.

On long-term memory, nicotine administered once at the doses of 0.3, 1.0 and 3.0 mg/kg after training did not manifest a significant effect on memory performance in normal rats, although a beneficial effect of nicotine on memory retention was observed after chronic (10 consecutive days) administration at the dose of 0.3 mg/kg i.p. A significant antiamnesic effect of a single dose of nicotine was recorded only in rats with their memory impaired by scopolamine, scopolamine + chlorisondamine or sulpiride. Sulpiride (10 mg/kg) itself, a specific dopamine D2 receptor blocker, did not show any significant effect on the performance in the passive avoidance or Y-maze tasks.

The different effect of an acute versus chronic doses of nicotine on short-term and long-term memory can be attributed to the fact that different brain regions are involved in storage and retrieval in these two categories of memory. In addition, the molecular mechanisms that underlie short- and long-term memory are different (DeZazzo and Tully, 1995).

Several effects of nicotine in the brain may be mediated through neuromodulatory potentiation of the release of a variety of neurotransmitters including acetylcholine, dopamine, GABA, norepinephrine, serotonin and glutamate (Levin and Simon, 1998; Levin and Rezvani, 2000; Yin and French, 2000). Until now, 11 different nicotinic acetylcholine receptor subtypes have been identified ($\alpha2-\alpha9$ and $\beta2-\beta4$) (Picciotto et al., 2000). Functionally, nicotinic acetylcholine receptors can be divided into those containing the $\beta2$ subunit combined with various α subunits, which have the highest affinity for nicotine (Picciotto et al., 2000), and those containing the $\beta4$ subunit combined with various α subunits, which have 10–100 times lower affinity for nicotine (Luetje and Patrick, 1991). The subtypes of nicotinic acetylcholine receptors most highly expressed in the brain are $\beta2\alpha4$ subunit containing receptors and $\alpha7$ subunit-containing α-bungarotoxin binding receptors (Levin and Simon, 1998; Picciotto et al., 2000). Both the $\alpha4\beta2$ combination and $\alpha7$ subunit have been especially implicated in learning and memory function. One hippocampal neuron can express more than one nicotinic acetylcholine receptor subtype (Levin and Simon, 1998). Nicotinic antagonists with differential blockade of $\alpha3\beta2$ (mecamylamine), $\alpha4\beta2$ (dihydro-beta-erythroidine) and $\alpha7$ (methyllycaconitine) nicotinic acetylcholine receptors impair working memory (Levin and Simon, 1998). The increase in both acetylcholine and glutamate release appears to be mediated through a $\alpha7$ subunit-containing nicotinic acetylcholine receptor as it can be blocked by α-bungarotoxin. Conversely, nicotine stimulates the firing of dopaminergic neurons (Picciotto et al., 2000; Pidoplichko et al., 1997) as well as the release of dopamine from striatal synaptosomes (Grady et al., 1992) through an $\alpha4\beta2$-containing nicotinic acetylcholine receptor. Because nicotine has a stimulatory effect on memory in rats with nicotine receptors blocked by chlorisondamine, this suggests that this nicotinic antagonist did not block all acetylcholine receptor subtypes. Identification of nicotinic acetylcholine receptor subtypes not blocked by CHL that are involved in cognitive functions requires future investigation. Our data show that nicotine continues to ameliorate long-term memory deficits induced by muscarinic, muscarinic + nicotinic acetylcholine or dopamine D2 receptor blockade. It is possible that the antiamnesic effect of nicotine is exerted by its action at nicotinic receptor subtypes not blocked by chlorisondamine or at nonnicotinic receptors. An understanding of this mechanism will require further investigation.

In conclusion, we found that nicotine, by activating the nicotinic acetylcholine receptors, improved short-term memory in normal rats and ameliorated the impairment of memory induced by muscarinic, nicotinic and dopamine D2 receptor blockade. Regarding long-term memory, a single dose of nicotine did not affect memory performance, although chronic nicotine administration improved memory performance in normal rats. Nicotine had an antiamnesic effect on long-term memory in rats with muscarinic, muscarinic and nicotinic or dopamine D2 receptor blockade. These results suggest that the antiamnesic effect of nicotine can also result from its actions at nicotinic receptors subtypes not blocked by chlorisondamine or at nonnicotinic receptors.

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