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Evidence that muscarinic receptors are involved in nicotine-facilitated spatial memory

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Abstract

A delayed nonmatching-to-position task (DNMPT) in a water maze (nine trials per day, 10 days) was used to investigate the role of muscarinic acetylcholinergic receptor (mAChR) in nicotine-facilitated spatial memory in Wistar rats. The index of spatial memory was determined by the number of correct choices made in choice swim (CS) and the time taken to complete the swim in the correct CS. Diazepam (6 mg kg^{-1} ip, daily) significantly impaired choice accuracy and increased the swim time for correct CS. In contrast, when nicotine (2 mg kg^{-1} sc, twice daily) and diazepam were administered simultaneously in the last three sessions of training, the choice accuracies of CS were similar to controls and significantly higher than in the diazepam group. However, the swim times for correct CS were longer in the nicotine + diazepam group than in controls. Atropine (30 mg kg^{-1} ip, daily) significantly decreased the choice accuracies of CS. The choice accuracies of CS swim times for correct CS in the atropine group did not differ significantly from those in the nicotine + atropine group, in which nicotine (2 mg kg^{-1} sc, twice daily) and atropine were given simultaneously. These results show that nicotine improves memory performance when the functions of mAChRs are normal; when the mAChRs are blocked, nicotine does not enhance spatial memory. Therefore, mAChRs are involved in the spatial memory-enhancing effect of nicotine.

Keywords: Spatial memory; Nicotine; Desensitization; Choice accuracy

1. Introduction

Alzheimer's disease (AD) is the most common cause of memory loss and dementia (Levey, 1996). Chronic nicotine patch administration improves learning rates in AD patients and might alleviate the cognitive impairment associated with the disease (Wilson et al., 1995). Nicotine agonists improve the performance of cognitive tasks in rats, while antagonists impair it. For example, chronic nicotine administration significantly improves memory performance on the radial-arm maze, whereas the nicotinic antagonist mecamylamine damages memory (Poincheval-Fuhrman and Sara, 1993; Attaway et al., 1999; Levin et al., 1994; Levin and Torry, 1996).

Nicotine acts on the nicotinic acetylcholinergic receptor (nAChR). After repeated administration, some effects of nicotine decrease or even disappear; this tolerance is caused by nAChR desensitization (de Fiebre and Collins, 1988). However, no tolerance is seen in the memory-improving effects of chronic nicotine administration (Levin and Simon, 1998). Therefore, other receptors must be involved in the memory-enhancing effect, because the nAChR is desensitized.

Nicotine stimulates acetylcholine release, so it might have effects mediated secondarily via muscarinic acetylcholinergic receptors (mAChRs; Tani et al., 1998). Our previous experiments have shown that nAChR desensitization facilitates mAChR function, indicating that nAChR desensitization does not produce an inactive state (Wang and Liu, 1991). MAChRs play key roles in the control of high-level cognitive processes, such as learning and memory (Nathanson, 1987), and muscarinic and nicotinic components interact: nicotine-induced improvement in working memory

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performance is reversed by acute administration of the muscarinic antagonist scopolamine (Levin and Rose, 1991). Thus, it is interesting to investigate whether mAChRs are involved in the enhancement of spatial memory effected by chronic nicotine administration.

To answer this question, the effects of repeated nicotine administration on acquisition of spatial memory were observed in Wistar rats, which were injected with diazepam or atropine and trained to learn a delayed nonmatching-to-position task (DNMPT) in a water maze.

2. Materials and methods

2.1. Animals

Male Wistar rats ($n = 37$), weighing 270 ± 20 g (Grade I, certificate No. 0043025), were provided by Experimental Animal Center of Beijing Institute of Pharmacology and Toxicology. The rats were housed under natural light–dark cycle conditions with food and water available ad libitum. The experimental protocol was approved by the Declaration of the National Institutes of Health *Guide for Care and Use of Laboratory Animals* (Publication No. 85-23, revised 1985).

2.2. Groups studied and treatments administered

For information regarding this, see Table 1.

2.3. Behavioral procedures

Delayed nonmatching-to-position task. The effects of nicotine on drug-induced deficit were tested in a water maze according to the protocol of Markowska et al. (1996), which includes pretraining and training. Briefly: a Plexiglas T-shaped partition was inserted into the water tank (1.3 m in diameter) to create a start section (A) and two choice sections (B, C). A sliding panel either remained centered, allowing access to both choice sections, or was moved to one side to block one section. An escape platform (5×5 cm^2) set 1 cm below the water level (water depth = 30 cm) was located in each of the two choice sections. Only one platform was available for the rat for each trial. For pretaining, to train the rat to swim and climb on to the platform, a straight swim procedure (one session, 10 trials) was used. A second shaping procedure (two consecutive days, nine trials per day), using the T partition, trained the rat to swim to the platform located in either choice section, with the starting point at the entrance to the choice section (Day 1) or the start section (Day 2). For training, each trial consisted of two parts: an information swim (IS) and a CS. For the IS, Section B was open and the rats were allowed 1 min to locate the escape platform with the starting point in the start section. After 10 s on the platform, the rat was placed in a holding cage for 1 min, then it performed the CS. For this, both choice sections were open but only the platform in Section C was available, and the rat was allowed 1 min to locate it. If the rat entered Section B, the sliding door was closed, confining the rat for 30 s. After this punishment, the sliding door was opened and the rat was allowed to locate the platform in Section C. If the rats did not find the escape platform within 1 min, they were directed to and placed on the platform, where they remained for 30 s. Nine trials were conducted per session; the intertrial interval was 10 min. A choice was labeled correct if the rat found the platform in Section C directly without punishment. Swim times for correct CS and numbers of correct CS were recorded. The choice accuracy (ratio) was the number of correct CS divided by the total number of CS.

Table 1
Groups studied and treatments administered

Group	Drug treatments		*n*
	Times/day	Administration	
Control (saline)	1	Intraperitoneal	7
Dia	1	Intraperitoneal	7
Dia+	1 (Dia)	Intraperitoneal	7
Nic	2 (Nic)	Subcutaneous	
Atr	1	Intraperitoneal	6
Atr+	1 (Nic)	Intraperitoneal	10
Nic	2 (Nic)	Subcutaneous	

2.4. The timeline

Rats were injected with drugs for 17 days. Pretraining (nine trials per day) was from Days 5 to 7, and training (nine trials per day) from Days 8 to 17.

2.5. Drugs and treatment

Atropine was purchased from Sigma. (−) Nicotine (base) was obtained from Merck. Diazepam was from Tianjin Amino. Nicotine and atropine were dissolved in 0.9% sodium chloride. Atropine (Atr, 30 mg kg^{-1}) or diazepam (Dia, 6 mg kg^{-1}) were administered 40 min before the start of the task. The first injection of nicotine (Nic, 2 mg kg^{-1}) was given 20 min after atropine or diazepam, and the second was 5 h after the first.

2.6. Data analysis

Choice accuracy and swim time for correct CS were analyzed by Splus2000 and SAS 6.12 using a series of within-subject repeated-measures multivariate analysis of variance (MANOVA) designs. The independent variables analyzed were drug and session. Scheffe's test was used for individual comparisons between the different groups.

3. Results

3.1. Choice accuracy of CS

The control rats learned quickly and performed accurately. On the fourth day, the choice accuracy of controls was 73.0%. From the 4th day to the 10th day, mean choice accuracy was 86.4 ± 4.1% (Fig. 1).

Drugs impaired performance [effect of drugs: $F(4,262) = 63.05723$; $P < .001$]. During training, the choice accuracy of CS was significantly lower in drug-treated groups than in the controls. Although choice accuracy increased with sessions [effect of sessions: $F(9,262) = 18.70149$; $P < .001$], the magnitude of this improvement was less in drug-treated groups than in the control [Drug × Session: $F(36,262) = 1.73755$; $P < .01$].

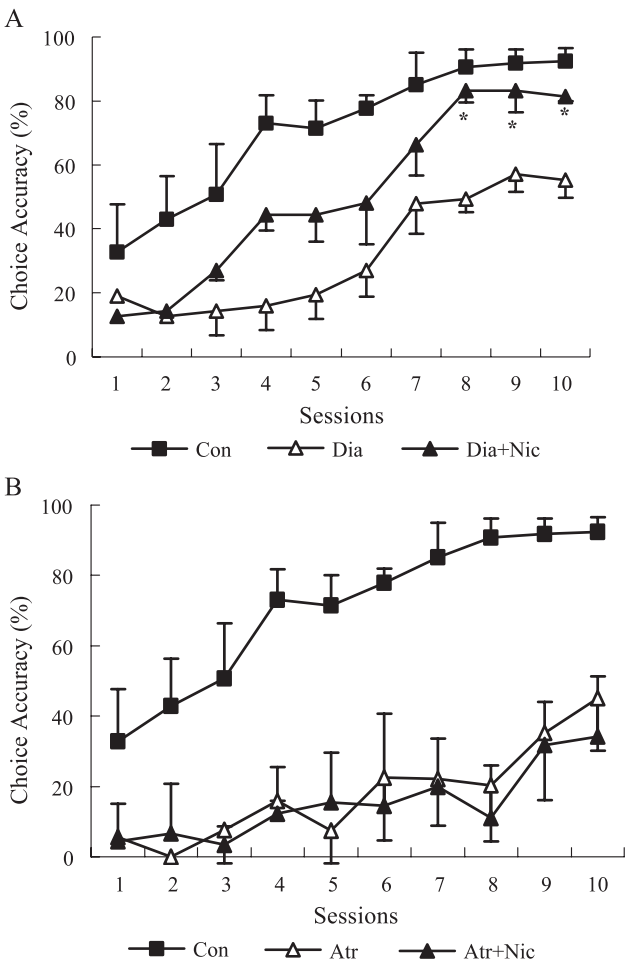

Fig. 1. DNMPT in the water maze. The effects of chronic administration of diazepam (6 mg kg^{-1} ip, daily), diazepam + nicotine (2 mg kg^{-1} sc, twice daily) (A), atropine (30 mg kg^{-1} ip, daily), or atropine + nicotine (2 mg kg^{-1} sc, twice daily) (B), on choice accuracy of CS are shown for 10 training sessions. Compared with control, the acquisition of spatial memory was slower in the drug-treated groups. In the last 3 days, there was no difference between the diazepam + nicotine and control groups, but the diazepam + nicotine group was faster than the diazepam group. Each point represents mean ± S.D.; $n = 7–10$. * $P < .05$, vs. diazepam. Scheffe's test. Con, control group (Con is the same in A and B); Dia, diazepam group; Dia + Nic, diazepam + nicotine group; Atr, atropine group; Atr + Nic, atropine + nicotine group.

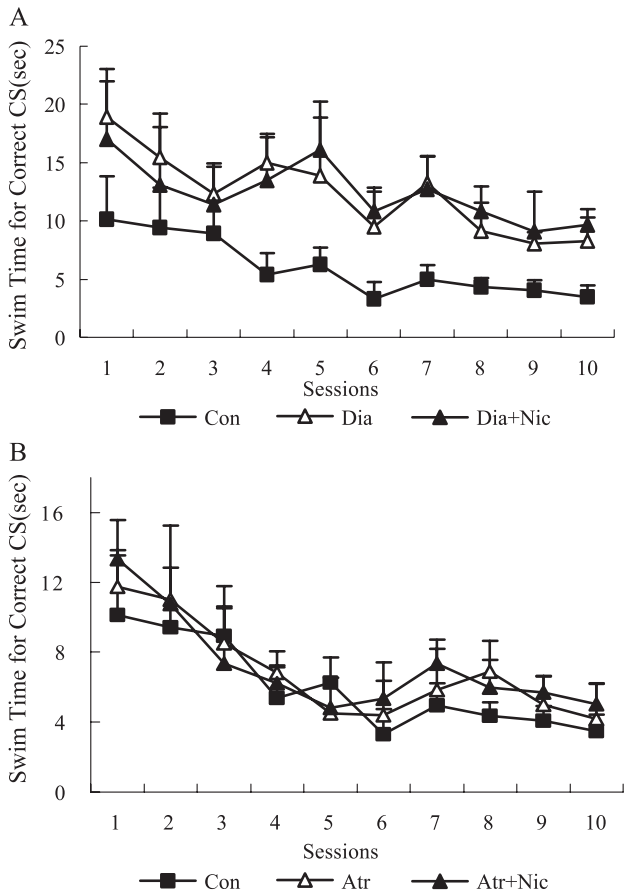

Fig. 2. DNMPT in the water maze. The effects of chronic administration of diazepam (6 mg kg^{-1}ip, daily), diazepam + nicotine (2 mg kg^{-1} sc, twice daily) (A), atropine (30 mg kg^{-1} ip, daily), or atropine + nicotine (2 mg kg^{-1} sc, twice daily) (B), on swim times for correct CS are shown for 10 training sessions. Compared with control, the swim times for correct CS were longer in the diazepam and diazepam + nicotine groups. Each point represents mean ± S.D.; $n = 7–10$ (see Fig. 1 for abbreviations).

The Scheffe's test showed that the choice accuracy in the diazepam group was lower than control (each session $P < .05$). In the first 7 days of training, the choice accuracy of the diazepam + nicotine group was also lower than control (each session $P < .05$). In the last 3 days, this difference became insignificant (each session $P > .05$), but the accuracy was higher in the diazepam + nicotine than the diazepam group (each session $P < .05$).

Atropine impaired memory. During training, choice accuracy was significantly lower than control in groups treated with atropine (each session $P < .05$) or with atropine + nicotine (each session $P < .05$). The difference of choice accuracy between the atropine + nicotine and atropine groups was not statistically significant (each session $P > .05$; Fig. 1).

3.2. Swim time for correct CS

During Session 1, control rats took 10.1 ± 3.7 s to find the platform. The swim time decreased steadily over repeated sessions [$F(9,340) = 7.39$; $P < .001$]. By Session 10, the swim time in control was 3.5 ± 0.9 s.

In groups treated with diazepam or diazepam + nicotine, the drugs increased the swim time for locating the platform [effect of drugs: $F(2,242) = 180.8989$; $P < .001$]. Although swim time decreased with sessions [effect of sessions: $F(9,242) = 7.7094$; $P < .001$], this decrease was less in the drug-treated groups than in controls [Drug × Session: $F(18,242) = 2.8171$; $P < .01$]. During training, swim times for correct CS were longer in the diazepam and diazepam + -nicotine groups than control (each session $P < .05$). The diazepam and diazepam + nicotine groups did not differ significantly (each session $P > .05$).

Drugs did not affect the swim time for correct CS in the atropine and atropine + nicotine groups [$F(2,205) = 1.14$, $P > .05$], and there were no significant differences from control (each session $P > .05$). Swim time decreased with sessions in the atropine and atropine + nicotine groups [effect of sessions: $F(9,205) = 14.55923$; $P < .001$; Fig. 2].

4. Discussion

We have established two models with damaged spatial memory and tested them by DNMPT in a water maze. In the diazepam model (6 mg kg^{-1}; Cain, 1997), the choice accuracies of CS increased with sessions but remained lower than control: long-term administration of diazepam impaired the spatial memory of the rats. Our results support findings from other laboratories that DZP produces memory impairment (Gorenstein et al., 1994).

Generally, the amnesic effects observed after administration of high doses (>5 mg/kg) of DZP are related to their sedative effects. However, the two phenomena can be dissociated under certain conditions. Constantinidis et al. (2002) found that an anxiolytic, nonsedative dose of diazepam produced spatial working memory impairment. Recent studies show that GABAergic inhibition in the frontal cortex, a center of working memory, controls the timing of neuronal activities in cognitive processes, thus shaping the flow of information in cortical circuits; hence, GABAergic inhibition plays a central role in working memory (Nakamura-Palacios and Roelke, 1997). DZP is a selective allosteric modulator of $GABA_A$ receptors (Auta et al., 1995). Thus, DZP perhaps impairs spatial memory by changing GABA receptor functions. The swim time for correct CS is longer in the diazepam model than in control, reflecting the motor impairment caused by this drug.

In the atropine model (30 mg kg^{-1}; Cain et al., 2000), although the choice accuracies increased with sessions, they were lower than control. Swim times for correct CS were similar in atropine-treated rats and control, which implies that impairment of spatial memory by atropine did not damage swimming ability. These results are consistent with others (Grauer and Kapon, 1996; Dhume et al., 1989; Sala et al., 1991), although the tasks were performed in different mazes. They support the view that mAChRs play important and possibly crucial roles in spatial memory. The cause of memory impairment by atropine is not known exactly. Possibly the muscarinic receptor is widely distributed in the central nervous system and participates in numerous functions relevant to adaptive behavior in the water maze.

To identify the nicotine-enhancing effect on memory in the two models, the drug was administered simultaneously with diazepam or atropine. The results showed that after the eighth session, the difference in the choice accuracy of CS between animals treated with diazepam and nicotine was not significantly different from control, and nicotine did not significantly affect swim time for correct CS. This indicates that the improved choice accuracy was not attributable to improved swimming ability, but more likely a consequence of improved memory, which means that nicotine improves memory performance in diazepam-injected rats. This agrees with previous experiments, which showed that chronic nicotine administration improved the performances of rats (Levin et al., 1993; Decker et al., 1992). Choice accuracy in atropine- and nicotine-treated rats was similar to that in the rats injected with atropine alone, and the number was lower than control. The swim time for correct CS was similar in the atropine and atropine + nicotine groups. These results suggest that nicotine did not enhance the performance of spatial memory in atropine-treated rats. If nicotine could improve the spatial memory of atropine-injected rats, at least, compared with atropine-treated animals, there would be the trends in the data, even if it is not statistically significant. But in our experiments (as shown in Figs. 1 and 2), the data does not show that trend. The difference between the two models is that in diazepam-treated rats, the functions of mAChRs are not impaired, while in atropine treated rats, the mAChRs are blocked.

Nicotine (2 mg kg^{-1}) injected subcutaneously for 5 days desensitizes nAChRs. For example, the experiment in our laboratory has showed that the rats desensitized on some aspects, such as body temperature, rotarod time, and seizure susceptibility (Wang and Liu, 1991). In this study, rats were injected with nicotine for 7 days before the task, so the effects of the drug were observed when the nAChRs had been desensitized. NAChRs desensitization affects the allosteric regulation of mAChRs and enhances the coupling between M receptors and the G proteins, facilitating the functions of the M receptors (Wang, 1997). The present results in conjunction with others indicate that when nAChRs are desensitized, facilitation of memory by nicotine depends on mAChRs. Thus, mAChRs are involved in the memory-enhancing effect of nicotine, although the specific brain sites and mechanisms involved are unknown. When drugs were administered acutely, nicotine did not attenuate scopolamine-induced deficits but it did attenuate mecamyl-

amine-induced deficits in spatial working memory, suggesting that nAChRs and mAChRs play distinct roles in memory (Levin et al., 1997). However, it is possible for nicotine to enhance memory via mAChRs: if mAChRs were blocked by scopolamine, nicotine could not attenuate the memory deficit.

In conclusion, using the DNMTP task to test acquisition of spatial memory, with choice accuracy of CS and swim time for correct CS as indicators, we found that when nAChRs were desensitized, nicotine enhanced performance in a spatial memory-deficient model if the function of mAChRs were normal, but failed if the mAChRs were blocked. Combining these data with our previous results, we deduced that when nAChRs were desensitized, mAChRs were involved in the memory-enhancing effect of nicotine. This finding is important for understanding the mechanism of nicotine-enhanced memory in AD.

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