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Effect of H_1-antagonists on spatial memory deficit evaluated by 8-arm radial maze in rats

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KEY WORDS maze learning; histamine H_1 receptors; pyrilamine; diphenhydramine; epinastine; tacrine

ABSTRACT

AIM: To evaluate effects of certain H_1-antagonists on spatial memory with 8-arm radial maze performance of rats. **METHODS**: Eight-arm radial maze performance was used to measure spatial memory in rats. **RESULTS**: Chronic treatments of classical H_1-antagonists, diphenhydramine (5 mg/kg) and pyrilamine (20 mg/kg) impaired acquisition memory process regarding both parameters of radial maze performance. In addition, the memory retrieval process was also impaired significantly by a single administration of diphenhydramine (5, 10 mg/kg) and pyrilamine (50 mg/kg). However, the newly developed H_1-antagonist, epinastine caused no appreciable effect on both acquisition and retrieval memory even at a high dose of 50 mg/kg. The memory deficit induced by diphenhydramine (10 mg/kg) or pyrilamine (50 mg/kg) was reversed by tacrine (1 mg/kg). **CONCLUSION**: Histamine H_1-receptors plays a certain role in spatial cognition, and its action may be due to both histaminergic and cholinergic neurons.

INTRODUCTION

It has been known that H_1-antagonists are widely used clinically to treat allergic diseases, especially type I hypersensitivity[1]. However, the classical H_1-antagonists have been implicated as causes of fatal traffic accidents and it has also been emphasized that even at the recommended doses, classical H_1-antagonists, such as diphenhydramine, chlorpheniramine, promethazine, and pyrilamine often have adverse effects on the central nervous system, such as diminished alertness, slowed reaction, induction of somnolence, and impairment of cognitive function[1,2]. We have previously demonstrated that histaminergic neuron system plays an important role in the control of both learning and memory[3-8], in which histamine regulates memory processes mainly mediated by presynaptic H_3-receptors and postsynaptic H_1-receptors[5,8]. Moreover, behavioral studies have found that the treatment with certain classical H_1-blockers impaired learning and memory in the step-through active avoidance response[9,10]. In these studies, however, the memory parameter used was transfer latency, which can be affected by behavioral toxicity such as decreases in locomotor activity or muscle relaxant activity. On the other hand, epinastine is the new second-generation H_1-antagonist, and has shown a less sedative action[11,12], however, the effects of epinastine on spatial memory have not yet been evaluated.

Therefore, in the present study, we use an 8-arm radial maze performance to further investigate effects of certain H_1-antagonists on spatial memory in rats.

MATERIALS AND METHODS

Animals Male Wistar rats (200-300 g, $n=62$, Charles River, Tokyo, Japan), maintained in individual cages with a 12-h light-dark cycle (lights on from 08:00-20:00). Water was given *ad libitum*. Experiments were carried out each day between 13:00-18:00.

Radial maze training The apparatus was made of clear Plexiglass, and consisted of a round central platform (30 cm in diameter) with 8 radiating arms attached to the platform at equal angles and distances. In addition, the testing room contained a white table, chair, curtain and several other distinctive visual objects and was well lighted by an overhead fluorescent lamp.

The experimental procedure was done as described previously[3,4,6-8]. In short, after familiarizing the rats

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with the radial maze for two days, all rats were trained with one trial per day (acquisition test). Drugs were administered before the acquisition test. In each trial, a single food pellet (45 mg each, Bio-Serv, Frenchtown, NJ, USA) was placed in the food cup in each of the 8 arms. A rat was placed on the central platform and allowed to make an arm choice to obtain food pellets until all 8 pellets had been eaten or 10 min had elapsed. The initial entry of an arm was scored as a correct choice, whereas a reentry to a previously visited site was scored as an error. Before the retrieval test, rats were trained continually until they reached a criterion of at least 7 different arms in the first 8 choices and all 8 within the first 9 choices. The animals were tested with either drug or vehicle after successfully completing the maze on 3 consecutive days. Twelve of 62 rats which were not able to solve the standard radial maze performance (including four dead) or just turned around the maze arm side by side were excluded. The retrieval trial was performed for 3 min or until the rat collected all pellets. The following indices of maze performance were used to represent accurate choice: (1) The number of total errors (TE) during a trial. (2) The number of initial correct choice (ICR) in an arm during the first 8 choices.

Drugs The drugs used in the study were diphenhydramine hydrochloride (Sigma), pyrilamine maleate (Sigma), epinastine hydrochloride (Boehringer Ingelheim KG, Ingelheim/Rhein, FRG), and tacrine (Sigma). Drugs were dissolved in saline and injected ip 30 min before test trial. Studies for drug effect were carried out once a week, on Thursdays or Fridays. The same animals were repeatedly used for two months, and they experienced all doses of either drugs.

Statistics analysis One-way analysis of variance with Dunnett's test was used for calculating a significant difference. Values are shown as $x \pm s$. Significant difference are express as $P < 0.05$ or $P < 0.01$ *vs* control group, respectively.

RESULTS

Effects of diphenhydramine, pyrilamine, and epinastine on acquisition process of spatial memory in 8-arm radial maze performance Ip injection of diphenhydramine (5 mg/kg) resulted in a significant increase in the number of TE and a decrease in the number of ICR from 1 to 4 test block in the acquisition memory process as evaluated by radial maze performance (Fig 1). Similarly, pyrilamine at a dose of 20 mg/kg created marked effects on both parameters of radial maze performance from 1 to 3 test block ($P < 0.05$). However no appreciable change was obtained for epinastine (50 mg/kg).

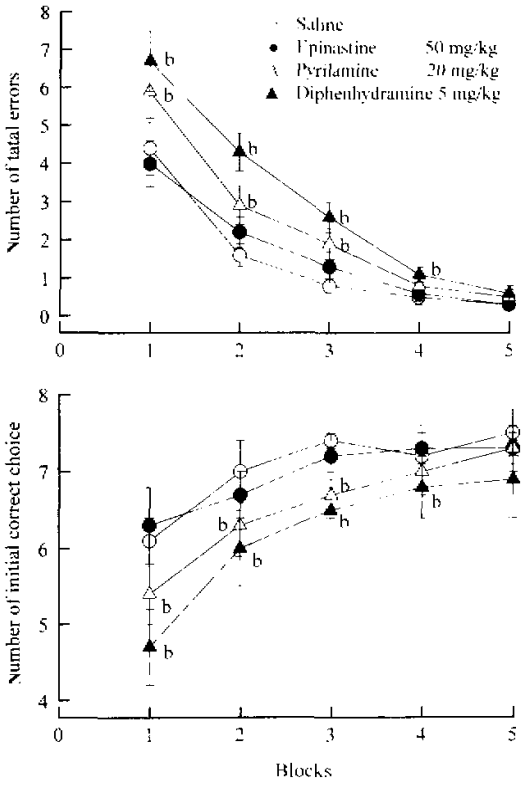

Fig 1. Effects of diphenhydramine, pyrilamine, and epinastine on acquisition of spatial memory in 8-arm radial maze performance. All drugs were injected ip 30 min before the test trial. The 15 d data analysis is shown in five 3-d time blocks. n = 21 – 23 rats. $x \pm s$. $^bP < 0.05$ *vs* saline-treated group.

Effects of diphenhydramine, pyrilamine, and epinastine on retrieval process of spatial memory in 8-arm radial maze performance Both diphenhydramine and pyrilamine produced an increase in the number of TE, and a decrease in the number of ICR in a dose-dependent manner (Fig 2). Diphenhydramine at a dose of 5 ($P < 0.05$) and 10 mg/kg ($P < 0.01$), and pyrilamine at doses of 50 mg/kg created significant effects ($P < 0.05$). On the other hand, epinastine produced no appreciable effect on both TE and ICR even at a high dose of 50 mg/kg.

Effect of tacrine on memory deficits induced

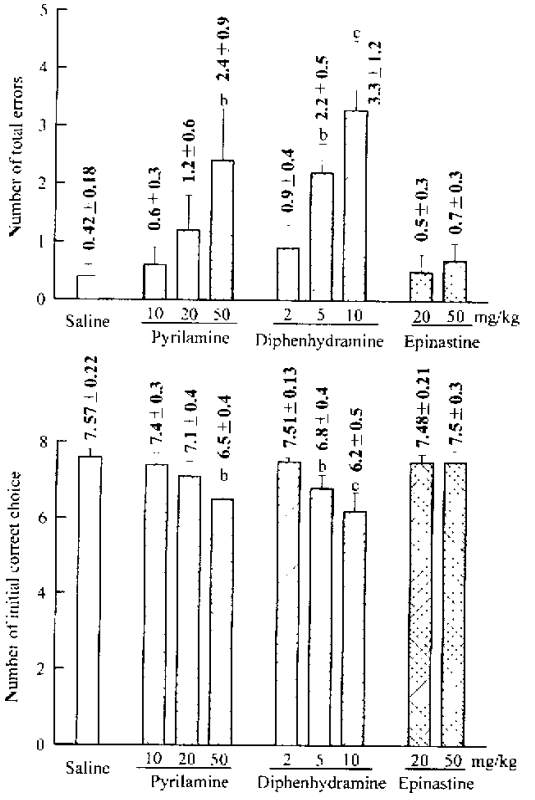

Fig 2. Effects of diphenhydramine, pyrilamine, and epinastine on retrieval of spatial memory in 8-arm radial maze performance. All drugs were injected ip 30 min before the test trial. $n = 21 - 22$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* saline-treated group.

by diphenhydramine (10 mg/kg) and pyrilamine (50 mg/kg) in 8-arm radial maze performance

The ip injection of tacrine, a representative acetylcholinesterase inhibitor reversed the spatial memory deficits induced by diphenhydramine (10 mg/kg) in a dose-dependent manner (Tab 1). Tacrine at a dose of 0.2 mg/kg showed no marked effect, however at doses of 0.5 and 1 mg/kg it significantly decreased the number of TE and increased the number of ICR ($P < 0.05$). Similarly, pyrilamine (50 mg/kg)-induced memory deficits were antagonized by tacrine at a dose of 1 mg/kg ($P < 0.05$).

Tab 1. Effect of tacrine on memory deficits induced by diphenhydramine (10 mg/kg) and pyrilamine (50 mg/kg) in 8-arm radial maze performance. Tacrine was injected simultaneously with diphenhydramine or pyrilamine. $\bar{x} \pm s$. $^bP < 0.05$ *vs* diphenhydramine + saline. $^cP < 0.05$ *vs* pyrilamine + saline.

Drugs	Doses (mg/kg, ip)	n	Number of total errors	Number of initial correct responses
Saline	–	23	0.18 ± 0.13	7.82 ± 0.26
Diphenhydramine + Saline	10 –	22	3.2 ± 1.0	6.2 ± 0.5
Diphenhydramine + Tacrine	10 0.2	22	2.4 ± 0.5	6.6 ± 0.4
	0.5	20	1.2 ± 0.4^b	6.9 ± 0.3^b
	1.0	22	1.0 ± 0.6^b	7.30 ± 0.27^b
Pyrilamine + Saline	50 –	22	2.3 ± 0.7	6.4 ± 0.4
Pyrilamine + Tacrine	50 0.2	18	2.0 ± 0.5	6.7 ± 0.3
	0.5	22	1.4 ± 0.5	7.1 ± 0.5
	1.0	22	0.7 ± 0.4^c	7.2 ± 0.3^c

DISCUSSION

It has been known that H_1-antagonists are among the most widely used drugs in the world. However, even at the clinical doses, the classical H_1-antagonists usually result in adverse effects on the nervous system, which are considered to be due to the blockage of H_1-receptor in the central nervous system[1,2,10]. In the present study, ip injection of diphenhydramine or pyrilamine resulted in spatial memory deficits. In addition, we have previously reported that 2-thiazolylethylamine, one of the representative H_1-agonists, ameliorated the memory deficits induced by not only diphenhydramine and pyrilamine but scopolamine and MK-801 in both passive avoidance response and radial maze performance in rats[4,6,7,9]. Yanai *et al* have recently found in humans using the PET technique that oral injection of chlorpheniramine (2 mg/kg), a selective and classical H_1-antagonist, occupied (76.8 ± 4.2) % of the available histamine H_1-receptors in the brain, meanwhile resulting in a memory deficit, wherein there appeared good correlation between H_1-receptor binding and memory deficits[11]. These findings strongly supported the evidence[3,4,6,7] that the postsynaptic H_1-receptors in the brain play an important role in spatial cognition.

It has been indicated that there is a close relationship between histaminergic and cholinergic systems[6,7,13,14]. Tacrine, which is a representative acetylcholinesterase in-

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hibitor used in Alzheimer's disease[13] has been reported to potently inhibit histamine-*N*-methyltransferase, the enzyme responsible for histamine metabolism, as compared to acetylcholinesterase and to enhance brain histamine levels *in vitro* and *in vivo*[13,14]. In the present study, we used tacrine as a tool to indicate whether it is involved in H_1-antagonists-induced spatial memory deficit. It was found that pyrilamine- and diphenhydramine-induced memory deficits were reversed completely by tacrine. Classical H_1-antagonists are well known to possess not only H_1-receptors but also muscarinic receptors. Therefore, the impairment of spatial memory by diphenhydramine and pyrilamine may be due to both an antihistaminergic and anticholinergic action. Our results support the previous evidence in which 2-methylhistamine-induced memory facilitation was attenuated by pretreatment with hemicholinium[9].

There has been a resurgence of interest in histamine H_1-antagonists since the recent discovery and development of antihistamines with low, if any, side effects of sedation, which are thought to be due to the occupation of central histamine H_1-receptor in the brain. Previous studies have reported that the second generation H_1-antagonists, such as azelastine, oxatomide, and ketotifen caused no apparent effect on sedation[10]. It is indicated that these lower neuronal side effects of second H_1-antagonists are associated with their difficulty in crossing BBB. Epinastine has been lately introduced as a second generation H_1-antagonist with less sedative effect on the central system, intended for the treatment of allergic disorders such as asthma[11]. It binds H_1-receptors with a high affinity and selectivity and its antihistamine properties have been demonstrated[11,16], however, it is also reported to poorly penetrate into the brain[12,16]. In our present study, we also found that epinastine caused no marked effect on the memory processes. More data is required to further explain this observation. Previous results show that the second generation H_1-antagonists result in no appreciable effect on learning or memory as evaluated by step-through active avoidance response in rats[10]. H_1-receptor occupancy in the frontal cortex of epinastine has been observed to be less than 20 % of total H_1-receptors[12]. Therefore, the low occupancy of H_1-receptor by epinastine in the brain, and its difficulty in crossing BBB, may be closely related to its low incidence of effects on spatial memory process.

Finally, we may conclude that histamine H_1-receptor plays an important role in spatial cognition, and its action may be due to both histaminergic and cholinergic neurons.

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组胺 H_1 受体阻断剂对大鼠八臂迷宫空间记忆的影响

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关键词 迷宫学习; 组胺 H_1 受体; 苯海拉明; 依匹斯汀; 他克林

目的: 研究和阐明组胺 H_1 受体阻断剂对大鼠空间记忆的作用机制. **方法**: 采用八臂迷宫学习的程序研究大鼠的空间记忆. **结果**: 在学习过程中, 腹腔内注射第一代组胺 H_1 受体阻断剂苯海拉明(5 mg/kg)或美吡拉明(20 mg/kg)显著性地引起空间记忆障碍. 苯海拉明(5, 10 mg/kg)、美吡拉敏(50 mg/kg)则浓度依赖性且显著性地引起空间记忆再生过程的障碍. 相反, 新型组胺 H_1 受体阻断剂依匹斯汀(50 mg/kg)对大鼠的学习和记忆再生过程均无明显作用. 他克林(1 mg/kg)可改善苯海拉明(10 mg/kg)、美吡拉敏(50 mg/kg)所致的记忆障碍. **结论**: 中枢组胺 H_1 受体参与了调节大鼠空间记忆的作用, 而且其作用主要与组胺神经及胆碱能神经相关.

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