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Facilitating effects of histamine on spatial memory deficit induced by scopolamine in rats

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KEY WORDS maze learning; scopolamine; histamine; histamine H_1 receptors; memory disorders

ABSTRACT

AIM: To investigate whether or not histamine was involved in scopolamine-induced spatial memory deficits evaluated in 8-arm radial maze performance of rats. **METHODS**: Eight-Arm radial maze performance was used to measure spatial memory in rats, and the brain regions were subsequently dissected and histamine contents were determined by HPLC. **RESULTS**: Intracerebroventricular (icv) injection of histamine (100 or 200 ng) or thioperamide (50 μg), and intraperitoneal (ip) injection of histidine (1000 mg/kg) ameliorated memory impairment induced by scopolamine regarding both parameters of radial maze performance. 2-Thiazolylethylamine, but not 4-methylhistamine showed the similar effect to histamine. Both histamine (200 ng, icv) and histidine (1000 mg/kg, ip) were equally effective in increasing the histamine content in the cortex, hippocampus, and hypothalamus. **CONCLUSION**: These results suggest that brain histamine plays an important role in learning and memory, and its action may be due to cholinergic neurons.

INTRODUCTION

The central cholinergic system has been established as playing an important role in normal cognitive function[1]. The impairment of central cholinergic transmission by pharmacological antagonism or neuroanatomical lesions has indicated to be associated with cognitive deficits in both rodents and man[1-3]. On the other hand, behavioral studies indicate that histamine ameliorates impaired memory retrieval induced by aging or hippocampal lesions in rats using passive and active avoidance tests[4-6]. Depletion of hippocampal histamine content is shown to cause adverse effects in rats both in active avoidance task and radial maze performance[7,8].

Recently, the relation between histaminergic neuron and cholinergic neuron with regard to learning and memory has attracted a great deal of attention[9-12]. Khateb *et al*[11] and Gorelova *et al*[12] have recently discovered that histamine can result in an excitatory effect on the cholinergic neurons. It has been reported that the treatment of histamine ameliorates learning deficits induced by scopolamine in the passive avoidance response and the elevated plus-maze test in mice[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. It has been demonstrated that the 8-arm radial maze paradigm is more useful to study learning and memory compared with other methods[8,13].

In the present study, we use 8-arm radial maze performance to further investigate whether or not histamine is involved in spatial memory deficit induced by scopolamine in rats.

MATERIALS AND METHODS

Animals The animals used in this study were male Wistar rats (♂, 200-280 g, $n = 70$, Charles River, Tokyo, Japan), maintained in individual cages with a 12-h light-dark cycle (lights on from 8:00 - 20:00). Water was given *ad lib*. Experiments were carried out each day between 13:00 - 19:00.

Surgical procedure Rats were anesthetized with sodium pentobarbital (35 mg/kg, ip), and fixed on a stereotaxic apparatus (Narishige, SR-5, Tokyo, Japan), and a guide cannula made of stainless steel tubing 700 μm in outer diameter, was implanted into the right lateral ventricle according to the following coordinates measured

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from bregma[14]; AP: −0.9 mm, L: 1.5 mm, H: 3.8 mm from the skull. At least 7 d were allowed for recovery from the surgery. All procedures involving animals were conducted in accordance with the guidelines for the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

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. Each arm was 50 cm long and 12 cm wide, surrounded by a wall 4.5 cm high. The distal end of each arm contained a food cup to hold a standard food pellet (45 mg each, Bio-Serv, Frenchtown, NJ, USA). The entire maze was elevated 40 cm above the floor. 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[8,13]. To familiarize the rats with the radial maze, prior to training they received one daily habituation trial for two days. Pellets were scattered over the entire maze surface, and 3 or 4 rats were simultaneously placed in the radial maze and allowed to explore for 10 min and to take food pellets freely. After adaptation, all rats were trained with one trial per day. In each trial, a single food pellet was placed in the food cup in each of the 8 arms. A rat was placed on the central platform and allowed to make arm choice to obtain food pellets until all 8 pellets had been eaten or 10 min had elapsed. 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 before the test. The animals were tested with either drug or vehicle after successfully completing the maze on 3 consecutive days. Forteen of 70 rats which were not able to solve the standard radial maze performance or just turn around the maze arm side by side were excluded. Fifty six of 70 rats which were able to solve the standard radial maze performance were used in the following drug test. The test trial was performed for 3 min or until the rat collected all pellets. 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. The following indices of maze performance were used to represent accurate choice: (1) The number of total errors during a trial (TE). (2) The number of initial entry of an arm during the first 8 choices (ICR).

Determination of brain histamine contents Histamine contents in the brain were determined as described previously[6-8]. After behavioral tests, the rats with 17 – 23-wk ages were sacrificed by decapitation, the brain was quickly removed and placed on an ice-cold stainless steel plate. The brain regions were subsequently dissected and histamine contents were determined by HPLC (CCP & 8010 series, Tosoh, Tokyo, Japan).

Drugs The drugs used in the study were histamine dihydrochloride (Wako, Osaka, Japan), *L*-histidine monohydrochloride (Wako, Osaka, Japan), thioperamide hydrochloride (provided by Eisai, Tokyo, Japan), 2-thiazolylethylamine dihydrochloride (SmithKline Beecham, London, UK), 4-methylhistamine dihydrochloride (SmithKline Beecham, London, UK), and scopolamine hydrobromide (SmithKline Beecham, London, UK). Drugs were dissolved in saline and injected icv in a fixed volume of 5 μL over a period of 60 s at a constant speed with a continual infusion pump (KN-201, Natsume, Tokyo, Japan). Scopolamine and histidine were injected ip. Studies for drug effect were carried out once a week, on Thursday. Thirty min before the learning behavior test, the rodents received ip injection of scopolamine. The same animals were repeatedly used, and they experienced for all doses of either drugs.

Statistics analysis One-way analysis of variance with Dunnett's test was used for calculating a significant difference. Values were shown as $\bar{x} \pm s$.

RESULTS

Effects of histamine and histidine on memory deficits induced by scopolamine in radial maze performance Tab 1 shows the effects of histamine (icv) and histidine (ip) on radial maze performance deficits induced by scopolamine (0.2 mg/kg). Scopolamine produced a marked increase in the number of TE, and a decrease in the number of ICR. The icv injection of histamine antagonized the effect of scopolamine in a dose-dependent manner; while no significant effect was observed at a dose of 50 ng, at doses of 100 ng and 200 ng significant decreases were observed in the number of TE ($P < 0.05$), and at a dose of 200 ng the number of ICR was significantly increased ($P < 0.05$). Similar results were obtained with histidine; ie, a significant effect was observed on the above parameters at a dose of 1000 mg/kg ($P < 0.05$).

Effects of thioperamide on memory deficits induced by scopolamine in radial maze performance Similar to histamine, thioperamide, a represen-

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tative and selective H_3-antagonist, dose-dependently antagonized the memory deficits induced by scopolamine (Fig 1), a significant effect was observed on both parameters at a dose of 50 μg ($P<0.05$).

Effects of 2-thiazolylethylamine and 4-methylhistamine on memory deficits induced by scopolamine in radial maze performance The icv injection of 2-thiazolylethylamine, a representative H_1-agonist reversed the spatial memory deficits induced by scopolamine (Tab 2). At doses of 50 and 100 ng, it caused a decrease in the number of TE and an increase in the number of ICR dose-dependently, and at a dose of 200 ng a significant decrease was observed in the number of TE and the numbers of ICR were increased ($P<0.05$). On the other hand, 4-methylhistamine, a representative H_2-agonist showed no appreciable effect on spatial memory deficits induced by scopolamine.

Effects of histamine and histidine on brain histamine contents Rats were sacrificed for histamine contents analysis, 10 min or 3 h after icv injection of histamine or ip injection of histidine. As shown in Tab 3, both histamine (200 ng, icv) and histidine (1000 mg/kg, ip) were equally effective in increasing the histamine content in the cortex, hippocampus, and hypothalamus ($P<0.01$).

Tab 1. Effects of histamine and histidine on memory deficits induced by scopolamine 0.2 mg/kg and evaluated by radial maze performance in rats. Histamine was injected icv 20 min after scopolamine, and histidine was injected 2.5 h before scopolamine. $\bar{x}\pm s$. $^bP<0.05$ *vs* scopolamine + saline-treated group.

Drugs	Doses	n	Total errors	Initial correct responses
Saline	–	25	0.17±0.08	7.84±0.18
Scopolamine + Saline	0.2 mg/kg, ip –	25	3.5±1.0	6.2±0.4
Scopolamine + Histamine	0.2 mg/kg, ip 50 ng, icv	19	2.2±0.6	6.5±0.4
	100 ng, icv	23	1.3±0.4^b	7.0±0.3
	200 ng, icv	25	1.0±0.5^b	7.34±0.18^b
Scopolamine + Histidine	0.2 mg/kg, ip 200 mg/kg, ip	25	2.5±0.6	6.03±0.27
	500 mg/kg, ip	25	1.9±0.5	6.6±0.5
	1000 mg/kg, ip	25	1.4±0.5^b	7.32±0.21^b

Tab 2. Effects of H_1 and H_2 agonists on memory deficits induced by scopolamine 0.2 mg/kg and evaluated by radial maze performance in rats. 2-Thiazolylethylamine and 4-methylhistamine were injected icv 20 min after scopolamine. $\bar{x}\pm s$. $^bP<0.05$ *vs* (scopolamine + saline)-treated group.

Drugs	Doses	n	Total errors	Initial correct responses
Saline	–	25	0.18±0.06	7.82±0.18
Scopolamine + Saline	0.2 mg/kg, ip –	25	3.5±1.0	6.2±0.4
Scopolamine + 2-Thiazolyl ethylamine	0.2 mg/kg, ip 50 ng, icv	21	2.5±0.6	6.5±0.4
	100 ng, icv	23	1.8±0.4^b	6.8±0.4
	200 ng, icv	25	1.33±0.28^b	7.24±0.18^b
Scopolamine + 4-Methylhistamine	0.2 mg/kg, ip 50 mg/kg, ip	16	3.5±0.7	6.3±0.4
	100 mg/kg, ip	19	2.9±0.5	6.6±0.5
	200 mg/kg, ip	20	3.4±0.6	6.0±0.3

Tab 3. Influence of histamine and histidine on brain histamine content. Histamine was injected icv 10 min and histidine was injected ip 3 h before the analysis. $\bar{x} \pm s$. $^cP < 0.01$ *vs* saline-treated group.

Brain regions	Histamine contents (ng/g tissue)		
	Saline $n = 25$	Histamine (200 ng) $n = 20$	Histidine (1000 mg/kg) $n = 16$
Cortex	29.7 ± 1.8	70 ± 5^c	63 ± 7^c
Hippocampus	25.2 ± 1.5	67 ± 5^c	56 ± 4^c
Hypothalamus	289 ± 17	631 ± 49^c	646 ± 60^c

DISCUSSION

In the present study, icv injection of histamine or ip injection of histidine improved the spatial memory deficits induced by scopolamine (0.2 mg/kg) in radial maze performance, at a dose which has been previously reported to impair memory in radial maze[15,16]. On the other hand, running time per choice was not influenced by the drug treatment (data not shown). Therefore, it is reasonable to presume that the ameliorating effect of histamine on the spatial memory deficit induced by scopolamine was unrelated to the changes in locomotor activity.

It is reported that there is a close relationship between histaminergic and cholinergic systems[9-12]. Khateb *et al*[11] and Gorelova *et al*[12] found that *in vitro* the treatment of histamine ameliorated cholinergic neurons activity in the nucleus basalis, medial septum, and diagonal band of Broca. It has been also reported that histamine enhanced acetylcholine release in the cortex and hippocampus *in vivo* experiments[17,18]. Therefore, the acetylcholine released by histamine may be, at least in part, contributing to the observed improvement of the scopolamine-induced memory deficits. Our results support the previous evidence in which histidine and thioperamide facilitated the memory deficit induced by scopolamine in passive avoidance response and in an elevated plus-maze test in mice[9,10].

In our previous studies, we have reported that postsynaptic H_1-receptors and presynaptic H_3-receptors play an important role in learning and memory in both passive avoidance response and radial maze performance in rats[4-8]. In the present study, 2-thiazolylethylamine ameliorated the memory deficits induced by scopolamine. In contrast, no effect was observed with 4-methylhistamine. Similar to histamine, thioperamide

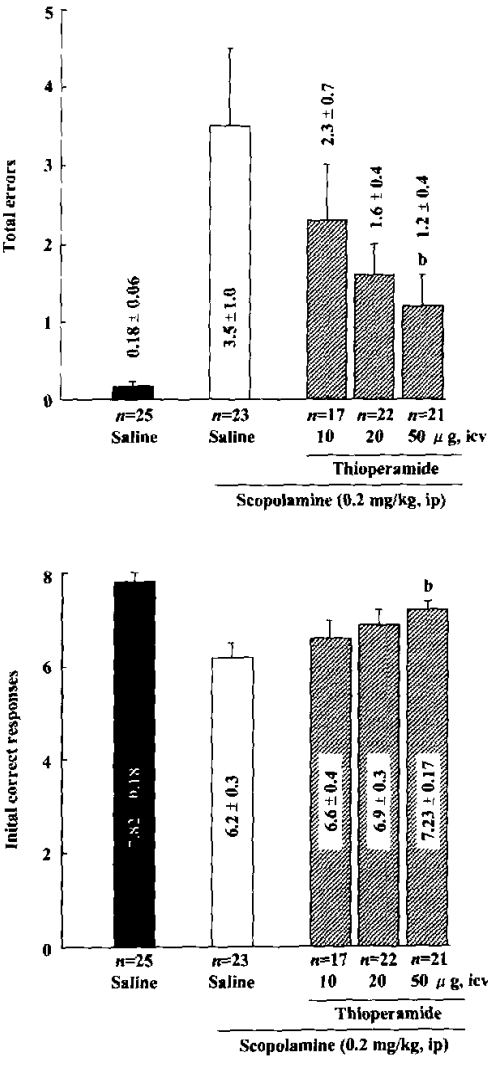

Fig 1. Effect of thioperamide icv on memory deficits induced with scopolamine (0.2 mg/kg) as evaluated by radial maze performance in rats. Thioperamide was injected icv 15 min after scopolamine. $\bar{x} \pm s$. $^bP < 0.05$ *vs* scopolamine-treated group.

ameliorated the scopolamine-induced memory deficits. Thioperamide has been reported to enhance both synthesis and release of histamine[19]. These evidences strongly suggest that the ameliorating effects of histamine may be mediated by postsynaptic H_1-receptors and presynaptic H_3-receptors.

It is well known that learning and memory are related not only to the cortex and hippocampus but also to the

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hypothalamus[20]. As described in the text, both histamine and histidine were effective in increasing histamine contents in the cortex, hippocampus, and hypothalamus. Previously, we have found a strong correlation between a decrease of histamine contents in the cortex, hippocampus, and hypothalamus and memory deficits induced by hippocampal lesion or treatment with α-FMH[6-8]. These findings support the contention that the histamine contents in the cortex, hippocampus, and hypothalamus are highly correlative to spatial cognition.

In conclusion, it seems reasonable to assume that there exists a close relationship between histaminergic and cholinergic neurons regarding spatial memory deficits induced by scopolamine as evaluated by the 8-arm radial maze task.

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组胺对东莨菪碱所致大鼠空间记忆障碍的改善作用

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关键词 迷宫学习; 东莨菪碱; 组胺; 组胺 H_1 受体; 记忆障碍

目的: 研究和阐明中枢组胺对东莨菪碱所致大鼠(空间)记忆障碍的作用机制. **方法**: 采用迷宫学习的程序研究大鼠的空间记忆, 并利用高效液相法测定脑内组胺含量. **结果**: 侧脑室内注射组胺(100, 200 ng)、2-β-噻唑乙胺(200 ng)及 4-[4′-(环己氨基硫代甲酰基哌啶)]-4H-咪唑(50 μg)或腹腔内注射组胺酸(1000 mg/kg)均可对抗东莨菪碱所致的记忆障碍. 相反, 4-甲基组胺(50-200 ng)却无明显作用. 组胺(200 ng)和组胺酸(1000 mg/kg)均可有效地增加大脑皮层、海马及下丘脑中的组胺含量. **结论**: 中枢组胺可以明显改善东莨菪碱引起的大鼠空间记忆障碍, 其作用主要与 H_1、H_3 受体相关.

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