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Effect of histamine H_3-receptor antagonist clobenpropit on spatial memory of radial maze performance in rats

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KEY WORDS memory disorder; scopolamine; histamine H_3-receptors; clobenpropit

ABSTRACT

AIM: The effect of histamine H_3-receptor antagonist, clobenpropit (VUF9153) on spatial memory deficits induced by scopolamine was investigated in 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 clobenpropit (50 μg) ameliorated memory impairment induced by scopolamine in both parameters of radial maze performance. The amelioration induced by clobenpropit was antagonized by an H_3-agonist, (R)-α-methylhistamine (5-10 μg). α-Fluoromethylhistidine (10, 20 μg), a histidine decarboxylase inhibitor, also effectively reversed clobenpropit-induced ameliorating effects. **CONCLUSION**: The brain histamine H_3-receptor antagonists are highly related to the spatial memory, and this action may be due to cholinergic neurons.

INTRODUCTION

A number of studies suggest that histamine plays an important role in learning and memory[1-7]. Behavioral studies indicate that histamine facilitates impaired memory retrieval induced by aging or hippocampal lesions in rats using passive and active avoidance tests[1-5]. Depletion of hippocampal histamine content is shown to recall adverse effect in rats both in active avoidance task and radial maze performance[4,7]. However the effect of histamine H_3-receptor in spatial cognition is less understood. Recently, it has been reported that scopolamine-induced learning deficits in the passive avoidance response and the elevated plus-maze test in mice are ameliorated by histamine[8,9]. In these studies, however, the memory parameter used is transfer latency, which can be affected by behavioral toxicity such as decrease 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 as compared with other methods[4,5,10].

In the present study, we use 8-arm radial maze performance to investigate whether histamine H_3-receptor is involved in spatial memory deficit induced by scopolamine in rats.

MATERIALS AND METHODS

Animals Wistar rats (♂, 200-280 g, Grade Ⅱ, purchased from Charles River, Tokyo, Japan, n = 58) were 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 with an outer diameter of 700 μm, was implanted into the right lateral ventricle according to the following coordinates measured from bregma[11]; AP: -0.9 mm, L: 1.5 mm, H: 3.8 mm from the skull. At least 7 days 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

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was 50 cm long × 12 cm wide, surrounded by a wall of 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, lighted by an overhead fluorescent lamp. In addition, several other distinctive visual objects were located around the room.

The experimental procedure was done as described previously[4,6]. To familiarize the rats with the radial maze, they received one daily habituation trial for two days prior to training. 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 center 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 reaching 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. The test trial was performed after 3 min or until the rat collected all pellets. The following indices of maze performance were used to represent accurate choice: (1) number of total errors (TE), (2) number of initial correct responses (ICR).

Determination of brain histamine contents Histamine contents in the brain were determined as described previously[3,4,7]. Each group consisted of 7 rats. After behavioral tests, the rats 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 Clobenpropit dihydrobromide (kindly provided by Prof Timmerman, Leiden-Amsterdam Center for Drug Research, Vrije Universiteit Amsterdam, the Netherlands), (R)-α-methylhistamine (Donated by Prof Schwartz, Unite de Neurobiologie et Pharmacologie, Centre Paul Broca, Paris, France) and α-fluoromethylhistidine (Merck Sharp & Dohme Research Lab, Rahway, NJ) 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 continuous infusion pump (KN-201, Natsume, Tokyo, Japan), and scopolamine hydrobromide (SmithKline Beecham, London, UK) dissolved in saline, was injected ip.

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

RESULTS

Effects of clobenpropit on memory deficits induced by scopolamine in radial maze performance Scopolamine 0.2 mg/kg produced a marked increase in the number of TE, and a decrease in the number of ICR. The icv injection of clobenpropit antagonized the effect of scopolamine in a dose-dependent manner; no significant effect was observed at doses of 10 and 20 μg, while at a dose of 50 μg a significant decrease was observed in the number of TE ($P < 0.05$), and the numbers of ICR were increased ($P < 0.05$). In addition, the drug treatment created no appreciable effect on running time per choice in 8-arm radial maze performance (Fig 1).

Effects of (R)-α-methylhistamine on clobenpropit-induced amelioration of memory deficits induced by scopolamine in radial maze performance The clobenpropit-induced amelioration of memory deficits induced by scopolamine were antagonized by (R)-α-methylhistamine, a representative H_3-agonist. At a dose of 2 μg, it caused no marked inhibition, however at a dose of 5 μg it significantly increased the number of TE ($P < 0.05$), and at a dose of 10 μg significant effects were observed in both parameters of radial maze performance ($P < 0.05$). Additionally as shown in Tab 1, (R)-α-methylhistamine had no significant effect on memory deficit induced by scopolamine in both parameters even at doses of 20 μg.

Effects of α-fluoromethylhistidine on clobenpropit-induced amilioration of memory deficit induced by scopolamine in radial maze performance The icv pretreatment with α-fluoromethylhistidine, a selective histidine decarboxylase inhibitor, dose-dependently antagonized clobenpropit-induced amilioration of memory deficit action (Tab 2), and at doses of 20 and 50 μg significant increases were observed in the number of TE ($P < 0.05$), and the numbers of ICR were decreased ($P < 0.05$).

Effects of clobenpropit on brain histamine contents Histamine contents were measured 15 min or 2 h after injection of clobenpropit or α-fluoromethylhistidine injection, respectively. Clobenpropit (20, 50 μg) alone created no appreciable influence on the his-

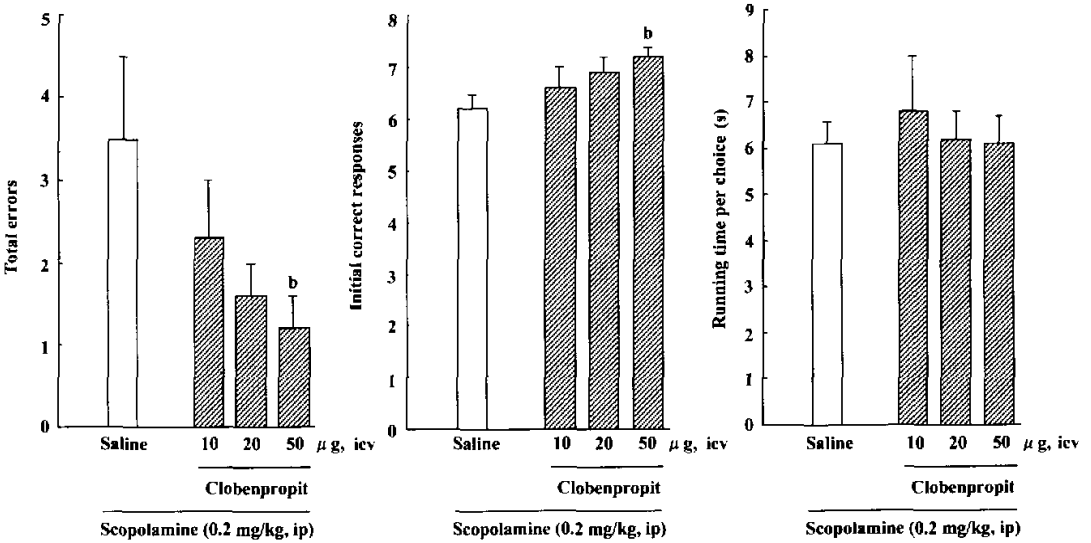

Fig 1. Effects of icv injection of clobenpropit on memory deficits of radial maze performance induced by scopolamine (0.2 mg/kg) in rats. Clobenpropit was injected icv 15 min after scopolamine injection. $n = 19-24$ rats. $^bP < 0.05$ vs scopolamine-treated group.

Tab 1. Effects of (R)-α-methylhistamine on clobenpropit- induced amelioration of memory deficits induced by scopolamine (0.2 mg/kg) in rats. $^bP < 0.05$ vs scopolamine + clobenpropit + saline-treated group.

Drugs	Doses	n	Total errors	Initial correct responses
Saline	–	25	0.19 ± 0.08	7.84 ± 0.14
Scopolamine + Saline	0.2 mg/kg, ip –	25	3.51 ± 1.33	6.10 ± 0.52
Scopolamine + Clobenpropit + Saline	0.2 mg/kg, ip 50 μg, icv –	22	1.24 ± 0.40	7.23 ± 0.21
Scopolamine + Clobenpropit + (R)-α-methylhistamine	0.2 mg/kg, ip 50 μg, icv 2 μg, icv	20	1.73 ± 0.53	6.72 ± 0.30
	5 μg, icv	22	2.51 ± 0.40^b	6.42 ± 0.32
	10 μg, icv	22	3.03 ± 0.50^b	6.11 ± 0.43^b
Scopolamine + (R)-α-methylhistamine	0.2 mg/kg, ip 2 μg, icv	19	2.64 ± 0.62	6.32 ± 0.42
	5 μg, icv	19	3.40 ± 0.80	6.21 ± 0.20
	10 μg, icv	21	2.90 ± 0.64	6.44 ± 0.32
	20 μg, icv	22	2.54 ± 0.73	6.53 ± 0.30

Clobenpropit and (R)-α-methylhistamine were injected icv 15 min after scopolamine injection.

tamine contents in the cortex, hippocampus, and hypothalamus. However, as shown in Tab 3, pretreatments of α-fluoromethylhistidine decreased histamine content in all of regions measured, at a dose of 10 μg it significantly decreased histamine content in the hippocampus and hypothalamus ($P < 0.05$). At a dose of 20 μg, it markedly decreased histamine contents in the cortex ($P < 0.05$), hippocampus ($P < 0.01$) and hypothalamus ($P < 0.05$).

DISCUSSION

It is generally known that H_3-receptors regulate the

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Tab 2. Effects of α-fluoromethylhistidine on clobenpropit-induced amelioration of memory deficits induced by scopolamine (0.2 mg/kg) in rats. $^bP<0.05$, $^cP<0.01$ *vs* scopolamine + clobenpropit + saline-treated group.

Drugs	Doses	*n*	Total errors	Initial correct responses
Saline	–	25	0.2 ± 0.1	7.8 ± 0.1
Scopolamine + Saline	0.2 mg/kg, ip –	25	3.5 ± 1.3	6.1 ± 0.5
Scopolamine + Clobenpropit + Saline	0.2 mg/kg, ip 50 μg, icv –	25	1.2 ± 0.4	7.3 ± 0.3
Scopolamine + Clobenpropit + α-Fluoromethylhistidine	0.2 mg/kg, ip 50 μg, icv 10 μg, icv	23	2.4 ± 0.6	6.5 ± 0.5
	20 μg, icv	20	3.5 ± 0.4^b	6.0 ± 0.4^b
	50 μg, icv	23	4.1 ± 0.9^c	5.4 ± 0.6^c

α-Fluoromethylhistidine was injected ip 1.5 h before scopolamine injection, and clobenpropit was injected icv 15 min after scopolamine injection.

Tab 3. Effect of clobenpropit on brain histamine contents. $^bP<0.05$, $^cP<0.01$ *vs* clobenpropit-treated group.

Drugs		*n*	Histamine contents (ng/g tissue) Cortex	Hippocampus	Hypothalamus
Saline			31.2 ± 1.5	23.4 ± 1.5	298 ± 34
Clobenpropit	(20 μg)	9	30.2 ± 1.4	24.0 ± 1.1	277 ± 21
	(50 μg)	9	27.8 ± 1.8	21.8 ± 0.9	290 ± 23
α-Fluoromethylhistidine					
	(10 μg)	8	27.2 ± 1.5	22.8 ± 1.0	282 ± 26
	(20 μg)	10	25.6 ± 1.6	20.1 ± 0.8	263 ± 24
Clobenpropit α-Fluoromethylhistidine	(50 μg) +				
	(10 μg)	7	20.3 ± 1.7	14.0 ± 1.0^b	200 ± 18^b
	(20 μg)	9	14.8 ± 2.5^b	10.6 ± 1.4^c	168 ± 28^b

α-Fluoromethylhistidine was injected ip 1 h 45 min before clobenpropit injection.

release and synthesis of neuronal histamine, and clobenpropit which is considered as a potent and selective histamine H_3-receptor antagonist[13] can activate the central histaminergic system increasing histamine release from nerve terminals[14,15]. Previous data[2,12] reported that clobenpropit can improve learning and memory in the passive avoidance response in both mice and rats. In the present study, it was found that clobenpropit reversed the memory deficits induced by scopolamine. Additionally, running time per choice was not influenced by the drug treatment. Thus, it can be reasonably presumed that the ameliorating effect of histamine on the spatial memory deficit induced by scopolamine is unrelated to the changes in locomotor activity. Therefore, clobenpropit induced reversal of scopolamine-induced spatial memory impairment may be due to accelerated histamine release from histaminergic presynapic terminals. The release of histamine by clobenpropit is, at least in part, contributing to the observed amelioration of the scopolamine-induced memory deficits.

It was also found that the ameliorating effects of clobenpropit was fully reversed by (R)-α-methylhistamine, a representative H_3-agonist[14]. (R)-α-methylhistamine alone showed no apparent effect on scopolamine-induced memory deficits. This observation suggests that the effects of clobenpropit are mediated through the autoreceptors located on histaminergic neurons, ie the H_3-receptors. Interestingly, we found that the ameliorating effect of clobenpropit was antagonized by α-fluoromethylhistidine, a potent inhibitor of histidine decarboxylase. α-Fluoromethylhistidine is known to markedly decrease endogenous histamine content from the nerve ter-

minals without affecting the levels of other neurotransmitters[16]. In the present study α-fluoromethylhistidine was also found to decrease the brain histamine contents and clobenpropit (50 μg) could not autoregulate enough release of histamine to counteract its effect. Therefore, the released histamine by clobenpropit is not enough to facilitate the scopolamine-induced memory deficits due to the part pre-inhibition of histamine synthesis by α-fluoromethylhistidine. These findings also confirm that histaminergic H_3-receptors are involved in mechanisms of spatial memory processes.

Recently, it has been reported that H_3-receptor is a heteroreceptor so in addition of controlling the central histaminergic transmission, *in vitro* evidence indicates that, stimulation of H_3-receptors can also regulate the release of acetylcholine[17]. It is also reported that histamine enhances acetylcholine release in the cortex and hippocampus *in vivo* experiment[18-20]. Therefore, clobenpropit induced release of acetylcholine may play an important role in the observed improvement of the scopolamine-induced memory deficits.

In conclusion, the results of the present study implicates that both histaminergic H_3-receptors and cholinergic neurons are involved in spatial memory processes, as observed in the 8-arm radial maze task, and there seems to exist a close interrelation between them.

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组胺 H_3 受体拮抗剂 clobenpropit 对大鼠放射状迷宫程序空间记忆的作用

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关键词 记忆障碍；东莨菪碱；组胺 H_3 受体；clobenpropit

目的：研究中枢组胺 H_3 受体拮抗剂 clobenpropit｛*S*-[3-(4(5)-咪唑基)-丙基-*N*-(4-氯苄基)]异硫脲，VUP9153｝对东莨菪碱引起的大鼠空间记忆障碍的作用．**方法**：采用8-方向放射状迷宫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 clobenpropit (50 μg)可明显对抗东莨菪碱导致的空间记忆障碍．相反，组胺 H_3 受体激动剂(R)-α-甲基组胺(5, 10 μg)可明显抑制 clobenpropit 的空间记忆改善作用．另外，clobenpropit 的作用被 α-氟甲基组胺酸(10, 20 μg)有效地抑制．**结论**：中枢组胺 H_3 受体与大鼠空间记忆的调节密切相关，而且其作用与胆碱能神经相关．

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