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Effects of Intracerebroventricular Injection of α-Fluoromethylhistidine on Radial Maze Performance in Rats

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CHEN, Z., Y. SUGIMOTO AND C. KAMEI. *Effects of intracerebroventricular injection of α-fluoromethylhistidine on radial maze performance in rats.* PHARMACOL BIOCHEM BEHAV **64**(3) 513–518, 1999.—The effects of α-fluoromethylhistidine (α-FMH) on spatial cognition were investigated using the eight-arm radial maze paradigm in rats. Intracerebroventricular (ICV) injection of α-FMH resulted in spatial memory deficits characterized by an increase in the number of total errors (TE) and a decrease in the number of initial correct responses (ICR). There was a strong correlation between increases in the number of TE and decreases in histamine contents of the cortex and hippocampus regions of the brain, which are known to participate in learning and memory. On the other hand, both histamine (50–100 ng, ICV) and thioperamide (10 μg, ICV) significantly ameliorated the memory deficit induced by α-FMH. However, metoprine showed no significant effect on the α-FMH–induced memory deficit. Pyrilamine and R-(α)-methylhistamine enhanced the memory deficit induced by α-FMH, at doses that had no appreciable effect when administered alone. In contrast, no significant influence on α-FMH–induced memory deficit was observed with zolantidine. © 1999 Elsevier Science Inc.

Eight-arm radial maze Spatial memory α-FMH Histamine (R)-α-Methylhistamine

WE previously demonstrated that histamine facilitated the impaired memory retrieval induced by aging or hippocampal lesions in rats using passive and active avoidance tests (4,10,13). Moreover, it has been demonstrated that histamine ameliorates scopolamine-induced learning deficits in the elevated plus-maze test in mice (16,17) and the water maze test in rats (24). On the other hand, H_1-antagonists were reported to impair memory recollection in rats in an active-avoidance paradigm (11). α-Fluoromethylhistidine (α-FMH), a potent inhibitor of histidine decarboxylase, was also shown to prolong the response latency in active-avoidance tasks when administered by either intraperitoneal (IP) or intracerebroventricular (ICV) injection (12). α-FMH was reported to markedly decrease endogenous brain histamine content from the nerve terminals without affecting the levels of other neurotransmitters (5,15,23). From these findings, it seems likely that learning and memory are intimately related with endogenous histamine content. In these studies, however, the memory parameter used was transfer latency, which is affected by behavioral toxicity such as decreases in locomotor activity or muscle relaxant activity. It has been demonstrated that the eight-arm radial maze paradigm is more useful to study learning and memory compared with other methods such as passive and active avoidance tasks (14,20,21), because it allows estimation of spatial cognition.

The aim of our investigation was to clarify whether endogenous histamine is involved in spatial memory using the eight-arm radial maze task in rats.

METHOD

Animals

The animals used in this study were male Wistar rats (200–280 g, Charles River, Tokyo, Japan), maintained in individual cages with a 12-h light–dark cycle (0800–2000 h). Prior to behavioral tests, the rats were gradually reduced over a period of 1 week to 80–85% of their free-feeding weight, and then kept on a restricted diet for the rest of the experiment. Water

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was given ad lib. Experiments were carried out each day between 1300–1900 h.

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 from bregma (22); 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 of the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, Okayama University.

Radial-Arm Maze Training

The apparatus was made of clear Plexiglas, and consisted of a round central platform (30 cm in diameter) with eight radiating arms attached to the platform at equal angles and distances. Each arm was 50 cm long × 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). 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.

To familiarize the rats with the radial maze, prior to training they received one daily habituation trial for 2 days. Pellets were scattered over the entire maze surface, and three or four 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 eight arms. A rat was placed on the center platform and allowed to make arm choice to obtain food pellets until all eight pellets had been eaten or 10 min had elapsed. Rats were trained continually until reaching a criterion of at least seven different arms in the first eight choices, and all eight within the first nine 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 for 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 (8,10). Each group consisted of seven 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

The drugs used in the study were α-FMH (generously provided by Dr. Kollonitsch of Merck Sharp & Dohme Research Laboratories, Rahway, NJ), histamine dihydrochloride (Wako, Osaka, Japan), thioperamide hydrochloride (provided by Eisai, Tokyo, Japan), pyrilamine maleate (Sigma, St. Louis, MO), zolantidine (a gift from SmithKline Beecham, London, UK), metoprine (a gift from Wellcome, Co, NC), and R-(α)-methylhistamine oxalate (Funakoshi, Tokyo, Japan). Drugs were dissolved in a vehicle consisting of 0.9% 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). Pyrilamine, zolantidine and metoprine were injected IP.

Statistics

The Mann–Whitney *U*-test was used to test the statistical significance for the ICR of the radial maze. One-way analysis of variance with Dunnett's test was used for TE and brain histamine analysis.

RESULTS

Effects of ICV Injection of α-FMH on Eight-Arm Radial Maze Performance

As shown in Fig. 1, α-FMH at doses of 20 and 50 μg had no significant effect. However, at a dose of 100 μg, this drug sig-

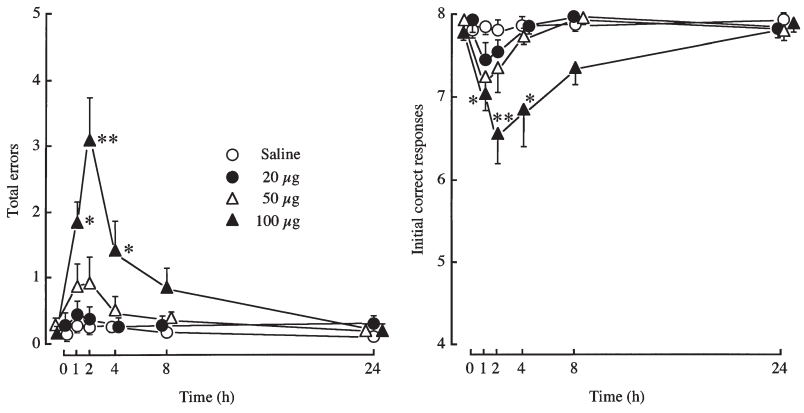

FIG. 1. Effects of α-fluoromethylhistidine on eight-arm radial maze performance in rats. α-Fluoromethylhistidine was injected intracerebroventricularly. Each value represents the mean ± SEM of 18–25 rats. *,**Significantly different from saline-treated group with $p < 0.05$ and $p < 0.01$, respectively.

TABLE 1
EFFECTS OF α-FLUOROMETHYLHISTIDINE ON RUNNING TIME PER CHOICE OF RATS IN RADIAL MAZE PERFORMANCE

Drugs	Doses (μg)	Running Time per Choice (s)
Saline	—	6.6 ± 0.7
α-Fluoromethylhistidine	20	7.0 ± 1.1
	50	6.6 ± 0.7
	100	6.4 ± 1.1

α-Fluoromethylhistidine or saline was injected 2 h before the trial. Each value represents the mean ± SEM of 18–25 rats.

nificantly increased the number of TE and decreased the number of ICR from 1 to 4 h after injection ($p < 0.05$). In addition, running time per choice was not influenced by injection of α-FMH even at a dose of 100 μg (Table 1).

Changes in Brain Histamine Contents After α-FMH Injection

Histamine contents were measured 2 h after injection of α-FMH. As shown in Table 2, at a dose of 20 μg, α-FMH showed no significant effect. However, at doses of 50–100 μg, this agent significantly decreased histamine contents in the cortex, hippocampus, and hypothalamus ($p < 0.05$, $p < 0.01$).

Correlation Between the Increase in Number of TE and Decrease in Brain Histamine Content 2 h After α-FMH Injection

To investigate the relationship between the increase in number of TE and decrease in histamine content in the brain induced by α-FMH, the regression line of Y (increase in number of TE) on X (decrease in histamine contents of the cortex, hippocampus, and hypothalamus) and the correlation coefficient (r) were calculated. As α-FMH showed its peak effect on behavior and the maximal reduction in histamine content 2 h after injection, we chose this time point for subsequent analysis. As shown in Fig. 2, the correlation coefficients of the increase in number of TE and decrease in histamine contents in the cortex, hippocampus, and hypothalamus were $r = 0.920$, $r = 0.931$, and $r = 0.859$, respectively.

Effects of Histamine, Metoprine, and Thioperamide on Memory Deficits Induced by α-FMH

The ICV injection of histamine dose dependently antagonized the spatial memory deficits induced by α-FMH. Although it showed no significant effect at a dose of 20 ng, a significant effect was observed in both parameters at doses of 50 ($p < 0.05$) and 100 ng ($p < 0.05$). On the other hand, metoprine had no significant effects on memory deficit induced by α-FMH, even at a dose of 20 mg/kg. In contrast, thioperamide antagonized α-FMH–induced spatial memory deficits at a dose of 10 μg ($p < 0.05$) (Table 3).

Influences of Histamine, Metoprine, and Thioperamide on Brain Histamine Depletion Induced by α-FMH

Histamine (100 ng, ICV) significantly restored α-FMH–induced histamine depletion ($p < 0.05$) (Table 4). However, metoprine (20 mg/kg, IP) had no apparent effect on the decrease in histamine content induced by α-FMH. On the other hand, thioperamide (10 μg, ICV) enhanced α-FMH-induced depletion of histamine in all regions examined ($p < 0.05$).

Effects of Pyrilamine, Zolantidine, and R-(α)-Methylhistamine on Spatial Memory Deficits Induced by α-FMH

As shown in Table 5, α-FMH (20 μg), at a dose that showed no appreciable effect when given alone, showed significant effects when administered in combination with pyrilamine (20 mg/kg) ($p < 0.05$) or R-(α)-methylhistamine (5 μg) ($p < 0.05$). On the other hand, a combination of α-FMH (20 μg) and zolantidine (20 mg/kg) showed no apparent effects compared with either given separately. Pyrilamine (20 mg/kg), R-(α)-methylhistamine (5 μg), and zolantidine (20 mg/kg) showed no effect on spatial memory when injected separately (Fig. 3).

DISCUSSION

In the present study, ICV injection of α-FMH was shown to result in an increase in the number of TE and a decrease in the number of ICR in the eight-arm radial maze test. On the other hand, running time per choice was not influenced by α-FMH. Therefore, it is reasonable to presume that the inhibition of spatial cognition induced by α-FMH was essentially

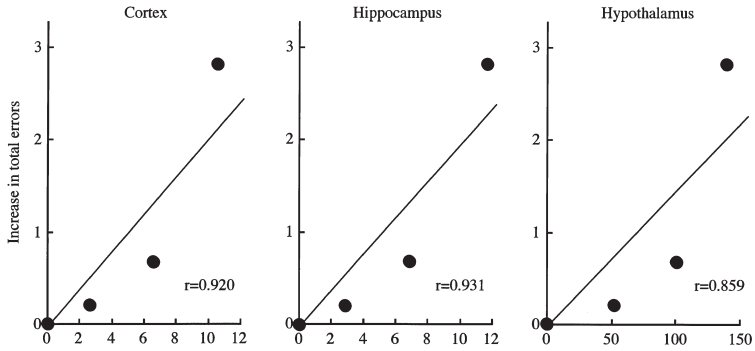

FIG. 2. Correlation between the increase in the number of total errors and decreases in histamine contents of the brain 2 h after α-FMH (100 μg) injection. Each value represents the mean ± SEM of 18–25 rats.

TABLE 2
CHANGES IN BRAIN HISTAMINE CONTENTS AFTER α-FLUOROMETHYLHISTIDINE INJECTION

Brain Regions	Histamine Contents (ng/g Tissue)			
	Saline	20 μg	50 μg	100 μg
Cortex	30.2 ± 1.8	27.8 ± 2.8	23.8 ± 1.2*	19.8 ± 1.7†
Hippocampus	25.6 ± 1.3	22.9 ± 1.3	18.8 ± 3.0*	14.0 ± 1.3†
Hypothalamus	303.1 ± 20.1	251.8 ± 35.8	201.8 ± 24.4*	162.3 ± 15.8†

α-Fluoromethylhistidine was injected 2 h before the the trial. Each value represents the mean ± SEM of 6–10 rats.
*†Significantly different from saline-treated group ($p < 0.05$ and $p < 0.01$, respectively).

TABLE 3
EFFECTS OF HISTAMINE, METOPRINE, AND THIOPERAMIDE ON MEMORY DEFICITS INDUCED BY α-FLUOROMETHYLHISTIDINE (100 μg) IN RATS

Drugs	Doses	Total Errors	Initial Correct Responses
Saline	—	3.1 ± 0.6	6.3 ± 0.3
Histamine	20 ng	2.4 ± 0.6	6.7 ± 0.3
	50 ng	1.4 ± 0.4*	7.4 ± 0.2*
	100 ng	1.0 ± 0.3*	7.5 ± 0.2*
Metoprine	10 mg/kg	2.3 ± 0.8	6.6 ± 0.4
	20 mg/kg	1.7 ± 0.6	6.9 ± 0.3
Thioperamide	2 μg	2.6 ± 0.6	6.6 ± 0.4
	5 μg	1.7 ± 0.8	7.0 ± 0.4
	10 μg	1.1 ± 0.4*	7.4 ± 0.3*

Each value represents the mean ± SEM of 18–25 rats.
*Significantly different from α-fluoromethylhistidine-treated group ($p < 0.05$).

unrelated to a decrease in locomotor activity. As described in the text, brain histamine content was decreased by α-FMH consistent with the observations of previous studies (6,7,12,24). A strong correlation was found between the increase in TE and decreases in histamine contents in the cortex and hippocampus. Previously, we reported a strong correlation between α-FMH–induced active avoidance deficit and a decrease of histamine content in the hippocampus (12). Learning and memory are related not only to the hippocampus and hypothalamus but also to the cortex (3,9,18). The above findings suggested that histamine contained in both the cortex and hippocampus is intimately involved with spatial cognition.

In the present study, we found that ICV injection of histamine led to amelioration of the spatial memory deficit induced by α-FMH. At the same time, appreciable restoration of histamine content was observed in all brain regions examined. Thioperamide improved the memory deficit induced by α-FMH, while R-(α)-methylhistamine showed potentiation of α-FMH–induced spatial memory deficits. A biochemical study revealed that thioperamide facilitated the decrease in histamine content induced by α-FMH in all brain areas examined. It has been demonstrated that thioperamide promotes histamine release and synthesis at presynapses, in contrast to R-(α)-methylhistamine (1,2,7,26). Therefore, released histamine may improve the α-FMH-induced spatial memory deficits.

On the other hand, metoprine, an inhibitor of *N*-methyltransferase, did not antagonize the spatial memory deficits induced by α-FMH. It is generally accepted that metoprine causes an increase in brain histamine contents in normal rats (8,25). However, as shown in this study, histamine content was not increased by metoprine after α-FMH injection. This may have been because presynaptic histamine content was already markedly decreased by administration of α-FMH. Therefore, the increase in histamine content as a result of metabolic inhibition by metoprine was not sufficient to ameliorate the memory deficit induced by α-FMH. It is likely, therefore, that α-FMH-induced spatial memory deficits of rats may be simply due to a decrease in histamine content at presynapses.

It has also been shown that certain H_1-antagonists cause retardation of both acquisition and retention processes in ac-

TABLE 4
INFLUENCES OF HISTAMINE, METOPRINE, AND THIOPERAMIDE IN BRAIN HISTAMINE DEPLETION BY α-FLUOROMETHYLHISTIDINE (100 μg)

Brain regions	Histamine Contents (ng/g Tissue)				
		α-Fluoromethylhistidine (100 μg)			
	Control	Saline	Histamine	Metoprine	Thioperamide
Cortex	30.6 ± 2.0	20.5 ± 1.9	31.2 ± 1.4*	24.1 ± 3.3	12.6 ± 2.2*
Hippocampus	25.4 ± 1.4	14.7 ± 1.5	26.3 ± 3.9*	17.9 ± 3.0	8.7 ± 2.2*
Hypothalamus	301.6 ± 22.7	168.4 ± 18.4	305.9 ± 27.8†	183.9 ± 19.2	128.1 ± 13.3*

Each value represents the mean ± SEM of seven rats.
*†Significantly different from saline-treated group ($p < 0.05$ and $p < 0.01$, respectively).

TABLE 5

EFFECTS OF PYRILAMINE, ZOLANTIDINE AND (R)-α-METHYLHISTAMINE ON SPATIAL MEMORY DEFICITS INDUCED BY α-FLUOROMETHYLHISTIDINE IN RATS

Total Errors			Initial Correct Responses		
	α-Fluoromethylhistidine			α-Fluoromethylhistidine	
Drugs	0	20 μg	Drugs	0	20 μg
Saline	0.2 ± 0.1	0.4 ± 0.2	Saline	7.8 ± 0.1	7.4 ± 0.2
Pyrilamine (20 mg/kg)	0.9 ± 0.5	1.9 ± 0.6*	Pyrilamine (20 mg/kg)	7.3 ± 0.3	6.7 ± 0.2*
Zolantidine (20 mg/kg)	0.3 ± 0.1	0.5 ± 0.3	Zolantidine (20 mg/kg)	7.4 ± 0.4	7.6 ± 0.2
(R)-α-Methylhistamine (5 μg)	1.4 ± 0.7	2.1 ± 0.7*	(R)-α-Methylhistamine (5 μg)	7.2 ± 0.4	6.5 ± 0.3*

Each value represents the mean ± SEM of 19–21 rats.
*Significantly different from α-fluoromethylhistidine-treated group ($p < 0.05$).

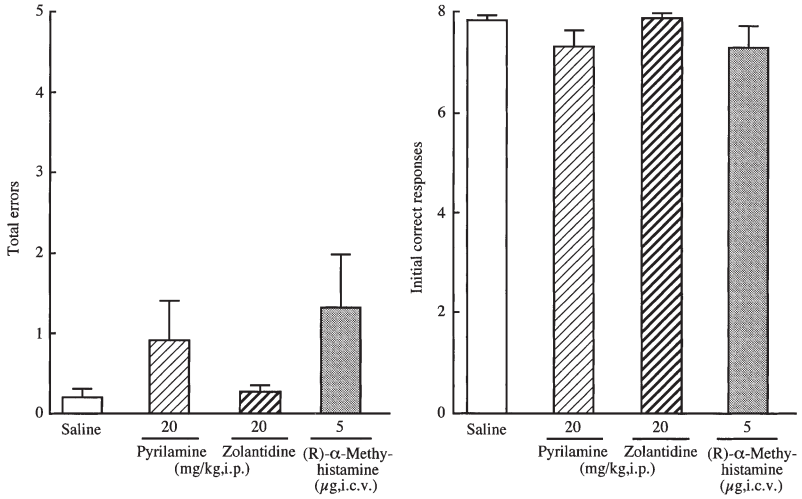

FIG. 3. Effects of pyrilamine, zolantidine, and (R)-α-methylhistamine on eight-arm radial maze performance in rats. Pyrilamine and zolantidine were injected intraperitoneally, (R)-α-methylhistamine was injected intracereobroventricularly. Each value represents the mean ± SEM of 19–22 rats.

tive-avoidance responses and radial maze performance in rats, and these effects are reversed by histamine or histidine (11,19). In the present study, pyrilamine, at a dose that showed no appreciable effect on eight-arm radial maze performance when administered alone, potentiated the memory deficit induced by α-FMH. In contrast, no effect was observed with zolantidine. These findings suggested that spatial cognition deficit induced by endogenous histamine depletion may be mediated by postsynaptic H_1-receptors.

In conclusion, endogenous histamine in the cortex and hippocampus plays an important role in spatial cognition of rats in the eight-arm radial maze task.

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