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Facilitating effect of histamine on spatial memory deficits induced by dizocilpine as evaluated by 8-arm radial maze in SD rats¹

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KEY WORDS histamine; maze learning; dizocilpine; memory disorders

ABSTRACT

AIM: To investigate whether or not histamine is involved in spatial memory deficits induced by dizocilpine (MK-801) as evaluated by 8-arm radial maze of rats. **METHODS:** 8-Arm (4-arm baited) radial maze was used to measure spatial memory in rats. **RESULTS:** Bilaterally intrahippocampal (ih) injection of MK-801 (0.3 µg/site) impaired working memory and reference memory in rats. Both histamine (50, 100 ng/site, ih) and intraperitoneal (ip) injection of histidine (100, 200 mg/kg) markedly improved the spatial memory deficits induced by MK-801. On the other hand, the ameliorating effect of histidine (100 mg/kg, ip) was completely antagonized by α-fluoromethylhistidine (α-FMH, 5 µg/site, ih), a potent and selective histidine decarboxylase (HDC) inhibitor, and H₁-antagonist pyrilamine (1 µg/site, ih), but not by H₂-antagonist cimetidine, even at a high dose (2.5 µg/site, ih). **CONCLUSION:** The hippocampal histamine plays an important role in the ameliorating effect on MK-801-induced spatial memory deficits, and its action is mediated through postsynaptic H₁-receptor.

INTRODUCTION

Histamine neurons are exclusively located in the tuberomammillary nucleus (TM) of the posterior hypothalamus receiving input mainly from the limbic system and project efferent nerve fibers to almost all parts of the brain^[1,2]. Histamine plays an important role as a neurotransmitter or neuromodulator in the mammalian

central neuron system (CNS)^[1-3]. The histaminergic neuron system seems to be involved in various physiological and behavioral functions through H₁-, H₂-, and H₃-receptors including sleep-wake cycles, emotion, appetite control, locomotor activity, stress behavior, neuroendocrine, and epilepsy^[2-4]. So far, a role for brain histamine in mechanisms regulating learning and memory has been well documented^[3-7]. For example, both histamine and its precursor, *L*-histidine, facilitate memory retrieval deficits induced by aging, hippocampal lesions and scopolamine as determined with passive and active avoidance task and 8-arm radial maze in rats^[5-7]. α-Fluoromethylhistidine (α-FMH), a selective and potent histidine decarboxylase (HDC) inhibitor, has been demonstrated to induce significant memory deficits in active avoidance task and 8-arm radial maze in rats^[8]. However, several reports showed conflicting findings

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as follows: chronic treatment with α -FMH resulted in facilitation of memory acquisition as evaluated by 8-arm radial maze of rats^[4], and a lesion in TM region showed an ameliorating effect in aged rats^[9]. The mechanisms of these differences seem to be very complex, which may be due to the different experimental methods, the species of animals, and drug treated doses and routes *etc.*

On the other hand, it has been demonstrated that *N*-methyl-*D*-aspartate (NMDA) receptors play an important role in learning and memory^[10,11]. NMDA receptor antagonist dizocilpine (MK-801) is widely used for a good tool to impair various kinds of learning behavior^[10,11]. Recently, the interactions between histaminergic system and NMDA receptor become more and more attractive^[3,12,13]. The polyamine site of the NMDA receptor has been suggested to be a binding site for histamine^[3]. Bekkers *et al* and Vorobjev *et al* discovered in cultured hippocampal pyramidal cells that histamine could dramatically enhance NMDA receptor-mediated synaptic transmission and facilitate the induction of long-term potentiation^[12,13]. However, few behavioral studies were made on the interaction of histamine and NMDA receptors in cognition process. *D*-cycloserine, a partial agonist of NMDA receptor, facilitates pyrilamine-induced memory deficits in a three-panel runway task^[14]. We have previously reported histamine reversed memory deficits of rats induced by MK-801 in 8-arm radial maze performance^[11]. However, little is known about the involvement of the hippocampal NMDA receptor in amelioration of histaminergic system and it remains to be unknown whether working memory, a kind of short-term memory, or reference memory, a kind of long-term memory, is prominently related to histamine. 8-Arm (4-arm baited) radial maze has been widely used to determine working memory and reference memory^[15].

Therefore, the objectives of our investigations are to use the radial maze task with 4-arms baited to further elucidate the roles of hippocampal NMDA receptor and histaminergic system in the regulation of spatial memory of rats.

MATERIALS AND METHODS

Animals All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animal. The animals used in this study were male Sprague-Dawley rats

(♂, 220-300 g, $n=90$, Grade II, Certificate No. 22-9601018, Experimental Animal Center, Zhejiang University), maintained in an air-conditioned room with controlled temperature (22-26 °C) and humidity (40 %-70 %), housed in individual cages with a 12-h light-dark cycle (lights on from 8:00-20:00). Animals were given free access to water and kept at 80 %-85 % of their free feeding body weight throughout the experiment. Experiments were carried out each day between 10:00-17:00.

Surgical procedure The rats were anesthetized with sodium pentobarbital (35 mg/kg, ip), and fixed on a stereotaxic apparatus (Narishige, SR-5, Tokyo, Japan). A guide cannula made of stainless steel tubing 700 μ m in outer diameter, was implanted into the bilateral dorsal hippocampus according to the following coordinates measured from bregma: AP: -4.3 mm, L: ± 1.9 mm, H: 3.8 mm from the skull. At least 10 d were allowed for recovery from the surgery. After the behavioral tests, Evans blue with a volume of 1 μ L was injected bilaterally into the dorsal hippocampus and the rats were sacrificed by decapitation. The accuracy of the injection site was carefully determined.

Radial-arm maze training The apparatus used was described in our previous report^[11]. To make rats familiar with the radial maze, prior to training, they received one daily habituation for 2 d. Food pellets (45 mg each, Bio-Serv, Frenchtown, NJ, USA) were scattered over the entire maze surface, and three or four rats were simultaneously placed in the radial maze and allowed to explore 10 min to take food freely. After adaptation, all rats were trained with 1 trial per day. In each trial, only 4 arms (1, 2, 4, and 7) were baited, and the sequence was never changed throughout the experiment. The rat was placed on the center platform that was closed off by a door. After 15 s, the door was opened and the rat was allowed to make arm choice to obtain food pellets until all 4 pellets had been eaten or 5 min had elapsed. The number of entries into the unbaited arms was regarded as the total error (TE). The number of entries into the never-baited arm was regarded as a reference memory error (RME), while re-entry into the arms where the pellet had already been eaten was regarded as a working memory error (WME). Rats were trained continually until reaching a criterion of at most 1 error per trial for 5 consecutive training trials. Fourteen of ninety rats which were not able to solve the standard radial maze performance or just turn around the maze arm side by side, or lose their guide cannula

before drug treatment, were excluded from the following drug test.

Drugs During the drug test, (+) MK-801 hydrogen maleate (Sigma, St Louis, MO, USA), histamine dihydrochloride (Sigma, St Louis, MO, USA), cimetidine dimaleate (Sigma, St Louis, MO, USA), pyrilamine dihydrochloride (Sigma, St Louis, MO, USA), and α -fluoromethylhistidine (Merck Sharp & Dohme Research Lab, Rahway, NJ) were dissolved in saline and injected into dorsal hippocampus in a fixed 1 μ L/site over a period of 120 s at a constant speed with a continuous infusion pump (KN-201, Natsume, Tokyo, Japan). L-histidine monohydrochloride (Sigma, St Louis, MO, USA) was dissolved in saline and injected ip. Studies for drug effect were carried out once a week, on Tuesday or Friday.

Statistical analysis All results were expressed as mean \pm SD. Differences were analyzed by One-way analysis of variance (ANOVA) with Dunnett's test using computer software (Sigmastat 1.01 for Windows 95, 1992, Jandel Corp, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Effect of MK-801 on memory retrieval as evaluated by 8-arm radial maze performance in rats Bilaterally intrahippocampal (ih) injection of MK-801 resulted in a dose-dependent increase in the number of errors in memory retrieval process. At a dose of 0.3 μ g/site, MK-801-treated rats showed significant increase in the number of TE, WME, and RME ($P < 0.05$, Fig 1).

Effects of histamine and histidine on MK-801-induced memory deficits in rats Injection (ih) of histamine antagonized the effect of MK-801 in a dose-dependent manner, no significant effect was observed at a dose of 25 ng/site, while at doses of 50 and 100 ng/site significant effects were observed in the number of TE, WME, and RME ($P < 0.05$, Tab 1). Histamine at a dose of 100 ng/site created a bell-shaped inhibition in the number of TE and WME. In addition, ip injection of histidine also produced a dose-dependent and significant inhibition against MK-801-induced memory deficits, at doses of 100 and 200 mg/kg ($P < 0.05$, Tab 1).

Effects of pyrilamine and cimetidine on memory amelioration of histidine on MK-801-induced memory deficits Injection (ih) of pyrilamine, a representative central H₁-antagonist, at a dose of 1 μ g/site, significantly antagonized the action of histidine-

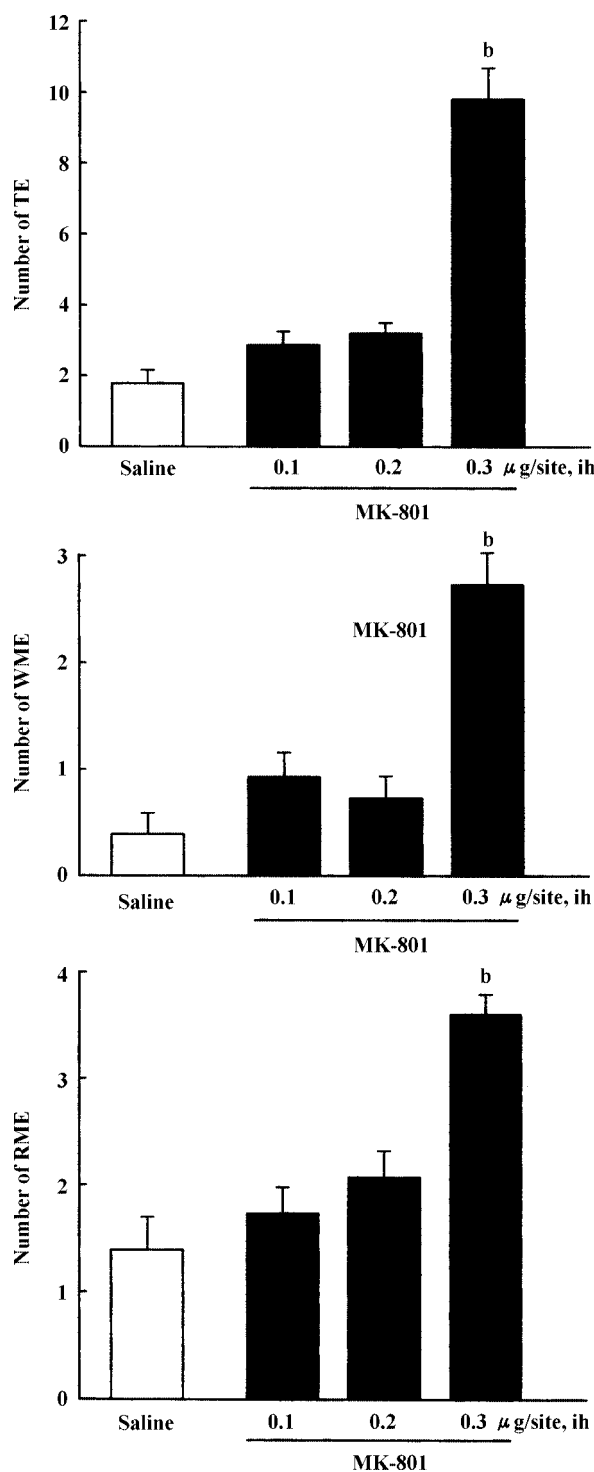


Fig 1. Effect of bilaterally intrahippocampal (ih) injection of MK-801 on spatial memory of rats in 8-arm radial maze. MK-801 was injected 10 min before radial maze test. $n = 15-18$ rats. Mean \pm SD. ^b $P < 0.05$ vs the saline-treated group. TE: total errors; RME: reference memory errors; WME: working memory errors.

induced ameliorating effect in the TE, WME, and RME ($P < 0.05$, Fig 2). On the other hand, cimetidine, an H₂-

Tab 1. Effects of histamine and histidine on memory deficits induced by intrahippocampal (ih) injection of MK-801 in 8-arm radial maze performance. MK-801 was injected 10 min before radial maze test. Histidine was injected 2 h before radial maze test. Histamine was injected 15 min before radial maze test. Mean±SD. ^b*P*<0.05 vs saline-treated group. ^c*P*<0.05 vs MK-801-treated group. TE: total errors; WME: working memory errors; RME: reference memory errors.

Drugs	Dose	<i>n</i>	TE	WME	RME
Saline	-	18	1.8±0.4	0.39±0.20	1.4±0.3
MK-801	0.3 µg/site, ih	15	9.8±0.9 ^b	2.7±0.3 ^b	3.60±0.19 ^b
MK-801+	0.3 µg/site, ih				
Histamine	25 ng/site, ih	15	8.5±0.6	2.3±0.5	3.60±0.16
	50 ng/site, ih	15	3.9±0.5 ^c	0.73±0.21 ^c	2.40±0.29 ^c
	100 ng/site, ih	16	4.6±0.4 ^c	1.44±0.29 ^c	2.50±0.22 ^c
MK-801+	0.3 µg/site, ih				
Histidine	50 mg/kg, ip	19	6.3±0.7	2.74±0.4	2.37±0.28 ^c
	100 mg/kg, ip	15	3.5±0.5 ^c	0.73±0.21 ^c	2.13±0.26 ^c
	200 mg/kg, ip	14	3.3±0.4 ^c	0.79±0.21 ^c	2.36±0.29 ^c

antagonist, had no appreciable effect on facilitation of histidine even at a high dose of 2.5 µg/site.

Effect of α-FMH on amelioration of histidine on MK-801-induced memory deficits in rats α-FMH, at a dose of 1 and 2.5 µg/site (ih) showed a tendency to reverse histidine-induced amelioration on memory deficits induced by MK-801, but no significant effect was obtained. At a dose of 5 µg/site, α-FMH created a significant increase in the number of TE, WME, and RME (*P*<0.05, Tab 2).

DISCUSSION

In the present study, bilateral ih injection of MK-801 induced serious spatial memory deficits in working memory and reference memory as evaluated by 8-arm (4-arm baited) radial maze in rats. This result was consistent with the previous studies, in which both working memory and reference memory were impaired by ip injection of MK-801 as assessed with 8-arm radial maze and water maze tasks^[10,11]. In addition, there ap-

Tab 2. Effect of bilaterally intrahippocampal (ih) injection of α-fluoromethylhistidine (α-FMH) on amelioration of histidine (100 mg/kg, ip) on MK-801-induced memory deficits in rats. MK-801 was injected 10 min before radial maze test. α-FMH was injected 1 h before MK-801 treatment. Histidine was injected 2h before radial maze test. Mean±SD. ^b*P*<0.05 vs saline-treated group. ^c*P*<0.05 vs MK-801-treated group. ^d*P*<0.05 vs histidine+MK-801-treated group. TE: total errors; WME: working memory errors; RME: reference memory errors.

Drugs	Doses	<i>n</i>	TE	WME	RME
Saline	-	18	1.8±0.4	0.39±0.20	1.4±0.3
MK-801	0.3 µg/site, ih	15	9.8±0.9 ^b	2.7±0.3 ^b	3.60±0.19 ^b
MK-801+	0.3 µg/site, ih				
Histidine	50 mg/kg, ip	19	6.3±0.7	2.7±0.4	2.37±0.28
	100 mg/kg, ip	15	3.5±0.5 ^c	0.73±0.21 ^c	2.13±0.26 ^c
	200 mg/kg, ip	14	3.3±0.4 ^c	0.79±0.21 ^c	2.36±0.29 ^c
MK-801+	0.3 µg/site, ih				
Histidine+	100 mg/kg, ip				
α-FMH	1.0 µg/site, ih	17	5.1±0.5	1.59±0.15	2.6±0.3
	2.5 µg/site, ih	15	5.1±0.6	1.6±0.4	2.60±0.21
	5.0 µg/site, ih	15	8.7±0.7 ^c	3.0±0.3 ^c	3.20±0.26 ^c

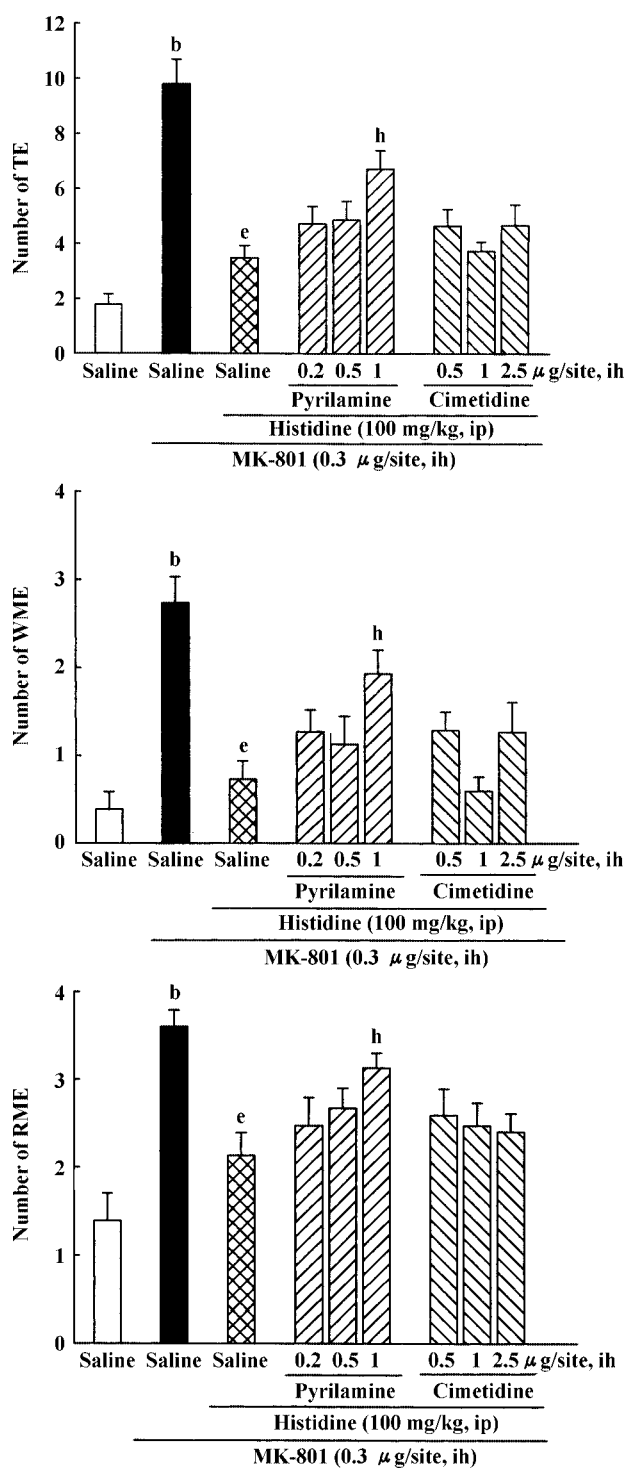


Fig 2. Effects of bilaterally intrahippocampal (ih) injection of pyrilamine and cimetidine on the amelioration of histidine (100 mg/kg, ip) on MK-801-induced memory deficits in rats. MK-801 was injected 10 min before radial maze test. Pylramine and cimetidine were injected 5 min before MK-801 treatment. Histidine was injected 2 h before radial maze test. $n=15-18$ rats. Mean \pm SD. ^b $P<0.05$ vs the saline-treated group. ^e $P<0.05$ vs the MK-801-treated group. ^h $P<0.05$ vs the histidine+MK-801-treated group. TE: total errors; RME: reference memory errors; WME: working memory errors.

peared no appreciable changes of the running time per choice (locomotor activity) and other behavioral symptoms, such as ataxia (data not shown), which usually occurring in cases of ip or intracerebroventricular (icv) treatment of MK-801^[10]. Therefore, the inhibition of hippocampal NMDA receptor by MK-801 in the present study mainly contributes to the spatial working and reference memory deficits in rats.

Both histamine and histidine created protective effects against MK-801-induced spatial memory deficits, including working memory and reference memory, which suggested histamine ameliorated both short-term and long-term spatial memory. It has been demonstrated that histamine in the hippocampus plays an important role in memory retrieval process, and ip injection of histidine could result in an increase in hippocampal histamine levels as same as icv injection of histamine^[6,16,17]. Moreover, histamine has been recently indicated to facilitate NMDA-mediated excitatory transmission, and to increase glutamate release via H₁- and H₂-receptors in the hippocampus^[12,13,18]. Therefore, our behavioral results provide more evidences that the glutamate released by hippocampal histamine contributes to the ameliorating effect on the MK-801-induced working memory and reference memory impairment in rats. Further biochemical studies are needed to elucidate its mechanism.

Interestingly, we found, histamine at a dose of 100 ng/site created a bell-shaped inhibition in the 8-arm radial maze. We have no more data to explain it. Similar findings were obtained by Sakai *et al*^[19] and Ghi *et al*^[20], who found biphasic effects of histamine on locomotor activity, dependent on doses administered. Kiyono *et al* also reported dual effects of histamine on waking-sleep from studies on EEG of rats^[21]. A lower dose of histamine (45 nmol) treatment in the hippocampus improved memory retrieval in contrast to a higher dose (90 nmol)^[17]. In addition, histamine at a dose of 10 μmol was found to inhibit the glutamate release in the hippocampus of rat^[22]. These findings suggest that the dual effects of histamine were probably mediated through glutamate systems^[22], and memory facilitation of histamine only existed within a certain range of low histamine level in the hippocampus. Recently, a facilitated learning acquisition of radial maze has been reported in histidine-deficient rats^[15].

α-FMH, which is a specific HDC inhibitor and created no effects in other neurotransmitters at low doses used in the study^[23] has been widely used as a

powerful tool for elucidating the brain histamine function. Previous report showed that α -FMH induced a severe impairment in memory retrieval in radial maze, correlated with a decrease in hippocampal histamine contents^[8]. We found that the memory amelioration of histamine was antagonized by α -FMH, at a dose of producing no appreciable effect on both working memory and reference memory when administered alone (data not shown). These results strongly confirm the ameliorating effect of endogenous histamine in hippocampus on spatial memory deficits induced by MK-801.

Postsynaptic H₁-receptors play an important role in learning and memory^[24,25]. Both clinical and animal studies indicated that H₁-receptor antagonists could produce disturbances in arousal, which affected the cognitive function^[25,26]. The human positron emission tomography (PET) studies indicated that marked decreases in the content of H₁-receptors existed in the frontal and temporal areas of Alzheimer's diseases patients^[26]. We found that the ameliorations of both spatial working memory and reference memory induced by histamine were reversed by ptyrilamine, a central H₁-antagonist. In contrast, no effect was observed with cimetidine. It was previously reported that icv injection of H₁-agonist, 2-thiazoly-lethylamine, but not H₂-agonist, 4-methylhistamine reversed the working memory deficits induced by MK-801 as evaluated by 8-arm radial maze in rats^[11]. These results consisted well with the study by Payne *et al*^[24], who has reported the ability of histamine to facilitate the NMDA receptor-mediated synaptic responses was reduced by histamine H₁-receptor antagonists, suggesting that amelioration of histamine is mediated by postsynaptic H₁-receptor, but not H₂-receptor.

In conclusion, histamine facilitates spatial memory deficits of both spatial working memory and reference memory induced by MK-801, and its action is mediated by postsynaptic H₁-receptors.

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