ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jan; 23 (1): 66 - 70
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Effects of brain histamine on memory deficit induced by nucleus basalis-lesion in rats[1]

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KEY WORDS avoidance learning; basal nucleus of Meynert; histamine; tacrine; memory disorders

ABSTRACT

AIM: To investigate whether or not brain histamine was involved in memory deficits induced by lesions of nucleus basalis magnocellularis (NBM) in rats. **METHODS**: Passive avoidance response was used to measure memory process in rats, and NBM was bilaterally lesioned by injection of ibotenic acid (6 μg/site). **RESULTS**: Icv injection of histamine (500 ng), and ip injection of histidine (1500 mg/kg), metoprine (10 mg/kg) or tacrine (3, 5 mg/kg) ameliorated memory impairment induced by NBM lesion regarding passive avoidance response. The ameliorating effect of histidine was antagonized by pyrilamine (2 - 5 mg/kg), a H_1-antagonist, but not by zolantidine, a H_2-antagonist. **CONCLUSION**: Histaminergic neurons play an important role in learning and memory via H_1-receptor, and its action may be due to cholinergic neurons.

INTRODUCTION

It is generally known that the severity of loss of cholinergic neurons in nucleus basalis of magnocellularis (NBM) is related to the degree of clinical dementia in patients with Alzheimer's disease. These findings have led to the development of cholinergic replacement strategies[1-3]. On the other hand, more and more studies suggest that histamine plays an important role in learning and memory[4-10]. Behavioral studies indicate that the increase of histamine facilitates impaired memory retrieval induced by aging or hippocampal lesions in rats[4-6], and depletion of hippocampal histamine content is shown to recall adverse effect in rats both in active avoidance task and radial maze performance[7]. Recently, the relation between histaminergic neuron and cholinergic neuron with regard to learning and memory has attracted a great deal of attention[8-14]. Not only histamine might affect cognition on its own, neurochemical studies suggest that histamine modulates the activity of cholinergic neurons[12]. It is indicated that histamine can result in an excitatory effect on the cholinergic neurons in the NBM and septal area[13,14]. It has also been reported that the increase of histamine ameliorates learning deficits induced by scopolamine in the passive avoidance response and radial maze performance in rodents[8,9,11]. Furthermore, it has been indicated that, AChE inhibitor tacrine not physostigmine, which used to treat Alzheimer's disease, could significantly decrease activity of histamine-*N*-methyltransferase (HNMT) and increase brain histamine contents. These findings suggest that the histaminergic action may be involved in cholinergic neurons. However, whether or not histamine is involved in the memory deficits induced by NBM lesion has been not yet referred.

Therefore, in the present study, we use step-through passive avoidance response to further investigate whether or not histamine could restore an NBM-lesion-induced memory deficit in rats.

MATERIALS AND METHODS

Animals The animals used in this study were male Sprague-Dawley rats (♂, 200 - 280 g, $n = 130$, Grade Ⅱ, Certificate № 22-9601018, Experimental Animal Center, Zhejiang University), maintained in individual cages with a 12-h light-dark cycle (lights on from 8:00 - 20:00). Water was given *ad libitum*. Experiments were carried out each day between 13:00 - 18:00.

Surgical procedure Rats were anesthetized with

[1] Project supported by the National Natural Science Foundation of China (№ 30000019) and Natural Science Foundation of Zhejiang Provice (№ 300062).
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Received 2001-05-08 Accepted 2001-09-25

sodium pentobarbital (35 mg/kg, ip), and fixed on a stereotaxic apparatus (Narishige, SR-6, Tokyo, Japan), and intracerebral injection of ibotenic acid (6 μg/site) were made by means of a 5 μL syringe with a 31 gauge needle into NBM (AP: −0.8 mm; L: 2.6 mm; H: −7.4 mm; relative to the bregma[15]), at a constant speed of 0.25 μL/min. NBM-sham-lesioned rodent received vehicle infusions of equal volume into NBM. 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: AP: −0.9 mm, L: 1.5 mm, H: 3.8 mm from the skull. When bilateral intracerebral injection of ibotenic acid was performed, severe aphagia was induced. Therefore, liquid food was given to rats by gavage. At least 7 d were allowed for recovery from the surgery. Eleven of 130 rats were dead for food rejection.

Step-through passive avoidance response Rats were tested on a step-through passive avoidance memory test 7 d after surgery. The training apparatus comprised a small lighted compartment (19 cm × 15 cm × 14 cm) and a large dark compartment (33 cm × 20.5 cm × 14 cm). The box was separated by a small 7 cm × 7 cm guillotine door. The light compartment was made of clear Plexiglas, and was illuminated by a lamp (100 W) from the outside. The dark compartment had series of stainless steel rode (3 mm in outside diameter), arranged side by side, 1 cm apart, through which the constant current was delivered. Rats were habituated to the box on two consecutive days[5]. On the first day, the rats were placed in the light compartment and allowed to explore the box. The latency to enter the dark compartment was recorded. As soon as rats entered into dark compartment, the door was closed, and the rat was kept in it for 15 s before being returned to cage. On the second training day, on entry into the dark compartment, the door was closed and rat was given 0.5 mA shock for 5 s. In the test trial, all the procedures were carried out similarly, except that electric shock was not given. The latency of rats to move into the dark compartment was recorded, up to a maximum time of 300 s.

Drugs Histamine dihydrochloride, *L*-histidine monohydrochloride, pyrilamine maleate, tacrine, and ibotenic acid were perchased from Sigma, St Louis, USA, and zolantidine dimaleate was perchased from SmithKline Beecham, London, UK. Metoprine was a gift from Dr NICHOL, Wellcome Research Laboratories, Research Triangle Park, NC. Histamine was dissolved in saline and icv injected 10 min before test trial 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). Histidine, metoprine, and tacrine were ip injected 3 h, 4 h, and 30 min before test trial. Studies for drug effect were carried out twice a week, on Tuesdays and Fridays, and training trials were performed 24 h before test trials. The same animals were repeatedly used, and they were experienced all doses of one drug.

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

Influence of ibotenic acid lesions of the NBM on passive avoidance response in rats Behavioral testing in the passive avoidance response demonstrated that the initial training latencies of sham and NBM-lesioned rats were equivalent (data not shown), a serious memory deficit was caused by bilateral injection of ibotenic acid (6 μg/site) on the 24 h retention test, a significant decrease in transfer latency from 1 d to 35 d after NBM lesion was observed compared with that before NBM lesion ($P < 0.05$) (Fig 1). In addition, a stable and significant decrease in latency was found in rats from 10 d to 28 d after NBM lesion. Therefore, the following experiments were carried out with these rats 10 d − 28 d after NBM lesion.

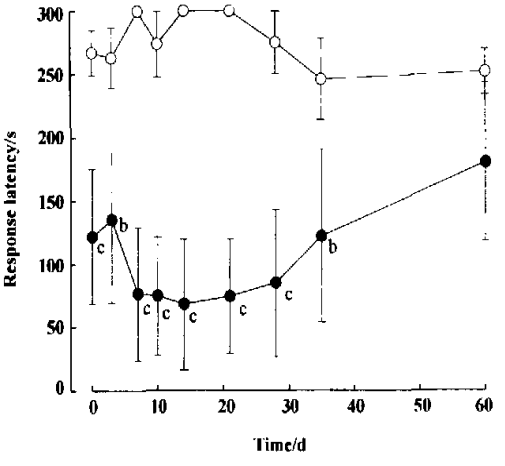

Fig 1. Influence of ibotenic acid (6 μg/site) lesion of the NBM on passive avoidance response in rats. NBM was lesioned by bilateral injection of ibotenic acid (6 μg/site). $n = 15$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* sham group. ○ Sham group. ● NBM lesioned rats.

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jan; 23 (1)
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Effects of tacrine and histamine on memory deficits induced by NBM lesions in rats Tacrine facilitated memory deficit induced by NBM lesion in passive avoidance response (Tab 1). Tacrine at doses of 0.3 and 1 mg/kg prolonged transfer latency of passive avoidance response dose-dependently, and significant effects were obtained at doses of 3 and 5 mg/kg ($P < 0.05$ or $P < 0.01$). Similar to tacrine, histamine icv reversed the memory deficits induced by NBM lesion. At doses of 100, 200, and 500 ng, it caused a prolongation of transfer latency dose-dependently, and at 500 ng it significantly prolonged the transfer latency ($P < 0.05$) (Tab 1).

Tab 1. Effects of tacrine and histamine on memory deficits as evaluated by step-through passive avoidance response induced by NBM lesions in rats. $\bar{x} \pm s$. **$^cP < 0.01$ *vs* sham-control group. $^eP < 0.05$, $^fP < 0.01$ *vs* saline-treated group in NBM lesion induced by ibotenic acid (6 μg/site).**

Drugs	Doses	*n*	Response latency/s
Sham-control		15	264 ± 39
Saline + saline	–	16	75 ± 34^c
Tacrine + saline	0.3 mg/kg, ip	12	77 ± 33
	1 mg/kg, ip	15	106 ± 29
	3 mg/kg, ip	14	180 ± 54^e
	5 mg/kg, ip	16	222 ± 47^f
Histamine + saline	100 ng, icv	13	98 ± 23
	200 ng, icv	13	140 ± 40
	500 ng, icv	13	194 ± 53^e

Effects of histidine and metoprine on memory deficits induced by NBM lesions in rats As shown in Fig 2, histidine ip at doses of 500, 1000, 1500 mg/kg, ameliorated the memory deficits induced by NBM lesions in a dose-dependent manner, and a significant effect was observed at 1500 mg/kg ($P < 0.05$). On the other hand, metoprine, a selective inhibitor of HNMT, resulted in a similar effect to histidine and it significantly prolonged transfer latency at a high dose of 10 mg/kg ($P < 0.05$).

Effect of H_1- and H_2-antagonist on histidine-induced ameliorating effect Pyrilamine, a selective and representative H_1-antagonist reversed histidine-induced ameliorating effect, dose-dependently, and a significant effect was observed at doses of 2 and 5 mg/kg ($P < 0.05$) (Tab 2). In contrast, zolantidine, a selective and representative H_2-antagonist, created no appreciable effects even at a dose of 20 mg/kg.

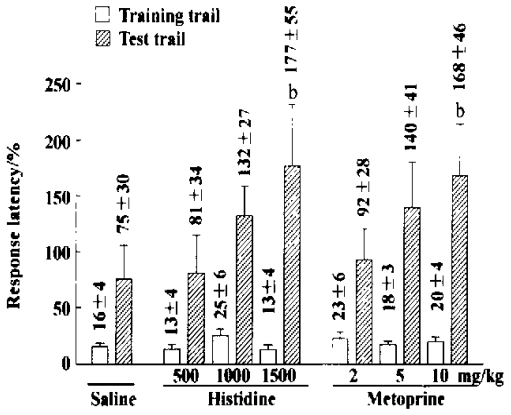

Fig 2. Effects of histidine and metoprine on memory deficits induced by NBM lesions in rats. Histidine or metoprine were ip injected 3 or 4 h before test trial. $n = 13 - 16$ **rats.** $\bar{x} \pm s$. **$^bP < 0.05$ *vs* saline-treated group in NBM lesion induced by ibotenic acid (6 μg/site).**

Tab 2. Effects of pyrilamine and zolantidine on histidine-induced ameliorating effect in NBM-lesioned rats induced by ibotenic acid (6 μg/site). $\bar{x} \pm s$. **$^bP < 0.05$ *vs* NBM + saline group. $^eP < 0.05$ *vs* histidine + saline group.**

Drugs	Doses	*n*	Response latency/s
NBM + saline		16	71 ± 36
Histidine + saline	–	16	177 ± 51^b
Histidine + pyrilamine	2 mg/kg, ip	16	139 ± 58
	5 mg/kg, ip	16	109 ± 38^e
	10 mg/kg, ip	14	90 ± 55^e
Histidine + zolantidine	5 mg/kg, ip	12	166 ± 42
	10 mg/kg, ip	13	182 ± 41
	20 mg/kg, ip	13	194 ± 53

DISCUSSION

It is well known that the severity of loss of cholinergic neurons in the NBM is related to the degree of clinical dementia, and lesions of the NBM by injection of ibotenic acid are commonly used to study the physiological role of brain cholinergic function and regarded as a suitable model of Alzheimer's disease[1,3]. In the present study, ibotenic acid lesion of the NBM produced severe memory deficits in passive avoidance response in rats, which was in agreement with the

previous data[16,17].

Tacrine resulted in an increase of brain acetylcholine contents, improved memory deficits of both memory parameters of passive avoidance response and radial maze performance induced by ibotenic acid NBM lesions[16]. Our data supported the previous findings. Tacrine is found to inhibit rather potently HNMT, the enzyme responsible for brain histamine metabolism *in vitro* and *in vivo*, and to enhance brain histamine contents in the cortex, hippocampus, hypothalamus, striatum, and thalamus[18,19]. Compared with tacrine, physostigmine which contributes no effect to clinical treatment for Alzheimer's disease[1,3], has less inhibitory effect (1/100) on HNMT[19]. Therefore, the action of tacrine in the present study might be, at least in part, related to the inhibition of brain histamine metabolism, which is suggested that brain histamine plays a certain role in treatment of Alzheimer's disease[7-10,20].

Both icv injection of histamine and ip injection of histidine showed protective effects against memory deficits in NBM-lesioned rats. Previous data showed that the activation of histaminergic neurons facilitated scopolamine-induced memory deficits in both plus-maze test and radial maze performance in rodents[8-10], and the 2-methylhistamine, a representative H_1-agonist, induced memory ameliorating effect was reversed by pirenzepine[21]. Recently, it was indicated that high densities of histaminergic neurons were found around cholinergic neurons, and the treatment of histamine ameliorated cholinergic neurons activity *in vivo* and *in vitro*, increasing acetylcholine release in the cortex, striatum, and hippocampus in the free moving rats[12-14,22,23]. Therefore, the acetylcholine released by histamine may contribute to the observed improvement of the NBM lesion-induced memory deficits. In addition, ibotenic acid lesions of the NBM damaged not only cholinergic neuron but also dopaminergic, serotoninergic neuron, *etc*, it has been reported that histamine can also regulate the release of dopamine, serotonin, *etc*[20,24]. Therefore, histamine induced release of dopamine, serotonin *etc*. may also play a certain role in the observed improvement of the NBM lesion-induced memory deficits.

Compared with tacrine, it is found that histamine has a weaker ameliorating effect, even at a high dose. Previous studies found histamine at lower doses could facilitate memory deficits induced by hippocampectory or scopolamine[5,8,9]. It may be due to the severe disruption of cholinergic activity induced by NBM lesion[16,17], histamine might not release enough acetylcholine to ameliorate memory deficit induced by NBM lesion until at a high dose.

In addition, we have previously reported that H_1-receptors play an important role in certain neuronal function, such as learning and memory, amygdaloid kindling, and regulation of regional cerebral blood flow in rats[7-10,25]. The present study was consistent with the previous findings, pyrilamine reversed ameliorating effect of the memory induced by histidine, in contrast, no effect was observed with zolantidine. These evidences strongly suggest that the ameliorating effects of histamine may be mediated by postsynaptic H_1-receptors.

In conclusion, it seems reasonable to assume that there exists a close relationship between histaminergic and cholinergic neurons regarding memory deficits induced by NBM lesion as evaluated by the step-through passive avoidance response in rats.

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ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Jan; 23 (1)
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中枢组胺对前脑基底核破坏所致大鼠记忆障碍的改善作用

R96 A

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关键词 回避学习; Meynert 基底核; 组胺; 他克林; 记忆障碍

目的: 研究和阐明中枢组胺对前脑基底核破坏所致大鼠记忆障碍的作用机制. **方法**: 采用被动回避反应研究大鼠的学习记忆过程. **结果**: 侧脑室内注射组胺(500 ng)、腹腔内注射组胺酸(1500 mg/kg)或他克林(3, 5 mg/kg)均可对抗前脑基底核破坏所致的记忆障碍. 选择性 H_1 受体拮抗剂吡拉明(2, 5 mg/kg)可剂量依赖性地抑制组胺的作用, 相反, H_2 受体拮抗剂卓兰替丁没有明显的作用. **结论**: 中枢组胺可以明显改善前脑基底核破坏所致大鼠记忆障碍, 其作用主要与 H_1 受体与胆碱能神经相关.

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