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Effects of huperzine A on nucleus basalis magnocellularis lesion-induced spatial working memory deficit

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KEY WORDS substantia innominata; huperzine A; physostigmine; cholinesterase inhibitors; kainic acid; choline acteyltransferase; memory; maze; learning

AIM: To study the effects of huperzine A on nucleus basalis magnocellularis (NBM) lesion-induced spatial working memory impairment. **METHODS**: A delayed-non-match-to-sample radial arm maze task was used to study spatial working memory. The choline acetyltransferase (ChAT) activity was determined by the conversion of [^{3}H]acetyl-CoA to [^{3}H]ACh. **RESULTS**: Unilateral NBM lesion by kainic acid 0.02 μmol impaired rat's ability to perform this working memory task as evidenced by fewer correct choices after different delay intervals and more total errors to complete the task. This behavioral impairment associated with a decrease in the activity of ChAT by about 40 % in the ipsilateral cerebral cortex. Huperzine A (0.2 mg·kg^{-1} ip 30 min before testing) ameliorated this spatial working memory impairment. Physostigmine (0.2 - 0.3 mg·kg^{-1} ip 20 min before testing) also attenuated the NBM lesion-induced memory deficit. **CONCLUSION**: The integrity of NBM is critical for spatial working memory processing, and this working memory impairment induced by NBM lesion can be ameliorated by huperzine A and physostigmine.

Senile dementia of the Alzheimer type (SDAT) is characterized by a progressive loss of memory for recent events (ie, working memory). Deficits in cholinergic markers such as choline acetyltransferase (ChAT) activity, acetylcholinesterase (AChE) activity, and high affinity choline transport have been observed in the cerebral cortex of those suffering from SDAT[1,2]. Cholinesterase inhibitors (ChEI) yielded clinical efficacy in the treatment of SDAT[3]. However, application of ChEI in therapeutic doses for SDAT leads to side effects, eg, livertoxicity from tacrine[4].

Huperzine A, an alkaloid first isolated from Chinese herb *Huperzia serrata* (Thunb) Trev[5], is a reversible AChE inhibitor. Its long half-life, good penetration through blood-brain barrier, and minimal side effects, as well as high therapeutic indices indicate that huperzine A is better than physostigmine and tacrine for the treatment of SDAT[6-8]. Huperzine A attenuated scopolamine-induced memory impairment[9]. To clarify its therapeutic potential in the treatment of AD, the present experiment was to study the effect of huperzine A on NBM lesion rats in delayed-non-sample radial maze paradigm in comparison to physostigmine.

MATERIALS AND METHODS

Rats Sprague-Dawley rats (♀, $n = 40$, 280 ± 15 g) were obtained from the Experimental Animal Center of Shanghai, Chinese Academy of Sciences (clean, certificate number 005). The rats were housed individually at 18 - 25 ℃ on a 12 h light/dark cycle. Water was available all the time. Since 5 d before behavioral training, food were limited to reduce the body weights to 85 %. Food limitation was maintained throughout the whole experiment, except for 7 d before and after surgery.

Radial-arm maze (RAM) The plastic octagonal center platform was 51.5 cm in diameter, and elevated 70.5 cm above the floor, with 8 radial arms. Each arm was 61 cm long and 12 cm wide. Plexiglas wall was 10 cm high extending along the length of each arm. Food well located 3 cm from the distal end of eash arm, was 1 cm deep and 2 cm in diameter.

Surgery Rats were anesthetized with sodium pentobarbital (40 mg·kg^{-1}, ip) and positioned on a stereotaxic apparatus. Kainic acid was dissolved in saline at 0.02 mol·L^{-1}, and infused 1 μL during 5 min into the right NBM. Stereotaxic coordinates were: 0.8 mm posterior to bregma; 2.6 mm lateral to midline; and vertical 6.8 mm from dura[10]. The needle was left in place for 3 min after the infusion. Saline-treated rats received 1 μL saline.

Behavioral training[11] Rats were initially trained on a standard RAM task for 2 wk. Then rats were trained to perform the task involving 2 episodes. In the first episode 4 arms were

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blocked. Taken from the apparatus after the 4 available arms had been chosen, the rat was placed in its home cage. Following a delay the rat was put again to the maze, which now had all 8 arms open but only those blocked arms during the predelay session contained food pellets. Rats were allowed to choose until the 4 remaining baited arms were visited or until a total of 10 postdelay choices were made. The delay interval between the 2 episodes was 5 min at first and increased stepwise to 2 h. The blocked arms chosen for each daily trial varied quasirandomly. Entry into an arm visited during the predelay session (retroactive error) or reentry into a postdelay chosen arm (proactive error) constituted a postdelay error. The number of correct choices (CC) in first 4 postdelay choices, postdelay errors (PDE), retroactive errors, proactive errors, and latency per arm choice served as dependent measures. The rats were trained to reach the criterion level performance (< 1 PDE in each trial for 3 consecutive trials).

Drug testing After rats achieved the criterion level, they were allowed to regain body weight 1 wk before surgery. Rats were treated with kainic acid or saline. One week following surgery, behavioral testing resumed. Rats were initially tested with delay intervals of 5, 30, or 60 min interposed between the 4th and 5th arm choices. These rats were used to examine the effects of huperzine A on reversing NBM lesion-induced memory deficit. Huperzine A was injected ip 30 min before the trial with a 15-min delay between the 4th and 5th arm choices, and physostigmine was injected 20 min before testing.

Enzyme assays The ChAT activity was measured[12]. The brain ChAT activities were assayed 1 wk after lesioning. The hippocampus, striatum, frontal cortex, and hypothalamus were homogenized in 19 vol of sodium phosphate buffer 75 mol·L^{-1}, pH 7.4. A 10-μL aliquot of homogenate was add to 10 μL of buffer substrate solution (sodium phosphate solution 75 mol · L^{-1}, pH 7.4; containing NaCl 600, $MgCl_2$ 40, physostigmine 2.0, choline iodide 10, acetyl-CoA 0.87 mol·L^{-1}; [^{3}H]acetyl-CoA 160.95 kBq; bovine serum albumin 0.05 %). After 30-min incubation at 37 ℃, 150 μL of 3-heptanone containing sodium tetraphenylboron 75 g · L^{-1} was added to extract [^{3}H]ACh. The [^{3}H]ACh in 100 μL aliquot of the organic phase was measured by liquid scintillation counter. The protein content was measured[13].

RESULTS

Effects of NBM lesion on performance of DNMTS Kainic acid-treated rats demonstrated an impairment in their performance compared to saline-treated rats. Analysis of CC indicated treated effects at 5-min ($P < 0.01$), 30-min ($P < 0.01$), and 60 min ($P < 0.01$) delay intervals compared to the saline-treated group. Similar increases in the number of PDE in the NBM lesion group at different delay intervals (5-min, 30-min, and 60-min, all $P < 0.01$ *vs* saline control at the same delay intervals) were observed (Fig 1).

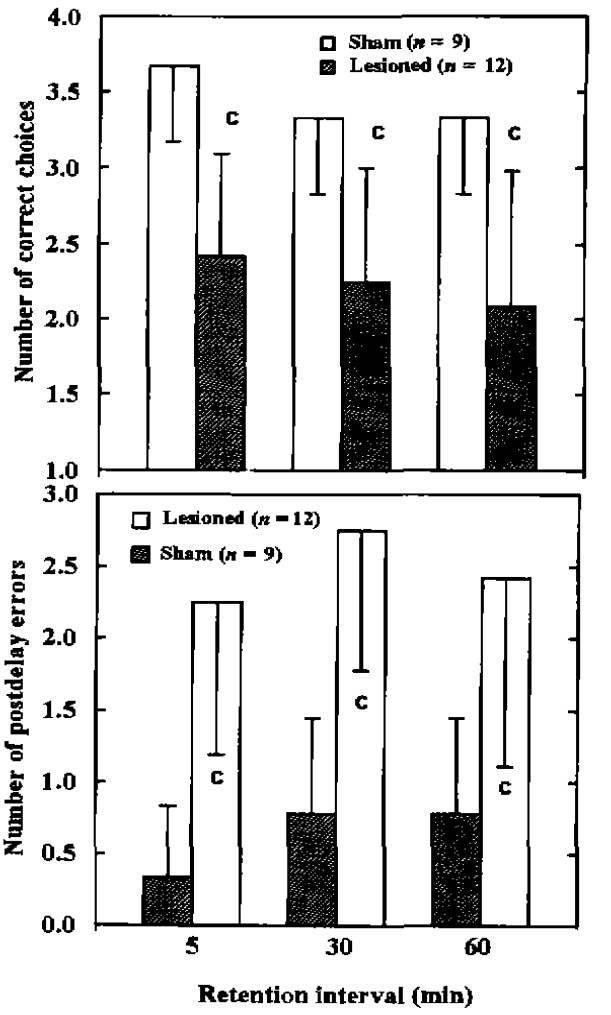

Fig 1. Number of correct choices made in the first 4 postdelay choices and number of postdelay errors after unilateral NBM lesion caused by kainic acid (0.02 μmol) on spatial working memory in rats with varying delay intervals imposed between the 4th and 5th arm choices. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **saline groups.**

The majority of errors during the postdelay period was made as reentries to arms visited originally in the predelay period (retroactive error) rather than to arms originally visited earlier in the postdelay stage (proactive error). There was an increase in the number of retroactive errors in NBM lesion rats at 5-min ($P < 0.01$), 30-min ($P < 0.01$), and 60-min delay intervals ($P < 0.05$). No significant treatment effects in proactive errors at same delay intervals were

seen (all $P > 0.05$). No significant difference in the kainic acid-treated rat's latency per arm choice to perform the RAM task was observed (all $P > 0.05$) (Tab 1).

Tab 1. Effect of unilateral NBM injection of kainic acid (0.02 μmol) on postdelay indices of radial-arm maze performance at varying delay intervals. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* saline groups (one-way ANOVA followed by Duncan's multiple-range test).

Delay	5-min	30-min	60-min	*n*
Retroactive errors				
Saline	0.3 ± 0.5	0.8 ± 0.7	0.8 ± 0.7	9
NBM lesion	2.1 ± 0.8^c	2.3 ± 0.6^c	1.9 ± 0.9^b	12
Proactive errors				
Saline	0	0	0	9
NBM lesion	0.2 ± 0.4	0.4 ± 0.5	0.5 ± 0.8	12
Latency per choice (s)				
Saline	8.4 ± 2.4	9 ± 3	9.1 ± 2.5	9
NBM lesion	9 ± 3	9.0 ± 2.8	10 ± 3	12

Effects of drugs on working memory deficits of NBM lesion rats Analyses of the PDE in NBM-lesioned rats revealed an impairment effect at 15-min delay interval compared to saline-treated rats ($P < 0.01$). Huperzine A 0.2 mg·kg^{-1} improved in the performance at 15-min delay interval compared to NBM lesion alone ($P < 0.01$) (Fig 2).

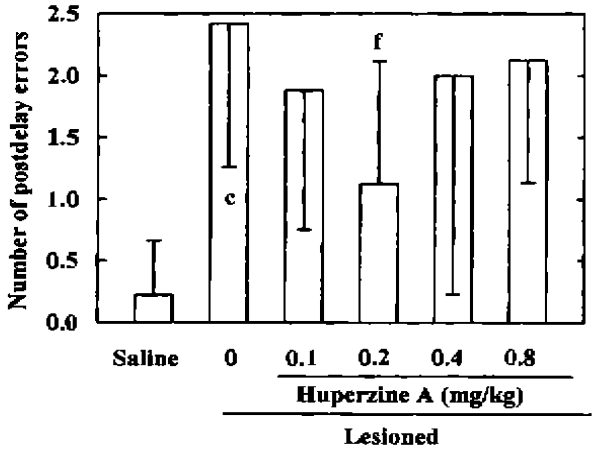

Fig 2. Effects of huperzine A on radial arm maze deficits induced by unilateral NBM injection of kainic acid (0.02 μmol). $n = 8 - 12$. $^cP < 0.01$ *vs* saline group. $^fP < 0.01$ *vs* vehicle-treated NBM-lesioned group.

Similarly, significant decreases in the number of PDE in 0.2 mg·kg^{-1} group ($P < 0.05$) and 0.3 mg·kg^{-1} group ($P < 0.01$) of physostigmine were noted (Fig 3).

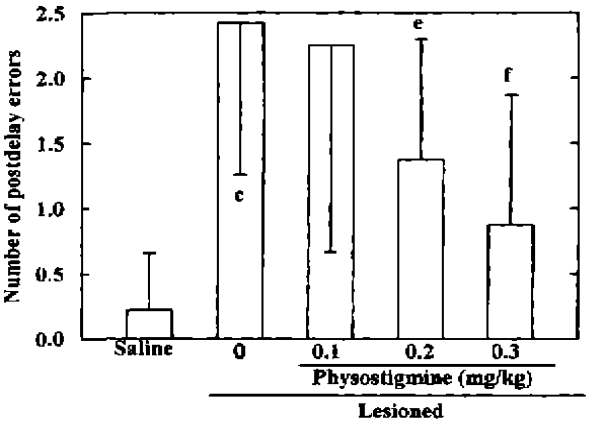

Fig 3. Effects of physostigmine on radial arm maze deficits induced by unilateral NBM injection of kainic acid (0.02 μmol). $n = 8 - 12$. $^cP < 0.01$ *vs* saline group. $^eP < 0.05$, $^fP < 0.01$ *vs* vehicle-treated NBM-lesioned group.

Neurochemical observations The kainic acid lesion caused reductions ($P < 0.01$) in ChAT activity in the ipsilateral cortex (about 40 %). In contrast, there were no significant decreases in ChAT activity in other brain regions (Tab 2).

Tab 2. Choline acetyltransferase activity after kainic acid lesion of right NBM $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* saline.

Region	μmol ACh formed·h^{-1}/g protein		
	Saline	Lesion	%
Hippocampus	41 ± 5	37 ± 3	90
Striatum	93 ± 12	86 ± 3	92
Cortex			
Ipsilateral	31 ± 4	18.1 ± 2.0^c	58
Contralateral	32.3 ± 2.5	34 ± 4	105
Hypothalamus	16.4 ± 2.9	17 ± 3	104

DISCUSSION

Because the basal forebrain cholinergic system is a major site of pathological degeneration in patients with SDAT, animal models of this disease have placed special emphasis on the behavioral effects of destruction of the homologous areas in rodents and nonhuman primates. The NBM in the rat is a group of large multipolar cells located in the ventromedial

region of the globus pallidus and is thought to be homologous to the nucleus basalis of Meynert in primates. It counts for 70 % – 80 % of the cholinergic innervation to the cortex. Lesion the NBM have been proposed as an animal model of AD[14,15].

In this study, we found that the unilateral NBM lesion with kainic acid produced an impairment in the delayed non-match-to-sample task and that the cholinergic network from the NBM to the frontal cortex was preferentially damaged. This impairment was associated with an inability to respond discriminantly during the postdelay session to arms chosen prior to the delay interval, as evidence by increase in retroactive errors and no change in proactive errors. Thus the NBM lesion caused deficits in the use of recently encoded information. This result differed from scopolamine which caused deficits in both retroactive errors and proactive errors (our unpublished data). The impairments of spatial working memory correspond with the decrease in cortical ChAT activities. These results suggest that the integrity of the NBM is important for normal spatial working memory.

In our experiment, huperzine A improved the spatial working memory impairment induced by unilateral NBM lesion in the delayed-non-match-to-sample radial arm maze task. The improving action of huperzine A was bell shaped, probably owing to the dose-dependent increase in ACh via direct cholinesterase inhibition in brain synapses. Physostimine also ameliorated this spatial working memory deficit. The result provides the view that huperzine A has a cognitive enhancing action, and suggests that the drug exerts stimulative action on cholinergic systems in the brain.

In summary, the NBM lesion rat provides a valuable model for examining the effectiveness of cholinergic drugs which might be used to treat the symptoms of AD. Huperzine A is a potent AChE inhibitor on attenuation the unilateral NBM lesion-induced spatial working memory deficit, suggesting that it may be of therapeutic value in cognition deterioration associated with senescence and in patients with SDAT.

REFERENCES

1 Bartus RT, Dean RL III, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science 1982; 217: 408 – 17.

2 Coyle JT, Price DL, DeLong MR. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 1983; 219: 1184 – 90.

3 Pendlebury WW, Solomon PR. Alzheimer's disease: therapeutic strategies for the 1990s. Neurobiol Aging 1994; 15: 287 – 9.

4 Watkins PB, Zimmerman HJ, Knapp MJ, Gracon SI, Lewis KW. Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. J Am Med Assoc 1994; 271: 992 – 8.

5 Liu JS, Zhu YL, Yu CM, Zhou YZ, Han YY, Wu FW, *et al*. The structures of huperzine A and B, two new alkaloids exhibiting marked anticholinesterase activity. Can J Chem 1986; 64: 837 – 9.

6 Wang YE, Yue DX, Tang XC. Anti-cholinesterase activity of huperzine A. Acta Pharmacol Sin 1986; 7: 110 – 3.

7 Yan XF, Lu WH, Lou WJ, Tang XC. Effects of huperzine A and B on skeletal muscle and electroencephalogram. Acta Pharmacol Sin 1987; 8: 117 – 23.

8 Tang XC, Xiong ZQ, Qian BC, Zhou ZF, Zhang CL. Cognition improvement by oral huperzine A: a novel acetylcholinesterase inhibitor. In: Giacobini E, Becker R, editors. Alzheimer disease: therapeutic strategies. Boston: Birkhäuser; 1994. p 113 – 9.

9 Xiong ZQ, Tang XC. Effect of huperzine A, a novel acetylcholinesterase inhibitor, on radial maze performance in rats. Pharmacol Biochem Behav 1995; 51: 415 – 9.

10 Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.

11 Chrobak JJ, Hanin I, Schmechel DE, Walsh TJ. AF64A-induced working memory impairment: behavioral, neurochemical and histological correlates. Brain Res 1988; 463: 107 – 17.

12 Leventer S, McKeag D, Clancy M, Wulfert E, Hanin I. Intracerebroventricular administration of ethylcholine mustard aziridinium ion (AF64A) reduces release of acetylcholine from rat hippocampal slices. Neuropharmacology 1985; 24: 453 – 9.

13 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265 – 75.

14 Archer T, Fowler CJ. Towards an animal model for the cholinergic lesion in Alzheimer's disease. Trends Pharmacol Sci 1985; 6: 61.

15 Smith G. Animal models of Alzheimer's disease: experimental cholinergic denervation. Brain Res Rev 1988; 13: 103 – 18.

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石杉碱甲对基底核大细胞部损毁所致工作记忆障碍的影响

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关键词 无名质；石杉碱甲；毒扁豆碱；胆碱酯酶抑制剂；卡因酸；胆碱乙酰转移酶；记忆；迷宫；学习

目的：研究石杉碱甲对基底核大细胞部(NBM)损毁诱导的工作记忆障碍的影响. **方法**：采用八臂迷宫延迟插板的程序研究空间记忆. 胆碱乙酰转移酶(ChAT)活力测定采用[^{3}H]乙酰辅酶 A 转变成[^{3}H]乙酰胆碱的方法. **结果**：单侧损毁 NBM (卡因酸 0.02 μmol)导致空间记忆障碍. 在不同的延迟间隔，大鼠完成程序产生的正确数减少和错误数增多. 损毁侧大脑皮层 ChAT 酶的含量下降了大约 40 %. 石杉碱甲(0.2 mg·kg^{-1}实验前 30 min ip)改善这种空间记忆障碍. 毒扁豆碱(0.2 −0.3 mg·kg^{-1}实验前 20 min ip)也有改善作用. **结论**：完整的 NBM 是空间记忆形成的关键. 石杉碱甲有效的改善 NBM 损毁导致的空间记忆障碍.

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Inhibitory effect of antisense basic fibroblast growth factor oligonucleotides on proliferation of cultured aortic smooth muscle cells induced by angiotensin Ⅱ in SHR rats[1]

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KEY WORDS basic fibroblast growth factor; antisense oligonucleotides; hyperplasia; vascular smooth muscle; cultured cells; thymidine; angiotensin Ⅱ; inbred SHR rats

AIM: To study the effect of antisense basic fibroblast growth factor (bFGF) oligonucleotides (ODN) transfection on the growth of cultured aortic smooth muscle cells (SMC) in spontaneously hypertensive rats (SHR). **METHODS**: Using cationic liposome-mediated method, antisense bFGF ODN were introduced into SMC, bFGF gene expression was detected by Northern blotting, cell hyperplasia was evaluated by [^{3}H] thymidine incorporation and cell counting. **RESULTS**: Transfection of antisense bFGF ODN (5 μmol·L^{-1}) almost completely inhibited enhanced bFGF mRNA expression and inhibited cell proliferation induced by angiotensin Ⅱ (Ang 1 μmol·L^{-1}). In basal state and Ang-stimulated state, [^{3}H]thymidine incorporation was inhibited by 26.5 % ($P<0.01$) and 42.0 % ($P<0.01$) and cell number was inhibited by 17.3 % ($P<0.01$) and by 22.2 % ($P<0.01$), respectively. **CONCLUSION**: The transfection of antisense bFGF ODN into cultured SMC effectively suppressed bFGF mRNA expression and inhibited the SMC proliferation induced by Ang.

Abnormal growth of vascular smooth muscle cells (VSMC) is critical to the pathophysiology of hypertension. Angiotensin Ⅱ (Ang) played a major role in the regulation of VSMC growth in hypertensive animal models[1].

The inhibition of VSMC hyperplasia by antisense oligonucleotides (ODN) transfer maybe lead to new therapy approaches for certain cardiovascular diseases[2] such as hypertension, atherosclerosis, and vascular restenosis following balloon angioplasty.

The delivery of antisense proto-oncogene *c-myb* ODN effectively suppressed intimal accumulation of rat carotid smooth muscle cells after catheter-mediated injury *in vivo* for at least 2 wk[3].

The delivery of antisense cell cycle regulatory genes (such as cell division cycle 2 kinase, cyclin-dependent kinase, and proliferating cell nuclear antigen) inhibited intimal formation in the rat carotid injury model for as long as 8 wk after a single transfer[4]. Previously, we had demonstrated that Ang enhanced basic fibroblast growth factor (bFGF)

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