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Effect of Nω-nitro-L-arginine on working memory of rats in eight-arm maze task

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AIM: To study the effect of Nω-nitro-L-arginine (NNA) (a NO synthase inhibitor) on memory in the rats. **METHODS**: A delayed non-match-to-sample (DNMTS) eight-arm maze task was used to study spatial working memory. **RESULTS**: Intraperitoneal injection of NNA 100 mg kg^{-1} before the trial or at the start of the delay did not affect the accuracy, while pretraining administration of scopolamine 0.25 mg kg^{-1} produced impairment in mnemonic performance as evidenced by fewer correct choices after the delay and more total errors to complete the task. Intracerebroventricular infusion of NNA (10, 50, 100 nmol) did not affect the accuracy. **CONCLUSION**: A single ip or icv injection of NNA is not sufficient to alter the memory formation and use in this DNMTS eight-arm maze task.

KEY WORDS memory; maze learning; Nω-nitro-L-arginine

Nitric oxide (NO) has been suggested as a possible retrograde messenger mediating logn-term potentiation (LTP)[1,2], a mechanism for vertebrate memory formation. It has been reported that NO is involved in the learning and memory[3,4]. Because NO synthase (NOS) is present in a wide variety of CNS neurons and NO likely has a variety of functions[5], the learning and memory impairments could have resulted from a nonspecific performance deficit. This is an important consideration when utilizing NOS inhibitors since these agents are potent vasoconstrictors; spontaneously hypertensive animals can exhibit impaired performance[6]. Tasks that allow for manipulation of retention intervals, and the use of post-training treatment protocols, by which agents can be delivered after the to-be-remembered event, are useful for elucidating effects in memory specific processes. The eight-arm maze[7] is an advantageous tool for the investigation of the neural basis of learning and memory in the rat. The insertion of a delay between choices permits an examination of the ability to maintain representations of previous arm entries over varying retention intervals.

Nω-nitro-L-arginine (NNA) competes with arginine for NOS and therefore blocks NO production[8,9]. The purpose of this study was to test whether ip or icv NNA injection affects spatial working memory. Scopolamine was selected for comparison with NNA.

MATERIALS AND METHODS

Rat Male Sprague-Dawley rats, weighing 280±s 19 g, were housed 2 per cage with food and water ad lib. Three d before the training, the food was removed for 24 h. On the next day, the food was available 1 h daily to maintain the body weight at about 90 % of the initial level.

Apparatus A gray plastic maze consisted of 8 arms (61 cm × 12 cm) radiating from an octagonal platform. Plexiglas wall was 10 cm high. Food wells, located 3 cm from the distal end of each arm, were 1 cm deep and 2 cm in diameter.

Training procedure Rats were initially habituat-

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ed by allowing them to explore the arms and to consume food pellets scattered in the whole maze. This procedure was repeated for 3 d. On d 4, rat was placed in the center of the maze and allowed to visit the 8 arms which were baited with a single pellet in each arm. After 12 RAM training trials, rats were trained to perform a task of 3 consecutive sessions with a delay imposed between the 4th and 5th arm choices. The training began from the 0-min delay session to the 15-min and then to the 1-h delay session. Plexiglas barriers were used to force the rats to select a set of 4 arms during the pre-delay session. The set of arms chosen for each daily session varied quasirandomly. Following the delay, rats were allowed to choose freely among all 8 arms. Entry into an arm visited during the pre-delay session constituted an error, as did repeated entries into post-delay choices. The rats were trained to reach the criterion level performance (0 or 1 error in each trial for consecutive 3 trials). Delayed non-match-to-sample (DNMTS) eight-arm maze task performance indices included the number of correct choices in the first 4 post-delay choices, the number of errors in the post-delay choices, and latency of per arm choice.

Medication All rats achieved the criterion level within 60 d. Following the completion of training, rats were tested in 2 experiments examining the effects of ip and icv NNA injection on performance of the DNMTS eight-arm maze task. In experiment A, rats were injected ip with NNA (Sigma Chemical Co). In experiment B, icv infusion of NNA. Using stereotaxic techniques, under sodium pentobarbital (40 mg kg^{-1}) anesthesia, a single hole was drilled through the skull 0.6 mm behind bregma, and 1.3 mm to the right of the midline. A 25-gauge cannula was lowered to a depth of 4.0 mm below the brain surface, and secured in position with stainless steel screw and dental cement. The cannula was connected via tubing to mini-osmotic pump.

RESULTS

In experiment A, NNA (100 mg kg^{-1}) was injected immediately after the onset of 1-h delay (Section A), or 1 h prior to the start of the trial (Section B), while scopolamine was injected 20 min before the start of the trial. There was no significant difference in the number of correct choices and errors between NNA and saline treatments. Scopolamine caused significant impairments in this working memory task as evidenced by fewer correct choices after the delay and more total errors to complete the task (Tab 1).

Tab 1. Effect of ip NNA 100 mg kg^{-1} on performance of DNMTS eight-arm maze task. $n=13$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.01$ *vs* **saline using ANOVA followed by Duncan's multiple-range test.**

	Correct choice	Errors	Latency
Section A			
Saline	3.5±0.5	0.5±0.6	8.2±2.8
NNA	3.3±0.6[a]	0.8±0.7[a]	18.5±9.0[b]
Section B			
Saline	3.6±0.5	0.5±0.6	7.7±2.2
NNA	3.2±0.5[a]	0.9±0.6[a]	18.3±7.6[b]
Scopolamine	2.0±0.5[b]	4.4±1.1[b]	14.5±3.6[b]

To rule out the possibility that the non-amnestic effect might be the inadequate inhibition of NOS in CNS, NNA was injected 15 min prior to the predelay session with rats tested 15-min retention interval. Icv NNA 10, 50, or 100 nmol in 1 μL were ineffective in preventing spatial working memory formation (Tab 2).

Tab 2. Effect of icv infusion of NNA on spatial working memory. $\bar{x}\pm s$. $^aP>0.05$ *vs* **saline.**

Drug	Rats	Correct	Errors
Saline	12	3.8±0.4	0.2±0.4
10 nmol	10	3.5±0.5[a]	0.6±0.7[a]
50 nmol	10	3.6±0.5[a]	0.6±0.5[a]
100 nmol	10	3.5±0.5[a]	0.5±0.5[a]

DISCUSSION

NOS inhibitors decrease motoric activity in certain behavioral protocols[10,11]. For example, injections of NNA 100 and 178 mg

kg^{-1} ip in normal rats produced a reduction in locomotion activity, suggesting a possible sedative effect[11]. In our experiment, although no alterations in choice accuracy occurred, an increase in latency of choices, indicative of a slowness in responding, was also observed in rats following injection of NNA. Changes in the *L*-arginine/NO pathway may influence feeding behavior[12,13]. In our study, decreased food intake was observed with NNA. Our findings indicate that ip injection of NNA impaired motoric performance at dose that did not affect choice accuracy. A single injection of NNA (50 mg kg^{-1} ip) reduced enzyme activity by 50 % in the brain[9]. Measurement of the inhibitory effect of NNA infusion on NOS activity demonstrated a 90 % inhibition of NOS in rat after 3 infusion of 10 nmol NNA. As the inhibition of NOS by NNA is essentially irreversible, these *in vitro* measurements of NOS activity reflect the degree of NOS inhibition *in vivo*[14]. Therefore, our failure to observe effects of NNA on the spatial working memory performance is unlikely due to the inadequate inhibition of NOS.

In our test, the DNMTS procedure prevents the use of efficient egocentric strategies (eg, clockwise or counterclockwise repetition of choice directions). Such strategies resemble sequential routines and do not require the use of extramaze cues in performing the task and minimize the mnemonic component of the task. In addition, we trained rats to a criterion to separate the effects on memory for recent events (working memory) from that required to learn new tasks. Our findings indicate that NNA did not affect choice accuracy. Such effects preclude assessment of the consequence of altering NOS activity that may be more critical to memory. Our results were consistent with those of Brennan and Kishimoto, who found that local inhibition of NOS activity in the accessory olfactory bulb did not prevent the formation of an olfactory memory[14].

The present study indicates that the spatial working memory is insensitive to NNA in the rat. The failure of NNA to inhibit memory formation and use suggests that caution should be exercised in assuming a direct role for NO in processes of spatial working memory.

REFERENCES

1 Bredt DS, Snyder SH. Nitric oxide, a novel neuronal messenger. Neurons 1992; **8**: 3—11.

2 Gribkoff VK, Lum-Ragan JT. Evidence for nitric oxide synthase inhibitor-sensitive and insensitive hippocampal synaptic potentiation.
J Neurophysiol 1992; **68**: 639—43.

3 Bohme GA, Bon C, Lemaire M, Reibaud M, Piot O, Stutzmann JM, *et al*. Altered synaptic plasticity and memory formation in nitric oxide synthase inhibitor-treated rats. Proc Natl Acad Sci USA 1993; **90**: 9191—4.

4 Hölscher C, Rose SPR. Inhibiting synthesis of the putative retrograde messenger nitric oxide results in amnesia in a passive avoidance task in the chick.
Brain Res 1993; **619**: 189—94.

5 Vincent SR. Nitric oxide: a radical neurotransmitter in the central nervous system.
Prog Neurobiol 1994; **42**: 129—60.

6 Wyss JM, Fisk G, Groen TV. Impaired learning and memory in mature spontaneously hypertensive rats. Brain Res 1992; **592**: 135—40.

7 Olton DS, Samuelson RJ. Remembrances of places passed, spatial memory in rats.
J Exp Psychol (Anim Behav) 1976; **2**: 97—116.

8 Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature 1991; **351**: 714—8.

9 Dwyer MA, Bredt DS, Snyder SH. Nitric oxide synthase: irreversible inhibition by *L*-N^G-nitroarginine in brain *in vitro* and *in vivo*.
Biochem Biophys Res Commun 1991; **176**: 1136—41.

10 Caton PW, Tousman SA, Quock RM. Involvement of nitric oxide in nitrous oxide anxiolysis in the elevated plus-maze.

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Pharmacol Biochem Behav 1994; 48: 689－92.

11 Domino EF, Yao L, Sheng JG, Grekin RJ, Malvin RL. Is nitric oxide involved in mediating rat open field behavior? Pharmacol Commun 1993; 3: 175－81.

12 Morley JE, Flood JF. Evidence that nitric oxide modulates food intake in mice. Life Sci 1991; 49: 707－11.

13 Morley JE, Farr SA, Suarez MD, Flood JF. Nitric oxide synthase inhibition and food intake: effects on motivation to eat and in female mice.
Pharmacol Biochem Behav 1995; 50: 369－73.

14 Brennan PA, Kishimoto J. Local inhibition of nitric oxide synthase activity in the accessory olfactory bulb does prevent the formation of an olfactory memory in mice. Brain Res 1993; 619: 306－12.

Nω-硝基-L-精氨酸对大鼠八臂迷宫工作记忆的作用

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目的：研究一氧化氮合成酶抑制剂 Nω-硝基-L-精氨酸(NNA)对大鼠空间工作记忆的作用. **方法**：采用八臂迷宫延迟插板的程序. **结果**：腹腔注射 NNA 100 mg kg^{-1}对大鼠八臂迷宫选择的准确性没有显著影响，只能增加反应的潜伏期. 东莨菪碱0.25 mg kg^{-1}使大鼠延迟后的错误选择显著增加. 脑室内注射 NNA (10, 50, 100 nmol/1 μL)没有影响准确性. **结论**：急性 NNA 给予对大鼠空间工作记忆的形成和使用没有显著影响.

关键词 记忆；迷宫学习；Nω-硝基-L-精氨酸

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Protection of methylflavonolamine against acute cerebral ischemia reperfusion injury in rats

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AIM: To examine the possible beneficial action of methylflavonolamine (MFA) on cerebral ischemia/reperfusion injury. **METHODS**: Acute cerebral ischemia-reperfusion injury was produced by 4-vessel occlusion and subsequent 1-h release. MFA, 20 mg kg^{-1}, was injected intravenously 5 min before occlusion and again before release. **RESULTS**: The brain water content in the reperfusion group (Rep) was elevated (82.7 %±1.1 % *vs* control 79.7 %±0.5 %, $P<0.01$), while MFA alleviated the brain edema (80.9 %±0.9 % *vs* Rep, $P<0.01$). The CK level of brain tissue in Rep decreased (4.7±1.4 *vs* control 8.4±1.2 U/mg protein, $P<0.01$), but MFA restored it (7.2±1.1 U/mg protein *vs* Rep, $P<0.01$). Reperfusion caused the rise of lipid peroxides (2.3±0.5 *vs* control 1.5±0.4 nmol/mg protein, $P<0.01$) and weakened the superoxide dismutase (SOD) (3.1±1.6 *vs* control 10.5±3.9 U/mg protein $P<0.01$), MFA reduced the rise of lipid peroxides (1.6±0.4 nmol/mg protein *vs* Rep, $P<0.05$) and protected the activity of SOD (7.9±1.6 U/mg protein *vs* Rep, $P<0.01$) in brain. **CONCLUSION**: MFA has the protective effects on cerebral ischemia/reperfusion,

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