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Intrahippocampal infusion of interleukin-6 impairs avoidance learning in rats[1]

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KEY WORDS interleukin-6; nitric oxide; avoidance learning; hippocampus; nitroprusside

AIM: To study the effect of intrahippocampal infusion of interleukin-6 (IL-6) on active avoidance in rats and the possible involvement of nitric oxide (NO). **METHODS:** Using a shuttle-box model, the effects of bilaterally intrahippocampal infusion of IL-6 3.2, 16, and 80 ng as well as sodium nitroprusside (SNP) 400 ng on active avoidance were studied on d 8 after administration. The levels of nitrite as an index of NO in the hippocampus were detected using a fluorometric assay 24 h after infusion of IL-6 3.2 or 80 ng. **RESULTS:** IL-6 16 and 80 ng impaired the acquisition performance of active avoidance by prolonging the latency of avoidance in training, but not the retention performance in testing. IL-6 80 ng and SNP 400 ng also resulted in a marked impairment in acquisition performances by decreasing the rate of avoidance, but not in retention performances. IL-6 80 ng markedly elevated the nitrite levels from 10.6 ± 0.7 in control rats to 13.6 ± 2.0 (nmol/g wet wt) ($P < 0.01$). IL-6 3.2 ng had no effect on active avoidance nor on nitrite levels. **CONCLUSION:** Intrahippocampal infusion of IL-6 impaired learning acquisition of active avoidance in rats.

Interleukin-6 (IL-6) is a multifunctional cytokine produced by essentially every injured tissue[1]. IL-6 levels are elevated in and around the senile plaques in cortex and hippocampus of Alzheimer's disease (AD) patients[2], suggesting the potential importance of IL-6 in AD pathology. There were no data concerning the effect of IL-6 on learning and memory in animals. To provide some experimental evidence in this respect, the present study was carried out.

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MATERIALS AND METHODS

Chemicals Human recombinant interleukin-6 (IL-6) was a gift from Chengdu Di'ao Pharmaceutical Co, Chinese Academy of Sciences. 2,3-Diaminonaphthalene (DAN) and sodium nitroprusside (SNP) were purchased from Sigma Chemical Co (USA). All other chemicals were of AR. IL-6 and SNP were dissolved in 0.9 % pyrogen-free saline, and DAN was dissolved in HCl 0.62 $mol \cdot L^{-1}$. SNP and DAN solutions were protected from light. Double-deionized water was used throughout the nitrite assay.

Rats Sprague-Dawley rats (♂, $n = 66$, $300 \pm s$ 20 g) were obtained from the Experimental Animal Center of Shanghai, Chinese Academy of Sciences (clean, certificate number 005). The rats were individually housed in plastic cages after surgery and maintained at a room temperature of 20-22 ℃ with a 12 L:12 D cycle (light on at 08:00). Water and rat chow were fed *ad lib*.

Surgery and intrahippocampal infusion Rats were anesthetized with sodium pentobarbital (50 $mg \cdot kg^{-1}$, ip) and placed on a stereotaxic apparatus with the incisor bar set 2.5 mm below the interaural line. Two holes were drilled through the skull to allow injections into the hippocampus. The coordinates of injection sites were 6.5 mm posterior to bregma, 5.0 mm lateral to the sagittal suture, and 6.3 mm ventral to the surface of the skull[3]. In Experiment 1, rats received bilateral infusions of saline, IL-6 1.6, 8, or 40 ng per side. In Experiment 2, rats received bilateral injections of saline, IL-6 40 ng, or SNP 200 ng. In Experiment 3, rats received saline, IL-6 1.6 or 40 ng injections. A 1-μL microsyringe was used for the infusion of 0.5 μL per side in 3 min.

Active avoidance task On d 8 after surgery, active avoidance was tested in a one-way shuttle box (50 cm × 30 cm × 15 cm) essentially as described[4]. Briefly, rats were trained to cross the hole from the shock side into the safe side within 10 s of conditioned stimulus (CS, 40 W bulb), otherwise they were punished with 5 s of unconditioned stimulus (US, 30 V footshock). Each rat received 15 trials daily for 4 consecutive days. One trial consisted of 15 s of intertrial interval followed by maximum 15 s of CS. The last 5 s of CS overlapped with the US, if necessary. Only the rats reaching the criterion of over 75 % avoidance in the last day of training were used for the retention testing conducted 2 d following the completion of training. Avoidance latency or avoidance rate were measured.

Histological localizations At the end of experiments, the rats were anesthetized with sodium pentobarbital and decapitated. The brain was fixed in 10 % form aldehyde. Frozen coronal sections 40 μm thick were stained with cresyl violet.

Hippocampal nitrite assay In Experiment 3, rats were decapitated 24 h after medication. Bilateral hippocampi were dissected on ice, weighed, and placed in plastic tubes containing 0.8 mL of ice-cold Tris (50 mmol·L^{-1}, pH 7.4). Tissues homogenates were spun at 25 000 × g for 30 min at 4 ℃. Supernatants were kept at −40 ℃ for the measurement of nitrite.

Determination of nitrite was done[5]. Briefly, samples were diluted with water and nitrite standards at 7 concentrations 0.05 − 1.0 μmol·L^{-1} kept on ice. One milliliter of samples or nitrite standards was mixed with 100 μL of freshly prepared DAN (0.05 g·L^{-1}). After a 10-min incubation at 20 ℃, the reaction was terminated with 50 μL of NaOH 2.8 mol·L^{-1}. Formation of 2, 3-diaminonaphthotriazole was measured using a Fluorescence Spectrophotometer (Hitachi G50-10 S) at λ_{ex} 365 nm and λ_{em} 450 nm. The detection limit was 10 nmol·L^{-1}.

Statistical analysis Data were expressed as $\bar{x} \pm s$. Differences between groups were evaluated by t-test.

RESULTS

Latency of active avoidance IL-6 16 and 80 ng resulted in marked prolongations in the latency of avoidance in d 3 −4 training and in d 2 −4 training, but no effect on the latency in the testing. IL-6 3.2 ng had no effect on the latency throughout training and testing (Tab 1).

Rate of active avoidance IL-6 80 ng and SNP 400 ng decreased the rate of avoidance throughout training and testing, but a significant reduction in the rate was seen only in d 3 training both for IL-6 and for SNP (Tab 2).

The needle tips were histologically located correctly within the hippocampus according to the atlas[6].

Levels of nitrite *in vivo* IL-6 80 ng significantly elevated the level of nitrite (nmol/g wet wt) in the hippocampus from 10.6 ± 0.7 in control rats to 13.6 ± 2.0 (n = 8, P < 0.01), but IL-6 3.2 ng had no effect on the level, which was 11.3 ± 1.2 in IL-6 3.2 ng rats.

DISCUSSION

The present results have shown that intrahippocampal infusion of IL-6 and SNP impaired acquisition of active avoidance in rats and this IL-6 administration elevated the level of nitrite as an index of NO in the hippocampus. Therefore, it may be postulated that overproduction of NO in the hippocampus probably mediates the impairment of active avoidance produced by IL-6. Moreover, present studies further ethopharmacologically support the hypothesis that IL-6 involves the pathology of AD.

Some studies have demonstrated the impairing effect of NO synthase (NOS) inhibitors on several

Tab 1. Effect of intrahippocampal infusion of IL-6 on the avoidance latency (s) in rats. n = 7, $\bar{x} \pm s$. [a]P > 0.05, [b]P < 0.05 *vs* control.

IL-6/ng	d 1	d 2	d 3	d 4	d 6
0	4.0 ± 2.3	2.3 ± 0.8	2.3 ± 0.7	2.1 ± 0.8	2.3 ± 1.3
3.2	4.4 ± 1.4[a]	2.3 ± 0.4[a]	2.9 ± 0.7[a]	2.6 ± 0.8[a]	3.1 ± 0.9[a]
16	4.8 ± 1.7[a]	3.2 ± 1.5[a]	3.4 ± 1.0[b]	3.1 ± 0.8[b]	2.7 ± 0.9[a]
80	4.4 ± 1.3[a]	3.6 ± 1.0[b]	3.4 ± 1.1[b]	3.2 ± 0.6[b]	3.3 ± 0.8[a]

Tab 2. Effect of intrahippocampal infusion of IL-6 on the avoidance rate (%) in rats. n = 7, $\bar{x} \pm s$. [a]P > 0.05, [b]P < 0.05 *vs* control.

Group	Dose/ng	d 1	d 2	d 3	d 4	d 6
Control	−	36 ± 20	65 ± 16	88 ± 9	84 ± 10	87 ± 10
IL-6	80	17 ± 17[a]	42 ± 38[a]	56 ± 26[b]	80 ± 16[a]	78 ± 15[a]
SNP	400	21 ± 8[a]	35 ± 40[a]	55 ± 37[b]	76 ± 22[a]	73 ± 22[a]

forms of behavioral tasks[7-10] and the memory-enhancing effect of SNP, a NO donor, at moderate doses on an inhibitory avoidance task[11], suggesting the beneficial effect of NO on learning and memory. In contrast, SNP at higher doses impaired the retention performance on passive avoidance[11], suggesting the amnesic effect. It seems that NO may have a dual effect on learning and memory depending on the levels. The present studies provide the first evidence that IL-6 and SNP impaired behavior of active avoidance.

Recently, nitrite has been reported to be a main oxidative product of NO and the assay for nitrite can be used as a measurement of NO produced *in vivo*[12]. To explore the effect of central administration of IL-6 on the formation of nitrite in the hippocampus, we detected the nitrite levels. The fact that the acquisition-impairing dose of IL-6 increased the nitrite content, whereas the non-amnesic dose of IL-6 had no effect on it, suggests a possible positive relation of high levels of NO to the IL-6 amnesia. Also, the ability of intrahippocampal infusion of SNP to impair the acquisition performance further supported the above mentioned suggestion. As for the mechanism by which IL-6 increased the NO formation, we have found in the *in vivo* microdialysis study that the nitrite increments are produced through an inducible effect of IL-6 on the NOS in the hippocampus (not published data). Collectively, the present results suggest that intrahippocampal injection of IL-6 impaired learning acquisition of active avoidance in rats and this IL-6 amnesia could be mediated by overproduction of NO in the hippocampus.

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海马内输注白细胞介素-6损害大鼠躲避反应学习

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关键词 白细胞介素-6；一氧化氮；躲避学习；海马；硝普盐

目的：研究海马内输注白细胞介素-6（IL-6）对大鼠主动躲避反应的影响和一氧化氮参与该过程的可能机制. **方法**：使用穿梭箱模型，在海马内输注IL-6和硝普钠(SNP)后 d 8 开始进行行为实验. 使用荧光测定方法，检测海马内输注 IL-6 后 24 h 时海马的亚硝酸盐水平. **结果**：IL-6 3.2 ng 对获得和保留均无影响. IL-6 16 ng 和 80 ng 显著延长了训练躲避潜伏期，从而损害了主动躲避反应的获得，但对其保留无影响. SNP 400 ng 和 IL-6 80 ng 通过减少躲避反应率也损害了主动躲避反应的获得，而对保留无影响. IL-6 80 ng 显著升高了海马亚硝酸盐水平，而 IL-6 3.2 ng 对之无影响. **结论**：IL-6 海马内输注损害大鼠主动躲避反应.