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Effect of desipramine on spontaneous activity of hippocampal CA1 neuron after transient cerebral ischemia in rats[1]

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KEY WORDS cerebral ischemia; desipramine; electrophysiology; hippocampus

AIM: To study the spontaneous firing of CA1 neurons in rat hippocampus after transient cerebral ischemia and the effect of desipramine (Des) on the post-ischemic electric activity of CA1 neurons. **METHODS**: Single-unit extracellular recordings were performed in rats on d 3 after 10 min of cerebral ischemia by occlusion of 4 arteries. Des and saline were injected into a tail vein. The histological changes of CA1 neurons was assessed by the neuronal density of the CA1 sector. **RESULTS**: The spontaneous firing rate of CA1 neurons on d 3 after ischemia was enhanced in comparison with the control value. Des (0.2 and 0.4 mg · kg^{-1}, iv, n = 5 & 6, respectively) reduced dose-dependently the increase of firing rate with maximal inhibition by 6 min (58 % & 85 %) to 9 min (69 % & 94 %) (*vs* vehicle group, $P<0.01$). About 50 % cells in CA1 region showed necrotic changes. **CONCLUSION**: Des antagonized the hyperexcitability of CA1 neurons after cerebral ischemia.

Transient cerebral ischemia causes delayed neuronal death (DND) in certain vulnerable region of the brain such as the pyramidal cells of the hippocampal CA1 subfield[1]. An excessive release of excitatory amino acid (EAA) activating on *N*-methyl-*D*-aspartate (NMDA) receptor and resulting disturbance of intracellular calcium homeostasis are thought to be critical factors in the pathogenesis of ischemic neuronal necrosis[2,3]. Desipramine (Des), a tricyclic antidepressant, interacted with the NMDA-receptor complex to block the action of NMDA[4], and protected mice against NMDA-induced lethality[5]. These findings raise the possibility of developing a novel class of NMDA-receptor antagonist or neuroprotective drug. The aim of this study focused to investigate the effect of Des on post-ischemic electric activity of CA1 neurons, so as to clarify the pharmacological feature of Des in antagonizing ischemic brain damage.

MATERIALS AND METHODS

Rats Sprague-Dawley rats (♀, n = 30) weighing 284 ± s 28 g were housed under a light-dark schedule (7 am — 7 pm) at 20 C with free access to food and water. Rats were divided into 2 groups: 10-min ischemia and sham-operated control. After the rats were anesthetized with ketamine (0.12 g · kg^{-1}, ip), both vertebral arteries were electrocauterized at the 1st cervical vertebrae and both common carotid arteries were exposed. On the following day, rats were anesthetized with ether and heparinized (100 IU · kg^{-1}, ip), 10-min cerebral ischemia was produced by bilateral carotid artery occlusion using small metal clips[6]. The EEG revealed a complete loss of activity within 100 s. Removal of the carotid clips permitted recirculation. Body temperature was kept at 37—38 C with a heating lamp.

Single-unit recording On the d 3 after cerebral ischemia, single-unit extracellular recordings were made under urethane (1.2 g · kg^{-1}, ip) anesthesia following insertion of a plastic cannula in a lateral tail vein for iv drugs and additional anesthetic. Using stereotaxic procedures a small burr hole was made in the skull and a glass electrode (tip diameter 2—3 μm; impedance 4—7 MΩ) was inserted into the CA1 region (bregma —3.8 to —4.0 mm, lateral 1.8—2.2 mm and depth 2.0—2.3 mm from the surface of the brain). The identification of CA1 pyramidal cells was ascertained by their electrophysiological characteristics[7]: 1) spontaneous firing pattern (single and complex, irregular); 2) duration of negative spike (0.2 — 0.4 ms); 3) rate of firing (mostly 0.5—5/s); and their localization (the electrode tip in the pyramidal cell layer). Microelectrode signals were amplified and monitored with a storage oscilloscope, or on line, analyzed with a personal computer for interspike interval histogram (ISH).

Des HCl (Sigma) 0.2 and 0.4 mg · kg^{-1} (in saline, pH = 6.0) was injected iv. The vehicle (0.9 % NaCl, 1 mL · kg^{-1}) was injected prior to Des for control. The unit activity was recorded until the rate appeared to be

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stable for 9-min as three 3-min ISH. Only one cell was recorded in each rat using Des ($n=11$).

Histological examination At the end of experiment, the terminal site of electrode was marked with the blue dye. Rats were perfused transcardially with 4 % formaldehyde in PBS 0.1 mol · L^{-1} (pH = 7.3). The brain sections (5 μm) were stained with cresyl violet and the neuronal density (ND) in CA1 region was examined with a light microscope.

Statistics Data were expressed as arithmetical $\bar{x}\pm s$. The changes of the unit activity and ND of CA1 neurons were assessed by the t test (two-tailed) and single-factor ANOVA. Statistical analyses were performed with Stat View 512 + Software (Brain Power Inc, CA) on a Macintosh SE computer.

RESULTS

Spontaneous activity of hippocampal CA1 neurons In 30 rats operated by sham or ischemia, the unit activities of 78 neurons of CA1 region were recorded. The firing rate of CA1 neurons in the control rats was 2.6±1.7 spikes · s^{-1} (n=42) which was consistent with previous report[7]. However, on d 3 after ischemia, the firing rate increased to 6.3±2.8 spikes · s^{-1} ($n=36$; $P<0.01$) which was characterized by an increment of complex firing pattern.

Effect of Des on CA1 spontaneous hyperactivity after ischemia In 11 post-ischemic rats, Des (0.2 and 0.4 mg · kg^{-1}) caused a decrease of CA1 spontaneous firing rate that was maximal during 6 to 9 min after injection (Fig 1).

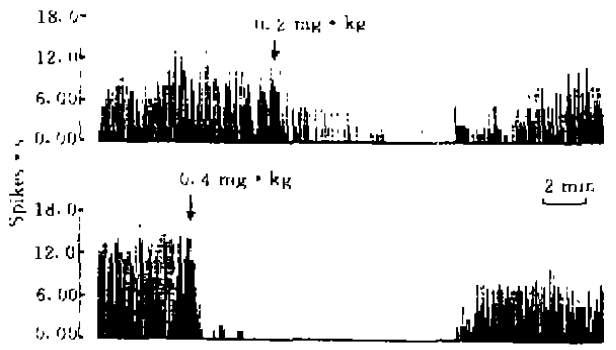

Fig 1. Effect of iv Des on spontaneous activity of hippocampal CA1 neurons in post-ischemic rats.

The inhibition of the unit activity was more remarkable by Des 0.4 mg · kg^{-1}, and recovered completely after 15—20 min. In contrary, vehicle failed to reduce CA1 firing rates to the significant degree (30 % of the predrug rate) in comparison with Des 0.2 mg · kg^{-1} (ANOVA, $P<0.01$) (Fig 2).

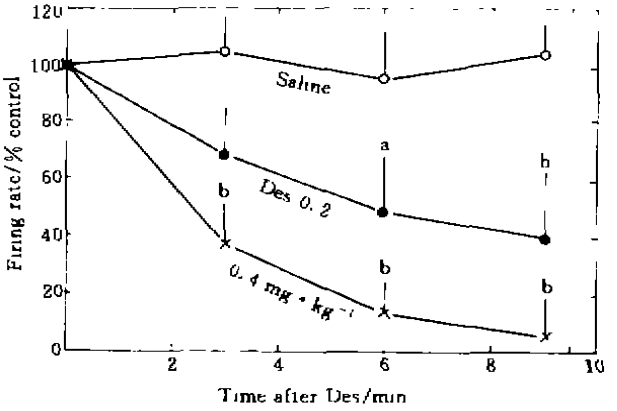

Fig 2. Dose-dependent effects of post-ischemically administered Des on the spontaneous firing rate of CA1 neurons. The rate (100 %) for saline (3 min before iv Des, $n=10$) and Des ($n=11$) groups were 5.4±2.4 and 5.8±2.6 spikes · s^{-1}, respectively.
$^aP<0.05$, $^bP<0.01$ *vs* control, $\bar{x}\pm s$.

Histology of hippocampal CA1 neurons after ischemia In sham-operated rats, CA1 neurons did not show marked abnormalities (ND; 157±21/mm, $n=7$). In d 3 rats after ischemia, about 50 % of stratum pyramidale (ND; 78±31/mm, $n=8$) in CA1 region was decreased significantly with condensed nuclei, shrunken cytoplasm, and pyknotic neurons.

DISCUSSION

The present study observed that the spontaneous firing rate of neurons in hippocampal CA1 was markedly increased on d 3 after 10 min of transient cerebral ischemia. This result was closely resemble to that of Chang[4], who described the increased spontaneous activity of CA1 neurons in a somewhat linear fashion from d 1 to d 3 after ischemia in the rat by two-vessel occlusion. In Mongolian gerbils, the hyperexcitability of hippocampal CA1 neurons was also observed consistently after 24 h of 5 min ischemia[3]. Thus, it is indicated that the hyperexcitability of CA1 neurons by transient ischemia keeps its post-effect during the reperfusion, which may be underlying the pathomechanisms of delayed neuronal death.

After transient ischemia a disturbed calcium homeostasis[5] and the impairment of protein syn-

thesis[10] in the vulnerable neurons are often involved during the chronic stage of reperfusion, and the time course of these delay disturbance is similar to that of the increased electric activity of CA1 neurons after ischemia. Some sodium/calcium channel blockers and EAA antagonists (such as dizocilpine maleate) decrease the excitability of CA1 neurons in parallel with their neuroprotective action[11]. These facts suggest that the increased excitabilities of CA1 neurons appear to participate in ischemic cell damages. Our histological examination also revealed the necrotic changes of CA1 neurons at d 3 after ischemia. So it is possible that the slowly developing pathologic process within the CA1 region that is triggered by events during the acute stage of ischemia could cause the increased firing rate of CA1 neurons and their eventual death.

The tricyclic antidepressant drug Des was effective to antagonize the increased electric activity of CA1 neurons after ischemia. This inhibitory result of Des may be deduced to endow the protective role on ischemic brain damage, since the increased excitability of CA1 neurons after ischemia was supposed to be linked to the delayed ischemia-induced neuronal death. On the other hand, by using single-channel recording it is also confirmed that Des, as a potent and selective NMDA receptor antagonist, is able to block the response of the membrane potential of hippocampal cell to NMDA[12]. The blockade of NMDA receptor effectively protected mice against NMDA-induced lethality[5]. However, some studies suggest that the inhibitory effect of Des may correlate with its well-known action of inhibition of norepinephrine (NE) uptake in neurons. Des inhibited the ischemia-induced NE release[13] and the activity of the locus coeruleus system[14], were available for decreasing the sensitivity of CA1 neuron to the excitatory transmitters or a depolarizing stimulus[15], which may be protective in the postischemic phase.

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REFERENCES

1 Kirino T, Tamura A, Sano K. Selective vulnerability of the hippocampus to ischemia-reversible and irreversible types of ischemic cell damage. In: Kogure K, Hossmann KA, Siesjo BK, Welsh FA editors. Molecular mechanisms of ischemic brain; vol 63. Progress in brain research. Amsterdam: Elsevier, 1985: 39-58.

2 Peruche B, Krieglstein J. Mechanisms of drug actions against neuronal damage caused by ischemia — an overview. Prog Neuro-Psychopharmacol Biol Psychiatry 1993; **17**: 21-76.

3 Sakamoto N, Kogure K, Kato H, Ohmoto H. Disturbed Ca^{2+} homeostasis in the gerbil hippocampus following brief transient ischemia. Brain Res 1986; **364**: 372-6.

4 Reynolds IJ, Miller RJ. Tricyclic antidepressants block N-methyl-D-aspartate receptors: similarities to the action of zinc. Br J Pharmacol 1988; **95**: 95-102.

5 Leander JD. Tricyclic antidepressants block N-methyl-D-aspartic acid-induced lethality in mice. Br J Pharmacol 1989; **96**: 256-8.

6 Pulsinelli WA, Brierley JB. A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 1979; **10**: 267-71.

7 Fox SE, Ranck Jr JB. Electrophysiological characteristics of hippocmpal complex-spike cells and theta cells. Exp Brain Res 1981; **41**: 399-410.

8 Chang HS, Sasaki T, Kassell NF. Hippocampal unit activity after transient cerebral ischemia in rats. Stroke 1989; **20**: 1051-8.

9 Suzuki R, Yamaguchi T, Choh-Luh Li, Klatzo I. The effects of 5-minute ischemia in Mongolian gerbils. II. Changes of spontaneous neuronal activity in cerebral cortex and CA1 sector of hippocampus. Acta Neuropathol (Berl) 1983; **60**: 217-22.

10 Bodsch W, Takahashi K, Barbier A, Grosse OB, Hossmann KA. Cerebral protein synthesis and ischemia. Prog Brain Res 1985; **63**: 197-210.

11 Wang TY. Excitability of neuron in acute cerebral hypoxia and ischemia. In: Chen WZ, Rui YC, editors. Cerebrovascular diseases, basis and clinic research. Shandong: Scientific Press, 1993: 1-11.

12 Sernagor F, Kuhn D, Vyklicky L, Mayer ML. Open channel block of NMDA receptor responses evoked by tricyclic antidepressants. Neuron 1989; **2**: 1221-7.

13 Gustafson I, Westerberg EJ, Wieloch T. Extracellular brain cortical levels of noradrenaline in ischemia: effects of desipramine and post-ischemic administration of idazoxan. Exp Brain Res 1991; **86**: 555-61.

14 Valentino RJ, Curtis AL, Parris DG, Wehby RG. Antidepressant actions on brain noradrenergic neurons. J Pharmacol Exp Ther 1990; **253**: 833-40.

15 Madison DV, Nicoll RA. Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. Nature 1982; **299**: 636-8.

地昔帕明对大鼠短暂脑缺血致海马 CA1 神经元自发放电的影响

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关键词 脑缺血; 地昔帕明; 电生理学; 海马

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大鼠

R743.3/R9

目的：观察短时脑缺血再灌后大鼠海马CA1神经元自发放电活动的改变以及地昔帕明(Des)对其放电频率的影响. **方法**：短暂性脑缺血(10 min, 4-VO法) 3 d后，细胞外记录CA1神经元单位放电的变化，尾静脉给药. 实验结束后对海马切片进行形态计量检查. **结果**：再灌d 3海马CA1细胞放电活动明显增加. Des (0.2 & 0.4 mg·kg^{-1}, iv)能显著减弱CA1区升高的放电频率，其最大抑制率分别在给药后6 min (58 % & 85 %)至9 min (69 % & 94 %)期间，与生理盐水对照值相比差异显著(ANOVA, $P<0.01$). 组织学显示该区约50 %锥体细胞呈缺血坏死. **结论**：Des能颉抗海马缺血后的高兴奋性活动.

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Effects of nitrendipine on capacity of calcium binding of erythrocyte membrane and total intraerythrocyte calcium content in SHR rats

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KEY WORDS nitrendipine; erythrocyte membrane; calcium; inbred SHR rats; inbred WKY rats

AIM: To study the effect of nitrendipine (Nit) on the capacity of calcium binding of erythrocyte membrane and total intraerythrocyte calcium content in spontaneous hypertensive rats (SHR). **METHODS**: Systolic blood pressure (SBP) of the consciouse rats was monitored by tailcuff method with a BP and HR recorder for MRS-Ⅱ rat. Erythrocyte membrane was prepared according to modified Bing's method. Calcium binding of membrane and total intraerythrocyte calcium content was determined by an automatic absorption spectrophotometer. The membrane protein was determined with a colorimetric methed. **RESULTS**: Nit (ig 10 mg·kg^{-1} *qd* ×20 d) induced a significant reduction in total intraerythrocyte calcium content (169±18 *vs* 87±14 μmol/L cell, $P<0.01$) accompanied by a marked fall of SBP (27.1±2.5 *vs* 16.7±1.0 kPa, $P<0.01$) but exerted no influence on the capacity of calcium binding of erythrocyte membrane under incubation in $CaCl_2$0 (basal calcium binding) or 40 mmol·L^{-1} (maximal calcium binding) (21.9±2.3 *vs* 22.7±2.1 and 55±14 *vs* 53±23 μmol/g protein, respectively, $P>0.05$). **CONCLUSION**: The antihypertensive effect of Nit is related to the reduction of intracellular calcium and possibly have no direct relation to the capacity of calcium binding of cell membrane.

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Abnormalities of calcium metabolism were found in essential hypertensive patients[1] and in SHR[2] with elevation of intracellular free calcium in erythrocyte and reduction of calcium binding to the erythrocyte[3-5]. Nitrendipine (Nit) is a calcium channel blocker and used in the treatment of hypertension[6]. The present study was designed to evaluate the effects of Nit on calcium binding of erythrocyte and total intraerythrocyte calcium content in SHR.

MATERIALS AND METHODS

Reagents Nit was purchased from Nanjing Pharmaceutical Co. Other reagents were of AR grade. All solutions were prepared with deionized distilled water. Containers were treated with 9.1 % nitric acid for 48 h, and washed with deionized water.

Rats SHR and Wistar-Kyoto (WKY) rats of 12-wk old, both sexes, were provided by the Animal Breeding Center of Fuwai Hospital.

The SHR rats were randomly divided into 2 groups. For the treated group, Nit 10 mg·kg^{-1} was given daily by gavage for 20 d. The control rats and WKY received only solvent of the same volume. All rats were maintained on the pellet food and tap water *ad lib*. Systolic blood pressure (SBP) of the conscious rats was monitored regularly