BIBLID: ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1992 Jul; 13 (4) : 315-318

Effect of dizocilpine maleate on cerebral anoxia and ischemic damage in rodents[1]

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ABSTRACT The Protective effects of dizocilpine maleate (DM) against anoxia in mice and ischemic damage in rats of 4-vessel occlusion (4-VO) were studied. DM 0.5 or 1.0 mg · kg^{-1} ip significantly prolonged the survival time of mice in closed containers. DM 0.5 and 1.0 mg · kg^{-1} ip 30 min prior to 4-VO obviously accelerated the electroencephalographic recovery, reduced the neuronal loss in the hippocampus, and increased the survival rate after 72-h reperfusion. These effects followed a dose-dependent manner. Our results indicate that selective non-competitive *N*-methyl-*D*-aspartate receptor blocker DM protects against anoxic and ischemic cerebral damage.

KEY WORDS dizocilpine maleate; anoxia; cerebral ischemia; hippocampus; electroencephalography

Dizocilpine [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine] maleate (DM) is a potent and selective *N*-methyl-*D*-aspartate (NMDA) type receptor blocker[1] with anticonvulsant and anxiolytic properties[2,3]. In addition, it is neuroprotective against ischemia, anoxic, and hypoglycemic attacks and the effects of endogenous neurotoxins[4]. It has been reported that DM has potent antagonistic effect against ischemic neuronal damage (IND) in several ischemic models of animals[5]. In the present study we observed the effects of DM on the anoxic model of mice and transient bilateral forebrain ischemia in the rat model of 4-vessel occlusion (4-VO). Also, we examined the dose-dependent effects of DM on EEG, behavior, and neuropathological alterations.

Received 1991 Aug 9 Accepted 1992 Apr 2
[1] Project supported by the National Natural Science Foundation of China (№ 0388028) and the Fund for Excellent Young University Teachers of the State Education Commission of China.

MATERIALS AND METHODS

Anoxia in mice Male INH mice, weighing 20 ± *s* 2 g, were used. 30 min after medication, each mouse was put in a closed glass vessel (125 ml) containing 25 g of calx natrica. The survival time was recorded.

Cerebral ischemia in rats Male Sprague-Dawley rats weighing 260 ± *s* 26 g were subjected to 4-VO[6]. Briefly, the rats were anesthetized with chloral hydrate (360 mg · kg^{-1}) and non-traumatic clasps were placed loosely around each common carotid artery without interrupting the blood flow. The vertebral arteries were occluded by electrocautery at the first cervical vertebra. Implantation of bilateral parietal cortex electrodes for recording was carried out according to our previous method[7]. The rats recovered from anesthesia in 24 h, during which they were fasted but allowed free access to water. Then the carotid artery clasps were tightened to produce occlusion in the conscious rats. Rats that did not become unresponsive within 30-60 s following clasp tightening and remained throughout occlusion were excluded. The carotid artery clasps were released 30 min later and the restoration of carotid artery blood flow was verified by direct visualization of the vessels. The ventral neck wound was closed with a suture. Rats that suffered from convulsion during the ischemic or postischemic period were excluded.

EEG recording EEG changes were recorded before, during, and after ischemia. The EEG became isoelectric within 2-3 min of 4-VO in those rats that became unresponsive. The rats without isoelectric EEG during ischemia were excluded.

Behavioral observation The ability of rats to move about and to climb before ischemia and after reperfusion were recorded. Behavioral changes after

medication were also observed.

Histopathological analysis The rats were decapitated at 72 h after cerebral reperfusion. Coronal sections (5 μm) from paraffin-embedded brain were cut at 3 different levels of the dorsal hippocampus. These coronal levels were chosen to span the complete longitudinal axis of the dorsal CA1 hippocampus. These sections were stained with HE and examined under light microscope. The observers were kept blind with the experimental conditions. Pyramidal cells in the CA1 zone of hippocampus were highly vulnerable to cerebral ischemia[7]. So, the hippocampal cell loss was used to assess the IND. Ischemic damage to neurons in the CA1 zone was graded on a scale of 0–3[8] with 0: normal hippocampus; 1: <10% of neurons damaged; 2: 10–50% of neurons damaged; 3: >50% of neurons damaged. Irreversible IND was accepted in any neuron showing a shrunk cell body with eosinophilic cytoplasm and a dark pyknotic nucleus, homogenizing cell change or naked nuclei.

Chemical reagents DM and pentobarbital were purchased from Merck E Co, USA and Serva Co, USA, respectively. Ketamine was made by Jintan Pharmaceutical Factory, Chungzhou, China. All drugs were dissolved in saline.

Statistics The Mann-Whitney *u*-test were used to compare different groups.

RESULTS

Protective effect of DM on anoxic mice DM 0.5 and 1.0 mg · kg^{-1} ip 30 min prior to the anoxia obviously protracted the survival time in the closed vessel. Ketamine 30 mg · kg^{-1} and pentobarbital 40 mg · kg^{-1} had the same effect (Tab 1).

Tab 1. Effects of dizocilpine maleate (DM) and ketamine on survival time of anoxic mice. $n=9$, $\bar{x}\pm s$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **saline.**

	Dose / mg · kg^{-1}	Survival time / min
Saline		18.0 ± 3.4
Pentobarbital	40	36.1 ± 8.9***
Ketamine	30	26.5 ± 7.8**
DM	0.5	24.7 ± 8.0**
	1.0	29.1 ± 5.6***

Behavioral observation The motor activity was attentively observed after DM and ketamine administration in mice and rats. The behavioral changes were severer in rats than those in mice. DM (0.1 mg · kg^{-1}, ip) produced circling. At doses of 0.5 and 1.0 mg · kg^{-1}, the circling was markedly accompanied by ataxia. The behavioral changes were dose-dependent.

In sham-operated group, rats ambulated and climbed without obvious difficulty. Rats subjected to 30 min of ischemia could tread 72 h later but were unable to climb normally. There were little difference between the control and drug-treated rats.

Effect of DM on body weight and survival in ischemic rats All experimental rats lost body weight. The mean difference between preoperative body weights and weights at 72 h following 30 min of 4-VO were 48 ± *s* 12 g ($n=22$). The body weight changes were not obviously different in each group. But DM ip 30 min prior to ischemia significantly increased the survival rate after 72 h reperfusion. This effect was dose-dependent (Tab 2). Ketamine 30 mg · kg^{-1} ip showed no protective effect.

Tab 2. Effects of dizocilpine maleate (DM) and ketamine on survivals of cerebral ischemic rats after 72 h reperfusion. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **saline.**

	Dose / mg · kg^{-1}	*n*	Survivals
Saline		14	5
Ketamine	30	8	4*
DM	0.1	10	6*
	0.5	11	9**
	1.0	9	9***

EEG In pre-ischemic period, DM ip produced prominent slowing and a correlated increase in the amplitude of EEG wave (Fig 1) which appeared to be dose-dependent over

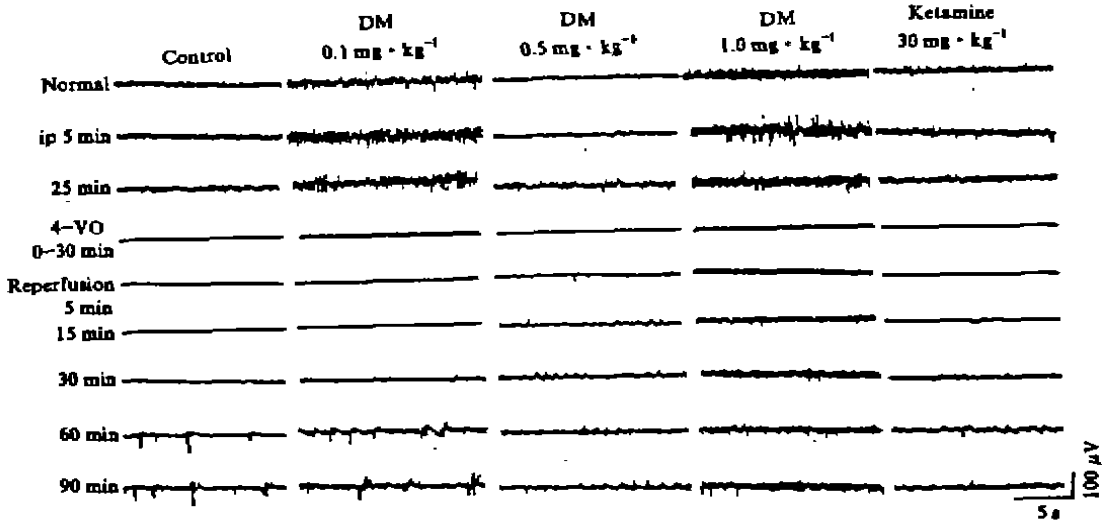

Fig 1. EEG in control, DM-, and ketamine-pretreated rats before, during and 90 min after a 30-min cerebral ischemia.

the range of 0.1-1.0 mg · kg^{-1}. These effects were definitely seen within 5 min after ip injection and reached the maximum in 10-20 min. Similar changes were seen in the ketamine group.

All rats showed prompt flattening of the EEG pattern with 4-VO. No electric activity was seen during the 30 min occlusion. Fig 1 showed the typical EEG patterns before, during the ischemia, and 90 min after reperfusion in the control and medicated rats. DM 0.5 and 1.0 mg · kg^{-1} ip 30 min prior to 4-VO obviously accelerated EEG recovery. But DM 0.1 mg · kg^{-1} and ketamine 30 mg · kg^{-1} exhibited no obvious effect.

Histopathological damage Neuronal damage was seen in CA1, CA3-4 and paramedian region of the hippocampus. The severities of histological damages to neurons in the left and right pyramidal zones of hippocampus were of similar degree. DM 0.1, 0.5, and 1.0 mg · kg^{-1} ip 30 min prior to 4-VO protected dose-dependently against the IND (Tab 3). Ketamine 30 mg · kg^{-1} did not produce protective effects.

DISCUSSION

In the present study, DM significantly prolonged the survival time of mice in anoxia and increased the survival rate of ischemic rats after 72 h reperfusion. DM accelerated EEG recovery after ischemia and reduced hippocampal neuronal loss. These effects were obviously dose-dependent. But another

Tab 3. Effects of dizocilpine maleate (DM) on ischemic neuronal damage of hippocampus CA1 in cerebral ischemic rats. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* saline.

	Dose / mg · kg^{-1}	n	Grade of neuronal damage
Sham-operation		7	0
Saline		7	2.7 ± 0.2
Ketamine	30	3	2.3 ± 0.6*
DM	0.1	3	2.3 ± 0.6*
	0.5	5	1.6 ± 0.7**
	1.0	5	1.0 ± 0.5***

dissociative anesthetic, ketamine, did not produce obvious protective effect in ischemic rats and only prolonged the survival time in anoxic mice. Previous studies indicated that DM can attenuate the IND in the global and focal ischemic model[9,10]. We combined EEG with neuropathological alterations to determine further the protective actions of DM in anoxic and ischemic models.

During anoxia and ischemia extracellular concentrations of excitatory amino acids (EAA) increased markedly in the brain[11]. Recently it has been proposed that overactivity of NMDA type EAA receptors could contribute to IND[12]. The potent protective effect of DM is possibly mediated by blocking NMDA receptors in the central nervous system[13]. The weak neuroprotective activity of ketamine is consistent with its low potency as NMDA receptor blocker[14].

We find that DM can induce locomotor in mice and rats. This effect is possibly mediated by increasing central dopaminergic neurotransmission[15].

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地佐西平对脑缺氧和脑缺血损害的作用

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提要 观察了地佐西平(DM)对小鼠脑缺氧和大鼠四血管结扎(4-VO)脑缺血模型引起的脑损害的作用. DM 0.5 和 1.0 mg · kg^{-1} ip 明显延长小鼠在密闭容器中的存活时间. 4-VO 前 30 min DM 0.5 和 1.0 mg · kg^{-1} ip 可明显促进脑电图的恢复, 减少海马神经元降解及提高复灌 72 h 后大鼠存活率. 结果表明, DM 可对抗脑缺氧和缺血引起的神经元损害.

关键词 地佐西平; 缺氧症; 脑缺血; 海马; 脑电描记术