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Neuroprotective effects of dextromethorphan against transient cerebral ischemia/reperfusion injury in gerbils[1]

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AIM: To study the neuroprotective effects of dextromethorphan against transient cerebral ischemia/reperfusion injury. **METHODS**: Transient cerebral ischemia/reperfusion injury in gerbils was produced by temporarily clamping the common carotid arteries (CCA) for 10 min. **RESULTS**: Intrahippocampal (ih) injection of 2.0 μL dextromethorphan (DM, 100 $\mu mol \cdot L^{-1}$) 5 min before ischemia quickened the recovery of EEG changes, the total power spectra of EEG, and the power of dominant frequency following reperfusion. The total power of EEG was increased to 92±30 ($P<0.01$) at 240 min following reperfusion. DM substantially reduced the severe ischemic neuronal damage (SIND) after 10 min of cerebral ischemia and 24 h of reperfusion. **CONCLUSION**: DM has neuroprotective effects against transient cerebral ischemia and reperfusion injury in gerbils.

KEY WORDS dextromethorphan; cerebral ischemia; electroencephalography; microinjections; hippocampus; microscopy

Considerable evidence showed that excitatory amino acids, in particular glutamate, play an important role in mediating ischemic neuronal damage[1,2]. Both *in vitro* and *in vivo* ischemia models demonstrate that antagonists of the *N*-methyl-*D*-aspartate (NMDA) subclass of glutamate receptor have neuroprotective properties[3,4].

The antitussive dextromethorphan (DM) is an opioid compound with few narcotic properties. Recently, DM has been shown to be a noncompetitive NMDA antagonist[5] that attenuates glutamate toxicity, NMDA-induced damage and hypoxic injury in neuronal culture and prevents ischemic neuronal damage in animal models of focal cerebral ischemia when administered before or after ischemia[6,7]. Oral DM in patients at risk for brain ischemia showed no clinical toxicity[8].

This study was to explore the neuroprotective effects of DM against transient cerebral ischemia in gerbils with electrophysiological and histological technics.

MATERIALS AND METHODS

Intrahippocampal injection Mongolian gerbils (♂, $n=20$, weighing 52±5 g, from the Dept of Experimental Animal, Shanghai Institute of Biological Products) were anesthetized with ether. The gerbil was mounted on a stereotaxic apparatus. After opening a small area of the skull on the left side, the dura was incised. A hole was drilled through the skull 2 mm posterior to the bregma and 2 mm left to the mid line for intrahippocampal injection of DM. A guide cannula (0.4 mm, ID) was inserted through the hole into the hippocampus (2.2 mm below dura), and sealed with a dummy injection cannula until the time of medication. After 2 d, DM (2.0 μL, 100 $\mu mol \cdot L^{-1}$) was injected into the CA1 sector in the left hippocampus through a delivery cannula inside the guide cannula 5 min before ischemia.

Preparation Two days after the above operation, the gerbil was anesthetized with ether again. Both common carotid arteries (CCA) were exposed through a midline cervical incision and were looped by silk sutures. Body temperature was maintained at

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37 — 38 C with a heating blanket. Cerebral ischemia was produced by clamping the CCA with small aneurysmal clips for 10 min. Reperfusion was started by removing the clips.

Gerbils were randomized into sham-operation, control (saline), and DM treatment groups. Saline (2.0 μL) or dextromethorphan (DM, 2.0 μL, 100 μmol · L^{-1}) was slowly injected into hippocampus 5 min before ischemia. In sham-operation groups, gerbils were subjected to the same procedures except for the occlusion of CCA.

EEG recordings and analyses After surgery, the gerbil head was immobilized in a stereotactic frame, and the skull was exposed. In the center of the right side of parietal bone, a 0.5 mm diameter silver electrode connecting to a SJ-42B multichannel physiologic recording system was inserted down to the external surface of dura mater to record the EEG. The reference electrode was placed to the midline of the occiput. EEG was recorded for 2 min before and 0, 15, 30, 60, 120, 240 min after ischemia , and stored on a magnetic tape (RMG-5304, Nihon Kohden). The EEG power spectra were analyzed by an IBM-PC equipped with an analog/digital converter. Linear spectra of consecutive EEG data sections (at 4-s periods, 200 Hz sampling rate) were computed using a fast Fourier transformation system. Integrated EEG power was calculated for selected frequency bands: δ 1.0 — 3.5 Hz, θ 3.5 — 7.5 Hz, α 7.5 — 13 Hz, and β 13 — 30 Hz.

Statistical analyses Data were analyzed by t test.

Histology The gerbil was deeply anesthetized with an overdose of sodium pentobarbital (50 mg · kg^{-1}, ip.) 24 h after reperfusion. Transcardial perfusion was performed with 50 mL of normal saline (NS) followed by 100 mL of 4 % paraformaldehyde in phosphate buffer (PB, pH 7.4, 0.1 mol · L^{-1}). The perfused brains were postfixed in the same fixative at 4 C for 24 h. The brain was then sectioned serially in coronal plane, embedded in paraffin. Sections were made at 8 μm thickness and stained with hematoxylin and eosin (HE). Sections were chosen for examination under a light microscope.

RESULTS

EEG changes During cerebral ischemia, the amplitude of EEG was severely inhibited, even became flattened and the total power spectra of EEG were decreased. After reperfusion, the recovery of EEG was very slow. The ih injection of 2.0 μL DM (100 μmol · L^{-1}) 5 min before ischemia remarkably quickened the recovery of EEG changes, the total power, and the power of δ ± θ dominant frequency of EEG (Fig 1, and Tab 1).

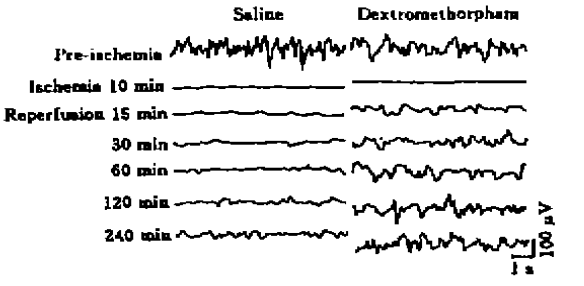

Fig 1. Effect of dextromethorphan (injected into hippocampus 5 min before ischemia) on EEG at cerebral ischemia and reperfusion in gerbils.

There were no significant EEG changes in sham-operation groups.

Pathological changes of neurones Twenty-four hours after 10 min cerebral ischemia, severe ischemic neuronal damage (SIND) was confined mainly to CA1 sector in the hippocampus. Ischemic neuronal changes such as edema, degenerative changes and necrosis (manifested as vacuolation, shrunken and dark triangular nucleus, and decrease in nuclear size and increase in basophilia of nucleus) were seen. Neurones in CA3, CA4, and dentate gyrus were almost intact. The ih injection of 2.0 μL DM (100 μmol · L^{-1}) substantially attenuated the SIND. In sham-operation groups, the morphology of neurones in hippocampus was normal (Fig 2, Plate 2).

Tab 1. Effects of dextromethorphan (DM. 2.0 μL. 100 μmol·L^{-1}. injected ih 5 min before ischemia) on EEG in cerebral ischemia/reperfusion gerbils. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* ischemia+NS group. $^{d}P>0.05$, $^{e}P<0.05$, $^{f}P<0.01$ *vs* pre-ischemia.

	Sham+NS ($n=4$)	Sham+DM ($n=4$)	NS ($n=6$)	DM ($n=6$)
		Total power of electroencephalogram		
Pre-ischemia	172±59	167±53	167±65	158±56^{a}
Ischemia 10 min	172±55	168±47	7±6^{f}	10±4^{fa}
Reperfusion 15 min	164±59	171±56	13±7^{f}	31±6^{fc}
30 min	178±68	176±46	21±10^{f}	59±11^{fc}
60 min	179±52	165±51	38±15^{f}	69±15^{fc}
120 min	163±58	167±52	43±15^{f}	81±26^{eb}
240 min	158±61	156±55	48±13^{f}	92±30^{ec}
		Power of δ+θ dominant frequency		
Pre-ischemia	83±32	87±35	77±40	79±36^{a}
Ischemia 10 min	83±29	86±29	0±0^{f}	1±2^{fa}
Reperfusion 15 min	86±33	96±34	4±3^{f}	13±7^{fb}
30 min	90±33	80±27	9±6^{f}	25±9^{fb}
60 min	80±21	80±28	13±7^{e}	29±12^{eb}
120 min	81±28	87±27	16±7^{e}	34±16^{eb}
240 min	83±25	88±33	24±8^{e}	43±18^{eb}

DISCUSSION

Mongolian gerbil is extensively used as a model for cerebral ischemia because of its unusual cerebral circulation which lacks inter communications between the carotid and vertebrobasilar circulations. Bilateral carotid occlusion causes complete forebrain ischemia, and transient carotid occlusion for 10 min induces delayed neuronal degeneration in the hippocampal CA1 sector of this animal[9,10]. According to previous reports, the pyramidal neurons in the CA1 sector receive rich glutamatergic innervations, namely the Schaffer collaterals from the CA3 sector, the commissural fibers from the contralateral hippocampus, and the perforant path fibers from the entorhinal cortex[11]. Thus, glutamate appears to be abundantly released in this region and to exert a toxic effect on the CA1 neurons.

The EEG change is a simple, direct, and immediate index for evaluating the brain conditions in cerebral ischemia. In cerebral ischemia the first change is a decrease of EEG activity, and the power spectrum of EEG, especially the power of dominant frequency in EEG, is well correlated with the degree of cerebral damage[12].

DM, a lipophilic compound, can easily pass through the blood-brain barrier and can be administered systemically. It is not inconceivable that the effects of DM when administered systemically are due simply to its metabolism to dextrorphan after *O*-demethylation in the liver. The dextrorphan also possesses neuroprotective property[13]. In our experiments, the EEG changes and the histologic findings have shown direct and strong evidences that focal administration of DM has an independent neuroprotective effects on transient cerebral ischemia.

It is attractive to postulate that the neuroprotective effects of DM are produced by the

antagonism of NMDA-receptor-mediated increase in calcium permeability, thereby preventing delayed neuronal necrosis[14]. However, other mechanisms unrelated to the NMDA receptors cannot be excluded. DM may improve postischemic hypoperfusion by favorably altering the regional cerebral blood flow (rCBF) or by decreasing the neuronal metabolic requirements[15].

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右美沙芬对沙鼠短暂脑缺血再灌注损伤的神经保护作用

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目的： 研究右美沙芬对短暂脑缺血再灌注损伤的神经保护作用. **方法**： 暂时夹闭沙鼠双侧颈总动脉造成短暂性脑缺血，10 min 后重新开放双侧颈总动脉使再灌注. **结果**： 缺血前 5 min 海马内微量注射右美沙芬(2.0 μL，100 μmol·L^{-1})能显著改善脑缺血10 min 再灌注后的脑电活动，脑电总功率及主频功率的恢复. 再灌注 240 min 后脑电总功率可恢复到 92±30. 右美沙芬还能明显减轻缺血10 min 后再灌注24小时的神经元缺血性损伤. **结论**： 右美沙芬对脑缺血引起的神经元损伤有神经保护作用，并能促进脑功能的恢复.

关键词 右美沙芬；脑缺血；脑电描记术；微量注射；海马；显微镜检查