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# Neuroprotective effects of phencyclidine on acute cerebral ischemia and reperfusion injury of rabbits[1]

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**ABSTRACT** Acute cerebral ischemia and reperfusion injury of rabbits was produced by permanently occluding the vertebral arteries and temporarily clamping the common carotid arteries for 30 min. Phencyclidine [1-(phenylcyclohexyl)piperidine, PCP] 40-80 $\mu g \cdot kg^{-1}$ icv 30 min before ischemia significantly attenuated the decrease of the total power of electroencephalogram (EEG) within 30 min of ischemia and improved the recovery of brain electric activity following reperfusion. PCP 20-80 $\mu g \cdot kg^{-1}$ dose-dependently suppressed the creatine kinase (CK) release during cerebral ischemia and reperfusion, and PCP 40-80 $\mu g \cdot kg^{-1}$ reduced brain ischemic damage. These improvements indicated that PCP has protective effects on acute cerebral ischemia and reperfusion injury.

**KEY WORDS** phencyclidine; cerebral ischemia; electroencephalography; creatine kinase; brain injuries

The "excitotoxin theory" suggested that the neurodegeneration resulted from periods of anoxia or ischemia was caused in part by an excess release of glutamate leading to over-activity at synapses utilizing excitatory amino acid transmitters[1]. Selective antagonists of the *N*-methyl-*D*-aspartate (NMDA) subclass of excitatory amino acid receptors attenuated glutamate induced neurotoxicity, ischemic neuron damage or hypoxic brain injury[2]. Of particular interest are the non-competitive NMDA antagonists such as ketamine, dextrorphan, dextromethorphan, and dizocilpine maleate (MK-801)[3-5]. These lipophilic compounds readily penetrate the central nervous system and can be administered systemically. PCP is also a non-competitive NMDA antagonist via blocking the NMDA receptor-channel complex by binding to the PCP site[6] and has been shown to reduce the infarct size after focal cerebral ischemia in rat[7]. The purpose of this study was to explore the effects of PCP on global cerebral ischemia in rabbit with multidisciplinary techniques.

## MATERIALS AND METHODS

Forty New Zealand white rabbits of either sex weighing 2.8 ± *s* 0.3 kg were used. Cerebral ischemia was produced by ligating bilateral common carotid arteries and bilateral vertebral arteries according to the model of bilateral hemispheric ischemia[8] and we made some modifications. Briefly, rabbits were anesthetized by iv 14% urethan and 0.7% α-chloralose 5 $mg \cdot kg^{-1}$ and both common carotid arteries and vertebral arteries were isolated via a ventral, midline cervical incision. Bilateral vertebral arteries were tied by silk thread and bilateral common carotid arteries were ligated by arterial clamps. Reperfusion following the 30-min ischemia was made by removing carotid clamps. Body temperature was measured with a rectal probe and kept between 36.5 and 37.5℃ with a warming pad. Mean arterial blood pressure (MABP) and

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heart rate (HR) were monitored continuously.

The rabbit head was immobilized in a stereotactic frame, and the skull was exposed. In the center of the right side of parietal, a 0.5 mm diameter silver electrode connecting to a SJ-42B multichannel physiologic recording system was inserted till dura mater of brain to record the EEG. The reference electrode was placed in midline of the occiput. Power spectra of EEG was analyzed with 7T08 Signal Processor.

Cerebrospinal fluid (CSF) samples (25 $\mu$l each) were slowly collected from the 4th ventricle before ischemia and 2 h after reperfusion. CK and lactate dehydrogenase (LDH) activity in CSF were determined colorimetrically by CK and LDH measurement kits, respectively.

Four hours after reperfusion, the rabbits were deeply anesthetized with 25 mg · $kg^{-1}$ sodium pentobarbital. Perfusion was performed through a lateral common carotid artery using 200 ml normal saline (NS) followed by 300 ml Bouin's solution. The rabbits were decapitated immediately after perfusion. Brains were stored in Bouin's solution for at least 7 d. The brains were then sectioned in coronal plane, embedded in paraffin, sectioned at 6 $\mu$m thickness, and stained with hematoxylin and eosin (HE). Sections were chosen for examination under a light microscope.

Rabbits were randomized into sham-operation, control (NS) or PCP treatment groups. PCP 20, 40, or 80 $\mu g \cdot kg^{-1}$ was slowly injected into a lateral cerebral ventricle (icv) 30 min before ischemia. In the control group, NS was injected icv in the same volume (10 $\mu l \cdot kg^{-1}$) as PCP. In sham-operation group, rabbits were subjected to the same procedure except for the 4-vessel occlusion.

Two-tailed $t$ tests and paired $t$ tests were used in the statistical analyses.

## RESULTS

**MABP and HR changes** In all ischemic groups, MABP immediately increased following artery occlusion and then rebounded to the control values after reperfusion began. In the groups treated with PCP 40 and 80 $\mu g \cdot kg^{-1}$, HR showed a tendency to decrease during cerebral ischemia and reperfusion (Tab 1). There was no significant difference in MABP or HR between the PCP and the NS groups.

**EEG changes** During cerebral ischemia, the amplitude of EEG were severely inhibited, even became flattened and the total power spectra of EEG was decreased. These changes in PCP treated groups were significantly different from those in NS control group. As shown in Fig 1 and Tab 1, icv PCP 40 or 80 $\mu g \cdot kg^{-1}$ before ischemia attenuated the decrease of the total power within 30 min of ischemia and improved the recovery following reperfusion. They also improved the recovery of EEG amplitude. The effect of PCP 80 $\mu g \cdot kg^{-1}$ was more potent than that of PCP 40 $\mu g \cdot kg^{-1}$, yet PCP 20 $\mu g \cdot kg^{-1}$ showed no influence.

**CK and LDH in CSF** After 30 min of cerebral ischemia and 2 h of reperfusion, in

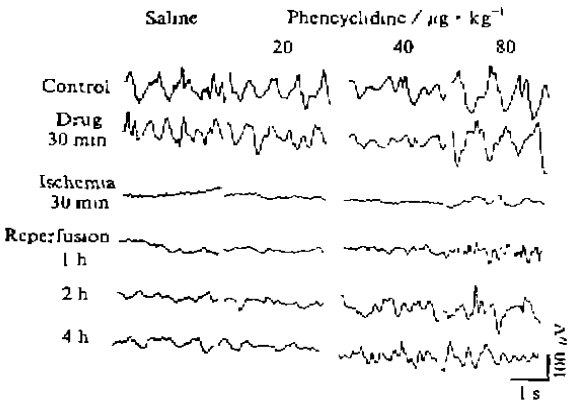


**Fig 1. Effect of phencyclidine on electroencephalogram at cerebral ischemia and reperfusion in rabbits.**

**Tab 1. Effects of phencyclidine on mean arterial blood pressure (MABP), heart rate (HR), and the total power spectra of EEG (relative unit, the total power in 30 min after anesthesia expressed with 1) in cerebral ischemia and reperfusion of rabbits. Sham 1: sham-operation + NS. Sham 2: sham-operation + PCP 40 $\mu g \cdot kg^{-1}$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* saline group.**

| | Sham 1 | Sham 2 | NS | Phencyclidine / $\mu g \cdot kg^{-1}$ 20 | 40 | 80 |
|---|---|---|---|---|---|---|
| Rabbits | 4 | 4 | 7 | 4 | 7 | 5 |
| 1. Mean arterial blood pressure (MABP kPa) | | | | | | |
| Control | 14.5 ± 2.1 | 15.2 ± 1.3 | 14.8 ± 1.1 | 13.9 ± 1.6* | 14.9 ± 1.0* | 14.7 ± 1.5* |
| Drug 30 min | 13.5 ± 1.6 | 14.2 ± 2.5 | 14.2 ± 1.4 | 13.8 ± 2.0* | 15.2 ± 1.8* | 15.0 ± 1.5* |
| Ischemia 30 min | 12.9 ± 1.9 | 13.7 ± 1.8 | 17.8 ± 1.8 | 19 ± 3* | 18.0 ± 1.2* | 17.6 ± 1.4* |
| Reperfusion 1 h | 12.6 ± 2.2 | 13.5 ± 0.7 | 13.3 ± 1.4 | 13 ± 3* | 13.7 ± 1.3* | 14.0 ± 0.3* |
| 2 h | 12.8 ± 1.2 | 13.7 ± 1.2 | 13.5 ± 2.6 | 13.4 ± 2.8* | 13.7 ± 1.3* | 14.1 ± 0.3* |
| 4 h | 13.0 ± 1.1 | 13.0 ± 1.4 | 13.3 ± 1.6 | 13 ± 4* | 13.9 ± 1.6* | 13.4 ± 1.0* |
| 2. Heart rate (HR / bpm) | | | | | | |
| Control | 300 ± 0 | 290 ± 17 | 311 ± 22 | 295 ± 30* | 298 ± 28* | 305 ± 35* |
| Drug 30 min | 300 ± 42 | 300 ± 0 | 296 ± 32 | 285 ± 31* | 291 ± 37* | 305 ± 48* |
| Ischemia 30 min | 307 ± 29 | 300 ± 0 | 296 ± 21 | 287 ± 34* | 279 ± 32* | 288 ± 24* |
| Reperfusion 1 h | 307 ± 29 | 310 ± 17 | 296 ± 31 | 300 ± 15* | 273 ± 40* | 285 ± 41* |
| 2 h | 300 ± 24 | 310 ± 17 | 299 ± 18 | 295 ± 12* | 279 ± 40* | 280 ± 31* |
| 4 h | 300 ± 17 | 302 ± 21 | 284 ± 15 | 300 ± 0* | 268 ± 54* | 270 ± 54* |
| 3. Total power spectra of EEG | | | | | | |
| Drug 30 min | 1.1 ± 0.4 | 0.9 ± 0.4 | 1.5 ± 0.9 | 1.3 ± 0.6* | 1.3 ± 0.6* | 1.5 ± 0.8* |
| Ischemia 30 min | 1.07 ± 0.22 | 1.1 ± 0.5 | 0.03 ± 0.03 | 0.008 ± 0.007* | 0.09 ± 0.11* | 0.17 ± 0.15** |
| Reperfusion 1 h | 1.0 ± 0.3 | 0.99 ± 0.22 | 0.12 ± 0.10 | 0.18 ± 0.06* | 0.53 ± 0.26*** | 0.41 ± 0.20** |
| 2 h | 1.0 ± 0.4 | 1.08 ± 0.20 | 0.27 ± 0.22 | 0.3 ± 0.4* | 0.54 ± 0.27* | 0.61 ± 0.27** |
| 4 h | – | – | 0.14 ± 0.14 | 0.14 ± 0.14* | 0.5 ± 0.4** | 0.6 ± 0.5** |

NS control group, the CK activity in rabbit CSF evidently increased (Tab 2). PCP suppressed dose-dependently the rise of CK activity. In rabbit CSF during cerebral ischemia and reperfusion the LDH activity tended to show an elevation, which disappeared in PCP 40 $\mu g \cdot kg^{-1}$ treatment group.

**Pathological changes of neurones** Severe brain damage was confined mainly to the cerebral cortex after 30 min of cerebral ischemia and 4 h of reperfusion. Edema, degenerative changes, and necrosis (manifested as vacuola-

**Tab 2. Effects of phencyclidine on creatine kinase activity (lg IU · $L^{-1}$) in rabbit cerebrospinal fluid during cerebral ischemia and reperfusion. Sham 1: sham-operation + NS, Sham 2: sham-operation + PCP 40 $\mu g \cdot kg^{-1}$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* pre-ischemia.**

| | Sham 1 | Sham 2 | Saline | Phencyclidine / $\mu g \cdot kg^{-1}$ 20 | 40 | 80 |
|---|---|---|---|---|---|---|
| Rabbits | 4 | 4 | 8 | 6 | 6 | 4 |
| Pre-ischemia | 1.9 ± 0.5 | 1.6 ± 0.6 | 1.3 ± 0.4 | 1.5 ± 0.7 | 1.6 ± 0.6 | 1.6 ± 0.4 |
| 2 h reperfusion after ischemia | 1.7 ± 0.6* | 1.3 ± 0.4* | 2.3 ± 0.8*** | 1.9 ± 0.7* | 1.7 ± 0.6* | 1.6 ± 0.6* |

tion, shrinkage, and triangulation of the nucleus and cytoplasm, and decrease in nuclear size and basophilia of nucleus) were seen. PCP 40 or 80 $\mu g \cdot kg^{-1}$ reduced the brain damage. The effect of PCP 80 $\mu g \cdot kg^{-1}$ was more potent than that of PCP 40 $\mu g \cdot kg^{-1}$. PCP 20 $\mu g \cdot kg^{-1}$ did not affect the pathologic changes during cerebral ischemia and reperfusion (Fig 2, Plate 1).

## DISCUSSION

It is generally considered that the experimental model, acute cerebral ischemia and reperfusion by occluding the 4 blood vessels, is reproducible, practical, and with a high incidence of brain ischemic neuronal damage[8]. As the ischemic brain damage induced by 30 min ischemia was reversible and 60 min ischemia was considered only suitable for pathologic study and not for evaluation of preventive agents[9], we chose ischemia for 30 min to observe PCP effects on cerebral ischemia and reperfusion injury. We used rabbits because their cerebral vasculature resembles that of humans[10].

EEG is a simple, direct, and immediate index to evaluate the brain condition in global cerebral ischemia. It has been reported that in global cerebral ischemia the first change is the decrease of EEG activity and the power spectrum of EEG is correlated with the degree of cerebral damage[11,12]. Since the experimental results showed that PCP markedly reduced the inhibition of cerebral electric activity and improved the recovery, it seemed that PCP protected cerebral function against damage during ischemia and reperfusion.

The elevation of CK and LDH activity in CSF indicated that CK and LDH release increased which expressed cell membrane permeability increased and the ischemic brain cell damage did exist[13]. PCP can suppress the rise of the activity of CK and LDH suggesting that PCP had neuroprotective effect. In the control group, CK activity significantly rose during cerebral ischemia and reperfusion, but LDH activity had only a tendency to increase and the levels of LDH was lower than those of CK in CSF. These results suggested that a rise of CK activity in CSF had a close relation to brain ischemic injury.

In our experiments, there were obvious morphologic changes of neurones in the cerebral cortex which indicated that there was severe damage after 30 min of cerebral ischemia and 4 h of reperfusion. The histologic findings showed that PCP reduced the neuronal edema and necrosis, a direct and strong evidence that PCP had neuroprotective effect in brain ischemic injury.

During cerebral ischemia and reperfusion, severe brain damage was chiefly confined to the cerebral cortex, especially in layers 3, 4 or 5. These regions are related to a higher density of NMDA receptors[14]. It has been reported that NMDA receptors play an important role in mediating the cortical ischemic neuronal damage[2,15]. Our results suggested that PCP is a neuroprotective agent against brain ischemic damage which may be mediated through NMDA receptors.

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## 苯环利定对兔急性脑缺血再灌注损伤的神经保护作用

R966.1

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**提要** 结扎兔双侧椎动脉和颈总动脉造成急性脑缺血. 30 min 后重新开放双侧颈总动脉使再灌注. 缺血前 30 min, icv 苯环利定 40-80 $\mu g \cdot kg^{-1}$ 能减轻缺血后脑电活动的抑制. 苯环利定还能抑制缺血后脑脊液中肌酸激酶活性的增加; 减轻神经细胞缺血性损伤. 实验结果表明苯环利定对脑缺血引起的神经损伤有保护作用, 并能促进脑功能的恢复.

**关键词** 苯环利定; 脑缺血; 脑电图; 肌酸激酶类; 脑损伤