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电刺激大鼠外侧网状核抑制福尔马林诱发的脊髓背角神经元反应

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摘要 观察了刺激外侧网状核(LRN)对皮下注射5%福尔马林诱发的背角神经元反应的作用. 在记录的19个神经元中，福尔马林诱发12个神经元产生双时相反应. 第一时相反应是在注射后立即产生，持续3—8 min，第二时相是在注射后20—35 min逐渐产生，持续30—60 min 刺激外侧网状核对两个时相反应均有抑制作用.

关键词 福尔马林；伤害性感受器；脊髓；网状结构；神经抑制

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Protective effects of 3, 6-dimethylaminodibenzopyriodonium edetate on global ischemia reperfused isolated rat hearts

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ABSTRACT The effects of 3,6-dimethylamino-dibenzopyriodonium edetate (IHC-72) on global ischemia reperfused rat hearts were investigated. In the isolated working rat heart, 40-min global ischemia followed by 30-min reperfusion resulted in increases of ventricular tachycardia (VT) and ventricular fibrillation (VF), increases of creatine kinase (CK) release and malondialdehyde (MDA) contents, but decreased superoxide dismutase (SOD) activity. Following ischemia and reperfusion, the accumulation of myocardial calcium increased. IHC-72 50 $\mu mol \cdot L^{-1}$ given 10 min before ischemia and during reperfusion decreased the cardiac CK release, VT, and VF, reduced the MDA contents, prevented the reduction of SOD activity and attenuated the accumulation of myocardial calcium and sodium *vs* control. These results indicated that IHC-72 protected myocardial reperfused injury.

KEY WORDS 3, 6-dimethylamino-dibenzopyriodonium; myocardial reperfusion injury; arrhythmia; superoxide dismutase; malondialdehyde; calcium

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It has been known that ischemia reperfused myocardial injury is related to the production of oxygen free radicals. The lipid peroxidation of oxygen free radicals leads to cell membrane damage, then the disturbances of intracellular calcium, sodium, and potassium[1]. Ischemia itself also leads to disorder of active and passive ionic homeostasis, and reperfusion may allow the calcium and sodium ions to flow along their concentration gradients into the myocardial cells[2]. There are evidences that the oxygen free radical scavengers or some calcium antagonists can protect ischemic reperfusion hearts from injury[3,4]. 3, 6-Dimethylamino-dibenzopyriodonium edetate (IHC-72) possesses anti-arrhythmic action in experimental models[5]. Our previous study showed that it inhibited the transmembrane movement of calcium and sodium in myocardial cells[6,7]. The present study is to

investigate whether IHC-72 can prevent lipid peroxidation and postischemia-reperfusion myocardial damage in isolated rat hearts.

MATERIALS AND METHODS

Drugs IHC-72, synthesized and given by Prof CHEN Shu — Ying (Department of Chemistry, Lanzhou University), was dissolved in distilled water. Verapamil (Ver, Beijing Pharmaceutic Factory, Beijing, lot № 850623).

Experimental procedures Sprague-Dawley rats, ♂, $n=64$, weighing 228± s 38 g, were lightly anesthetized with ip sodium pentobarbital 35 mg · kg^{-1}, and then heparin (200 IU · kg^{-1}) iv. Hearts were excised quickly and plunged into cold (4℃) solution until contraction ceased. To remove as much blood as possible, the hearts were flushed several times with saline solution via aorta. Then the hearts were immediately placed on Lagendorff equipment. Modified Krebs-Henseleit (K-H) solution[8] aerated with 95% O_2 + 5% CO_2, maintained at 37℃, pH 7.4, was used for perfusion. A constant perfusion pressure (7.8 kPa) was maintained during the experiment. An epicardial ECG was kept on throughout each experiment. This was obtained via 2 silver electrodes, one attached to the myocardium of right ventricular free wall, the other to the root of aorta.

Measurement of coronary flow (CF), creatine kinase (CK), superoxide dismutase (SOD), and malondialdehyde (MDA) After being stabilized for 15 min, the perfused rat hearts were divided into 4 groups, 8 in each; A) Control group, superfusing for 70 min with K-H solution; B) Ischemia/reperfusion group, 40-min global ischemia followed by 30-min reperfusion; C) IHC-72 (50 μmol · L^{-1}) group, and D) Ver (2 μmol · L^{-1}) group, the perfusion of the drugs was commenced 10 min prior to the global ischemia and continued during the experiment, other conditions being the same as in B. CF was measured by collecting the coronary effluent at 10 min before global ischemia and 5, 10, 20, and 30 min during reperfusion; myocardial leakage of CK in coronary effluent was assayed in a Monarch-716 biochemical autoanalyzer (USA) and calculated to IU · min^{-1}/g dry wt[9]. At the end of the experiment, ventricles were minced and homogenized with saline for the measurement of SOD activity[10] and MDA contents[11].

Measurement of calcium, sodium, potassium, and magnesium of myocardium Thirty-two rats were divided into 4 groups; A) Control, perfusing for 60 min with K-H solution; B) Ischemia/reperfusion, 40 min global ischemia followed by 20 min reperfusion; C) IHC-72, before global ischemia and during reperfusion hearts were perfused with K-H solution containing IHC-72 50 μmol · L^{-1}; D) Ver, perfused with K-H solution containing Ver 2 μmol · L^{-1}. Towards the end of the experiment, the hearts were flushed through with 10 ml of ice-cold sucrose 0.3 mol · L^{-1} and histidine 5 mmol · L^{-1}, pH 7.4. This procedure was to minimize the contribution of extracellular Ca^{2+} and Na^{+}. The atria were discarded and ventricles blotted, weighed, and then dried to constant weight at 100℃. The dried ventricles were digested in 3 ml of HNO_3 10.9 mol · L^{-1} for 48 h. Ca^{2+}, Na^{+}, K^{+}, and Mg^{2+} were determined by an atomic absorption spectrophotometer (Hitachi 180 — 80, Japan). The wavelengths used were 422.7, 589.6, 776.9, and 258.2 nm, respectively.

Statistical analysis The results were expressed as $\bar{x}\pm s$. The chi-square test was used to estimate the significance of the difference between the incidence of arrhythmia in the control group and that in the medicated group. Other comparisons were made using t test.

RESULTS

Effects on ischemia- and reperfusion-induced arrhythmias During ischemia there were no significant differences as compared with the control in the incidences of ventricular tachycardia (VT) and ventricular fibrillation (VF), while during reprfusion the incidences of VT and VF were increased by 100% and 87.5%, respectively. After perfusing with K-H solution containing IHC-72 50 μmol · L^{-1} or Ver 2 μmol · L^{-1}, the incidences of VT and VF were decreased; the durations of VT and VF were shortened (Tab 1).

Tab 1. Effects of IHC-72 and verapamil on incidence and duration of arrhythmia induced by ischemia/reperfusion in isolated rat hearts. VT = ventricular tachyardia, VF = ventricular fibrillation; $n=8$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{***}P<0.01$ *vs* control; $^{++}P<0.05$, $^{+++}P<0.01$ *vs* reperfusion group.

Group	Incidence/%		Persistent duration/min	
	VT	VF	VT	VF
Control	0	0		
Ischemia	25^{*}	12.5^{*}	0.4 ± 0.3	0.6 ± 0.4
Reperfusion	100^{***}	87.5^{***}	0.9 ± 0.7	1.7 ± 0.8
IHC-72	37.5^{+++}	25^{++}	$0.28\pm0.13^{++}$	$0.8\pm0.4^{++}$
Verapamil	35^{+++}	0^{+++}	$0.19\pm0.08^{++}$	

Effects on coronary flow (CF) and creatine kinase (CK) release of myocardium There were no significantly different effects on CF between the control, IHC-72, and Ver groups before ischemia. This meant that IHC-72 and Ver did not increase the CF under normal condition. But both ICH-72 and Ver increased the CF during reperfusion as compared with that of the control (Fig 1). At 5, 10, 20, and 30 min after reperfusion with K-H solution containing IHC-72 50 μmol · L^{-1} or Ver 2 μmol · L^{-1} the release of CK decreased markedly (both $P<0.01$ *vs* control, Fig 2).

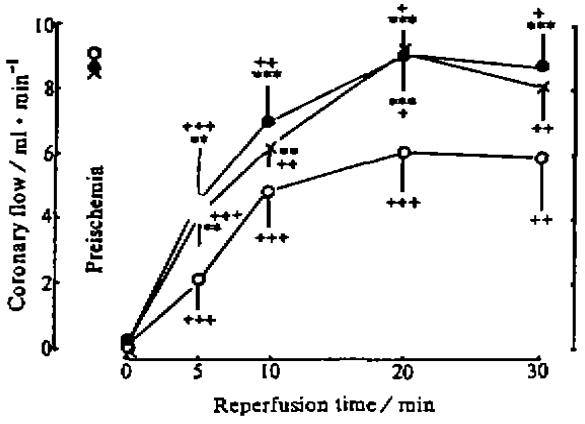

Fig 1. Effects of IHC-72 50 μmol · L^{-1}(●) and verapamil 2 μmol · L^{-1}(×) on coronary flow (CF) in isolated modified K-H solution perfused rat hearts. (○) Control. $n=8$, $\bar{x}\pm s$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* control; $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* preischemia.

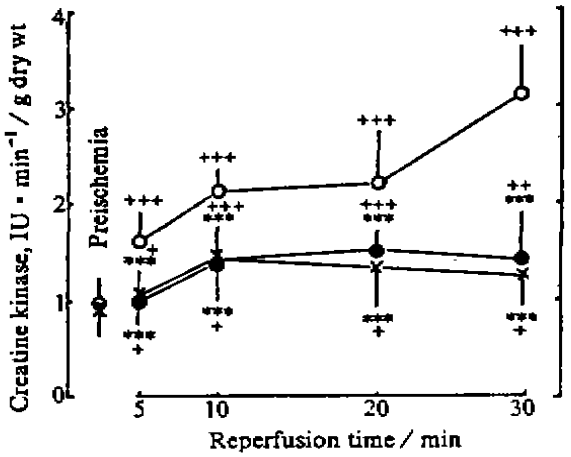

Fig 2. Effects of IHC-72 50 μmol·L^{-1}(●) and verapamil 2 μmol·L^{-1}(×) on CK release in isolated rat hearts subjected to ischemia and reperfusion. (○) Control. $n=8$, $\bar{x}\pm s$. $^{***}P<0.01$ *vs* control; $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* preischemia.

Effects on superoxide dismutase (SOD) activity and malondialdehyde (MDA) content in myocardium In the ischemia/reperfusion groups the SOD activity decreased, while MDA content increased significantly. IHC-72 increased the SOD activity and suppressed the rise of the myocardial MDA content at the concentration of 50 μmol·L^{-1} ($P<0.01$ *vs* reperfusion group). Ver 2 μmol·L^{-1} also had the same favorable effects on the ischemia/reperfusion damaged rat hearts (Tab 2).

Tab 2. Effects of IHC-72 and verapamil (Ver) on activity of superoxide dismutase (SOD) and content of malondialdehyde (MDA) in isolated rat hearts subjected to global ischemia followed by reperfusion. $\bar{x}\pm s$. $^{***}P<0.01$ *vs* **control;** $^{+++}P<0.01$ *vs* **reperfusion.**

Group	n	SOD, μmol/g wet wt	MDA, nmol/g wet wt
Control	8	68±10	7.0±1.2
Reperfusion	8	36±13***	9.4±1.9***
IHC-72	8	59±11+++	7.1±0.7+++
Ver	8	61±11+++	6.43±0.16+++

Effects on myocardial electrolyte contents of isolated reperfusion rat hearts In reperfusion group, myocardial Ca^{2+} and Na^+ increased ($P<0.01$ and $P<0.05$ *vs* control, respectively). Both IHC-72 50 μmol·L^{-1} and Ver 2 μmol·L^{-1} decreased the myocardial Ca^{2+} markedly ($P<0.01$, Tab 3). IHC-72 also decreased the Na^+ content of myocardium. But the contents of K^+ and Mg^{2+} in myocardium were not affected by IHC-72 50 μmol·L^{-1} or Ver 2 μmol·L^{-1}.

Tab 3. Influences of IHC-72 and verapamil on myocardial electrolyte contents of isolated rat hearts subjected to 40-min global ischemia and reperfusion for 20 min. $n=8$, $\bar{x}\pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control;** $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* **Isc/Rep (ischemia/−reperfusion).**

Group	Myocardial electrolyte content, μmol/g dry wt			
	Ca^{2+}	Na^+	K^+	Mg^{2+}
Control	5.7±0.6	95±19	251±56	43±6
Isc/Rep	7.4±1.0***	117±21**	254±62*	41±9*
IHC-72	4.1±1.1+++	91±14++	249±52+	42±9+
Verapamil	3.5±0.9+++	96±24+	270±40+	40±13+

DISCUSSION

The leakage of myocardial CK to coronary effluent has been used as a sign of ischemia/reperfusion myocardial injury[12] and the change of SOD activity and MDA contents reflected indirectly the amount of oxygen free radicals formed. The oxygen free radicals and disturbances of myocardial elements, particularly the intracellular Ca^{2+} overload, played an important role in the development of ischemia/reperfusion injury. Oxygen free radicals and intracellular Ca^{2+} over-load were mutually promoting, thus a vicious cycle was set up in the ischemia/reperfusion injury. Ver can protect the ischemia/reperfusion damaged heart, which is related to its antagonism against oxygen free radicals by reducing the inward flux of Ca^{2+}, inhibiting the formation of xanthine oxidase and protecting the activity of endogenus SOD[4]. Ver increased CF as a result of improving the ischemia/reperfusion induced cardiac dysfunction and possibly of dilating the coronary artery[13,14].

In the present study, we found that the effects of IHC-72 on ischemia/reperfusion damaged hearts were similar to those of Ver. The result of IHC-72 attenuating the accumulation of myocardial Ca^{2+} was consistent with our previous experiment[6]. These results suggested that IHC-72 possessed protective effects on the ischemia/reperfusion isolated rat hearts. Thus the mechanism of IHC-72 protecting ischemia/reperfusion damaged hearts may be similar to that of Ver.

REFERENCES

1 Pridjian AK, Levitsky S, Krukenkamp I, Silverman NA, Feinberg H. Intracellular sodium and calcium in the postischemic myocardium. *Ann Thorac Surg* 1987; **43**: 416—9.

2 Jennings RB, Sommers HM, Kaltenbach JP, West JJ. Electrolyte alterations in acute myocardial ischemic injury. *Circ Res* 1964; **14**: 260—9.

3 Bernier M, Hearse DJ, Manning AS. Reperfusion-induced arrhythmias and oxygen-derived free radicals: Studies with "anti-free radical" interventions and a free radical-generating system in the isolated perfused rat heart. *Circ Res* 1986; **58**: 331—40.

4 Li X, Chen JX, Suo JJ. Protective effects of Panax notoginseng saponins on experimental myocardial injury induced by ischemia and reperfusion in rat. *Acta Pharmacol Sin* 1990; **11**: 26—9.

5 Wang N, Zhao DH, Sheng BH. The anti-arrhythmic action of IHC-72 in experimental models. *Northwest Pharm J* 1989; **4**: 1—3.

6 Ji GJ, Zhao DH, Liu DQ, Sheng BH. Effects of IHC-72 on the ventricular muscle contraction induced by isoproterenol, calcium and histamine in guinea pigs. *J Fourth Milit Med Univ* 1992; **13**: 210—2.

7 Ji GJ, Zhao DH, Liu DQ, Sheng BH. Comparison of antiarrhythmic effects of IHC-72 (an iodonium-72), lidocaine and verapamil. *Acta Pharm Sin* 1992; **27**: 407—11.

8 Jin XZ, Liu GD. Prevention of regional ischemic-reperfused myocardial damages in isolated working rat hearts by puerarin. *J Med Coll PLA* 1987; **2**: 335—8.

9 Duma RJ, Siegel AL. Serum creatinine phosphokinase in acute myocardial infarction. *Arch Intern Med* 1965; **115**: 443—51.

10 Li YX, Fang YZ. A new assay for superoxide dismutase activity: Chemiluminescene method. *Biochim Biophys* 1983; **2**: 59—62.

11 Aust SD. Lipid peroxidation In: Greenwald RA, editor. *Handbook of methods for oxygen radical research*. Boca Raton: CRC Press, 1988: 203—7.

12 Jin XZ, Liu GD. Prevention of postischemic reperfusion damage on isolated working rat hearts by bopindolol and propranolol. *Acta Pharmacol Sin* 1989; **10**: 394—8.

13 Zsotér TT, Church JG. Calcium antagonists. Pharmacodynamic effects and mechanism of action. *Drugs* 1983; **25**: 93—112.

14 Liu JC, Chen P. The effect of myocardial protection by verapamil and/or coenzyme Q_{10} on isolated rat heart. *Chin Circ J* 1991; **6**: 394—7.

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3,6—(二甲氨基)—二苯骈碘杂六环依地酸盐对全心缺血再灌注离体大鼠心脏的保护作用

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摘要 离体大鼠心脏全心缺血再灌注后，室速(VT)和室颤(VF)发生率明显提高，肌酸激酶(CK)释放及丙二醛(MDA)含量增加，超氧化物歧化酶(SOD)活性降低，以及心肌钙聚集显著增加。IHC-72 50 μmol·L^{-1}显著降低VT和VF发生率，减少CK释放，降低MDA含量，提高SOD活性，以及减少心肌 Ca^{2+}，Na^{+}聚集。结果提示，IHC-72对缺血再灌注离体大鼠心脏有保护作用。

关键词 3,6—(二甲氨基)—二苯骈碘杂六环；心肌再灌注损伤；心律失常；超氧化物歧化酶；丙二醛；钙

超氧物歧化酶