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Effects of berbamine on hemodynamics and myocardial reperfusion injury in isolated working rabbit hearts

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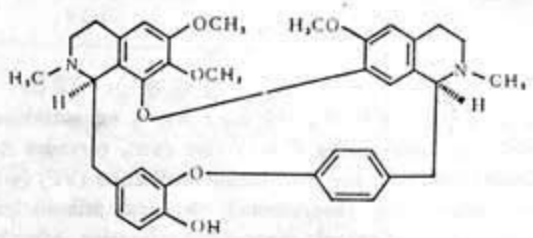
ABSTRACT The isolated working rabbit hearts perfused with Tyrode's buffer solution persisted working in good condition at least up to 70 min. Berbamine (Ber) 3 μmol · L^{-1} significantly changed the hemodynamic parameters and reduced cardiac functions. The effects of Ber appeared in a dose-dependent manner. Reperfusion following 30-min of global ischemia aggravated the myocardial damages induced by ischemia, and the parameters of cardiac functions in working hearts markedly reduced and did not restore even at 20 min of reperfusion. Ber 1 μmol · L^{-1} reduced the myocardial ischemic reperfused damages and restored all parameters to the level of preischemia within 10 min of reperfusion, and this situation of working hearts also lasted 40 – 50 min. The present results showed that Ber could protect myocardium against ischemic reperfused damages, promote the recovery of cardiac functions and prolong the efficient working period in isolated working rabbit hearts.

KEY WORDS berbamine; berbines; heart; ischemia; myocardial reperfusion injury; hemodynamics

Berbamine (Ber) is a dibenzylisoquinoline alkaloid isolated from plant and its chemical structure is similar to that of tetrandrine (Tet)[1], a calcium channel blocker[2]. According to the structure-activity relationship, Ber also has the same pharmacological effects as Tet. The previous studies showed that Ber possessed hypotensive actoin[3], anti-arrhythmias[4], reduction in the contractility and electric activity in isolated guinea pig papillary muscles and human pectinate muscles preparations[5,6]. Ber also showed the protective action against myocardial[7] and cerebral ischemia[8]. The present study was to investigate the hemodynamic and protective effects of Ber on normal myocardium and myocardial ischemic reperfused damages in isolated working rabbit hearts.



Berbamine (6,6',7-trimethoxy-2,2'-dimethyl-berbaman-12-ol). $C_{37}H_{40}N_2O_6$. $M_r = 608.74$

MATERIALS AND METHODS

Drug Ber was provided by The Institute of Applied Ecology of The Chinese Academy of Sciences. Its purity was over 96 %. Ber crystals were dissolved in 1 % distilled water before use, pH 5.3–5.4.

Isolated working heart preparation Japanese white rabbits of either sex weighing 2.3 ± SD 0.4 kg provided by The Animal Center of Harbin Medical University were used to prepare the working heart models. The thoracic and pericardial cavities were opened and pulmonary artery was cut off. A cannula of 3.5 mm internal diameter was inserted into aorta and retrogradely perfused with cold Tyrode's buffer solution at a rate of 8 ml · min^{-1}, and then the pulmonary veins of both sides were ligated and another cannula of 3.0 mm internal diameter was inserted into left artrium for normal perfusion[9]. The heart preparations were removed into a homo-

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isothermal perfused chamber circulated by warm water (37℃). After 10–15 min equilibration, the retrograde perfusion was converted into normal perfusion. The perfusing pressure of left atrium and afterload of left ventricle were maintained at 1.96 and 9.78 kPa respectively. Coronary flow was maintained at about 5 % of normal working heart via retrograding perfusion during 30–min low–flow global ischemia. The experiments were divided into 4 groups: (1) control· (2) perfusion + Ber; (3) ischemia + reperfusion; (4) Ber + ischemia + reperfusion.

Parameters of cardiac functions Two catheters were introduced into the left ventricle and aorta separately along the left ventricular and aortic cannulae, the other ends of the 2 catheters were connected with Nihon Kohden Polysystem (RM–6000) via a transducer (PT–200T). The indices including heart rate (HR), aortic pressure (AR), left ventricular pressure (LVP), left ventricular end–diastolic pressure (LVEDP), the maximal rate of left ventricular pressure change ($\pm dP/dt_{max}$), the time from peak pressure to maximal negative dP/dt(TP_{max}–max neg dP/dt), the time to peak pressure (TPP) as well as the pressure at maximal negative dP/dt($P_{max\ neg\ dP/dt}$) were recorded. Cardiac output (CO), stroke volume (SV) and aortic flow (AF) were measured with an electromagnetic flow meter (MF–27) and coronary flow (CF) was collected. These values were corrected by the wet weight of hearts. Cardiac index (CI), stroke index (SI), the time constant of isovolumic diastolic pressure decay (T) and the heart performances, including stroke work (SW) and left ventricular work (LVW), were calculated.

Statistical analysis All experimental data were expressed in terms of $\bar{x} \pm SD$. and evaluated by t test for paired values.

Buffer solution The composition of Tyrode's buffer solution was as follows: NaCl 136.9, KCl 5.4; $CaCl_2$ 1.8; NaH_2PO_4 0.42; $NaHCO_3$ 11.9; glucose 5.0 mmol · L^{-1}. The temperature of buffer solution was kept at 36 ± 1 ℃ and equilibrated with 95% O_2 + 5% CO_2, pH 7.2–7.4.

RESULTS

Effects of Tyrode's buffer on working hearts The control experiment of Tyrode's buffer was performed to evaluate the changes of cardiac functions of working hearts during perfusion. The results showed HR 131 + 11 bpm, AP 9.3 + 1.6 kPa, LVP 12.6± 0.8 kPa, LVEDP 0.37 ± 0.13 kPa, $+dP/dt_{max}$ 450 ± 11 kPa · s^{-1}, $-dP/dt_{max}$ 316 ± 23 kPa · s^{-1}, CO 100 ± 12 ml · min^{-1} and T 23 ± 3 ms at 0 min of perfusion. At 70 min of perfusion, the % changes of above parameters were HR 3.1, AR −4.2 , LVP −6.3, LVEDP 10.8, $+dP/dt_{max}$ −14.0, $-dP/dt_{max}$ −13.3, CO −6.0 and T 6.0, having no statistical significance ($P > 0.05$) *vs* the parameters at 0 min of perfusion. The perfusion for 90 min markedly reduced the parameters, suggesting the start of natural failure of working hearts.

Effects of ber on working hearts (Tab 1) Ber was added to the buffer to provide the final concentrations of 1, 3, and 10 μmol · L^{-1} , the interval between two doses was 8 min. There was an inhibitory effect of Ber on the working hearts and this effect of Ber appeared in a dose–dependent manner.

In the hearts treated with Ber 1 μmol · L^{-1}, the parameters of working hearts were not changed significantly. Ber 3 μmol · L^{-1} reduced the contractility (AP, AF, LVP and $+dP/dt_{max}$), relaxation (LVEDP, $-dP/dt_{max}$, T and TP_{max}–max neg dP/dt), cardiac pump functions (HR, CO, CI, SI, and CF) and performances (SW and LVW), but not SV and TPP. The effects of Ber 10 μmol · L^{-1} on working hearts were very marked.

Effects of ischemic reperfusion on working hearts The ischemic reperfused control experiments were performed to estimate the extent of recovery of cardiac functions during

Tab 1. Effects of berbamine on hemodynamics and cardiac pump function in 6 isolated working rabbit hearts. $\bar{x}$ ± SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Parameters	Berbamine (μmol · L^{-1}) 0	1	3	10
HR (bpm)	125 ± 18	124 ± 14*	113 ± 10*	101 ± 11**
AP (kPa)	9.7 ± 1.3	9.8 ± 1.4*	9.2 ± 1.4*	8.2 ± 1.9**
LVP (kPa)	12.4 ± 1.2	11.9 ± 1.4*	10.6 ± 1.16**	9.6 ± 0.7***
LVEDP (kPa)	0.38 ± 0.10	0.41 ± 0.11*	0.54 ± 0.14***	0.68 ± 0.09***
$+dP/dt_{max}$ (kPa · s^{-1})	446 ± 43	421 ± 32*	377 ± 27**	280 ± 18***
$-dP/dt_{max}$ (kPa · s^{-1})	300 ± 19	274 ± 21*	205 ± 19**	170 ± 21***
AF (ml · min^{-1})	73 ± 9.3	67 ± 8.9*	53 ± 11**	37 ± 11***
CF (ml · min^{-1})	20 ± 4.9	19 ± 5.2*	15 ± 4.9**	8.3 ± 2.9***
CO (ml · min^{-1})	93 ± 9	89 ± 9*	69 ± 8***	47 ± 12***
SV (ml)	0.75 ± 0.08	0.73 ± 0.08*	0.69 ± 0.09*	0.55 ± 0.16**
CI (L · min^{-1} · m^{-2})	0.80 ± 0.09	0.79 ± 0.87*	0.60 ± 0.06***	0.39 ± 0.09***
SI (ml · bpm · m^{-2})	6.5 ± 0.8	6.4 ± 0.8*	5.9 ± 0.6*	4.6 ± 1.2**
T (ms)	24.8 ± 1.6	26.4 ± 1.4*	37.7 ± 3.6***	43 ± 7.8***
TPP (s)	0.16 ± 0.03	0.16 ± 0.08*	0.19 ± 0.05*	0.24 ± 0.03***
$P_{max\ neg\ dP/dt}$ (kPa)	7.4 ± 0.7*	7.7 ± 0.5*	7.6 ± 0.5*	7.7 ± 0.9*
TP_{max}–max neg dP/dt (s)	0.12 ± 0.03	0.13 ± 0.04*	0.16 ± 0.04**	0.20 ± 0.06***
SW (g · cm · s^{-1} · m^{-2})	8.1 ± 1.6	7.4 ± 1.3*	5.9 ± 0.6***	4.0 ± 1.4***
LVW (kg · m · min^{-1} · m^{-2})	1.01 ± 0.17	0.93 ± 0.11*	0.59 ± 0.08***	0.33 ± 0.11***

HR: Heart rate; AP: Aortic pressure; LVP: Left ventricular pressure; LVEDP: Left ventricular end–diastolic pressure; $+dP/dt_{max}$: Maximal rate of change of left ventricular pressure; AF: Aortic flow; CF: Coronary flow; CO: Cardiac output; SV: Stroke volume; CI: Cardiac index; SI: Stroke index; T: Time constant of isovolumic diastolic pressure decay; TPP: Time to peak pressure; $P_{max\ neg\ dP/dt}$: Pressure at $-dP/dt_{max}$; TP_{max}–max neg dP/dt: Time from peak pressure to $-dP/dt_{max}$; SW: Stroke work; LVW: Left ventricular work.

Tab 2. Effects of berbamine on contractility and relaxation of postischemic reperfused myocardium in 5 isolated working rabbit hearts. C) Control hearts; T) Treated hearts. $\bar{x}$ ± SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* before.

Parameters		Before	Reperfusion (min) 1	5	10	20
AP (kPa)	C	9.2 ± 0.5		7.2 ± 1.4***	8.1 ± 0.8**	7.7 ± 0.8**
	T	9.2 ± 0.3	7.7 ± 0.4***	7.7 ± 0.6***	8.1 ± 0.7*	8.42 ± 0.18*
LVP (kPa)	C	12.2 ± 1.5		8.7 ± 1.4**	9.6 ± 1.0**	9.1 ± 1.3**
	T	13.0 ± 1.5	7.0 ± 0.77***	10.0 ± 1.9**	11.8 ± 1.6*	12.3 ± 1.2*
AF (ml · min^{-1})	C	70 ± 15		33 ± 11***	47 ± 15***	49 ± 9**
	T	73 ± 7	34 ± 15.8***	56 ± 12**	66 ± 19*	70 ± 19*
$+dP/dt_{max}$ (kPa · s^{-1})	C	444 ± 22		338 ± 38***	358 ± 36***	358 ± 38**
	T	445 ± 19	301 ± 22***	381 ± 21**	437 ± 23*	439 ± 18*
LVEDP (kPa)	C	0.34 ± 0.09		0.74 ± 0.19***	0.80 ± 0.13***	0.97 ± 0.28***
	T	0.36 ± 0.06	0.77 ± 0.08***	0.63 ± 0.19**	0.52 ± 0.17*	0.41 ± 0.15*
$-dP/dt_{max}$ (kPa · s^{-1})	C	294 ± 17		198 ± 21***	217 ± 27***	217 ± 40**
	T	306 ± 29	183 ± 14***	253 ± 25***	285 ± 16*	298 ± 16*
T (ms)	C	25.1 ± 2.6		35 ± 4.3***	32.0 ± 1.6***	30 ± 3.0***
	T	23.9 ± 2.3	37.9 ± 4.6***	27.8 ± 2.6***	24.1 ± 1.9*	24.4 ± 2.3*
$P_{max\ neg\ dP/dt}$ (s)	C	0.13 ± 0.04		0.19 ± 0.07**	0.16 ± 0.07**	0.15 ± 0.03*
	T	0.12 ± 0.04	0.18 ± 0.04***	0.14 ± 0.04**	0.12 ± 0.05*	0.12 ± 0.06*

reperfusion. The results showed that the postischemic reperfusion significantly reduced contractility and relaxation (Tab 2) as well as cardiac pump functions (Fig 2). The parameters of cardiac functions were reduced to approximately zero at start of reperfusion and began to restore at 5 min of reperfusion, but all parameters did not restore to the preischemic level even at 20 min of reperfusion ($P<0.05$ or 0.01).

Protective effects of Ber on contractility and relaxation (Tab 2) Ber 1 μmol · L^{-1} showed marked protective effects. The contractility (AP, AF, LVP and $+dP/dt_{max}$) was decreased by only 16, 53, 46 and 32 % ($P<0.01$) at 1 min of reperfusion and restored to 92, 96, 95 and 99% ($P>0.05$) at the end of reperfusion. The % changes of relaxation ($-dP/dt_{max}$, LVEDP, T and TP_{max}–max neg dP/dt) in the control hearts were −25% ($P<0.05$), 185 and 22 % ($P<0.01$) and 15 % ($P>0.05$) respectively even at 20 min of reperfusion, while the above parameters in the Ber–treated hearts already restored to 97, 87, 98 and 100 % ($P>0.05$) of preischemia respectively at the same period.

Protective effects of Ber on cardiac pump functions and performances Fig 1 showed that the postischemic reperfusion decreased CO, CI, SV, HR, CF and LVW, but reperfusion for 20 min did not recover these parameters to the preischemic level. In this experiment, however, Ber 1 μmol · L^{-1} improved the cardiac pump functions and promote the restorations of HR, CF and LVW.

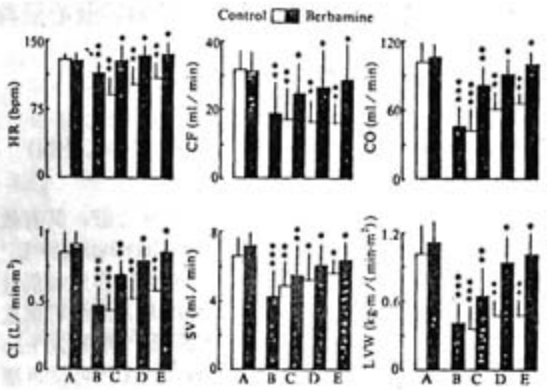

Fig 1. Effects of berbamine on the recoveries of isolated working rabbit hearts undergoing reperfusion. A, B, C, D and E) 0, 1, 5, 10 and 20 min after the start of reperfusion following 30–min global ischemia. $n=5$ hearts, $\bar{x} \pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* before.

DISCUSSION

In these experiments the catheter recording LVP was introduced into left ventricle. This method was thought to be much better than that of Langendorff hearts, because the myocardial injury and the leak of perfusate could be avoided.

The results indicated that Ber could reduce the cardiac function dose–dependently, and the effects of Ber were consistent with those of other calcium channel blockers. The possible mechanism of the inhibitory effect of Ber on working hearts was that Ber reduced the transmembrane Ca^{2+} influx via blocking the potential–dependent Ca^{2+} channel (PDC)[10,11]. But there is generally no simple explanation for CF reduction in Ber treated hearts.

Myocardial reperfusion would induce a double–edged sword to ischemic myocardium[12]. Similar results were obtained in the present works. The present study also proved the protective effects of Ber on myocardial ischemic reperfused damages in isolated working rabbit hearts and the result was consistent with that of varapamil[13,14]. The relaxation is more important in cardiac functiosn and easily damages by ischemic reperfusion and directly affects the cardiac pump function as well as coronary circulation. The present results indicated that impairments of relaxation and cardiac pump functions caused by ischemic reperfusion were more serious than that of contractility in the control hearts, but in the Ber–treated hearts, their restorations were

much better than that of contractility. In addition, the cardiac functions of control hearts did not recover from ischemic reperfused damages even at the end of reperfusion, in contrast, Ber-treated hearts appeared not only recovered rapidly, but the efficient working period prolonged as well. These results suggested that Ber 1 $\mu mol \cdot L^{-1}$ had direct protective effects on ischemic reperfused hearts, especially on relaxation and cardiac pump functions, and it was consistent with the results of *m*-nifedipine and nifedipine on isolated working guinea pig hearts[14].

Myocardial injury during ischemic reperfusion is thought to be associated with a cytosolic accumulation of Ca^{2+}. Most of the Ca^{2+} antagonist have been shown to prevent the massive overloading with Ca^{2+} which is thought to cause myocardial cell necrosis. The present study found that BA had similar pharmacological actions to Ca^{2+} channel blocking agents such as Tet and Ver. So the possible mechanism of the protective effects of Ber on myocardial ischemic reperfused damages was thought to be related to the Ca^{2+} channel blockade.

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小檗胺对兔离体工作心脏血流动力学及心肌再灌注损伤的影响

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提要 用 Tyrode 液灌流兔离体工作心脏，其有效工作时间至少可达 70 min，小檗胺(Ber) 3 $\mu mol \cdot L^{-1}$ 可明显改变工作心脏的血流动力学参数，作用呈剂量依赖性. 兔工作心脏全心缺血 30 min 后再灌注可加重心肌缺血损伤，心功能参数明显降低，再灌 20 min 仍不能恢复. 缺血前给予 Ber 1 $\mu mol \cdot L^{-1}$ 可使再灌心脏的心功能参数于再灌 10 min 内恢复到缺血前水平，并持续 40-50 min. 结果表明 Ber 能保护缺血再灌心肌，促进心功能恢复，延长心脏的有效工作时间.

关键词 小檗胺; 小檗因类; 心脏; 缺血; 心肌再灌注损伤; 血流动力学