Acta Physiologica Sinica
Feb. 2001, **53** (1), 7 ~ 12

Role of renal nerve in cardioprotection provided by renal ischemic preconditioning in anesthetized rabbits

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Abstract: The effects of renal ischemic preconditioning (RIP) on ischemia-reperfused myocardium were examined in the urethane-anesthetized rabbit to determine whether RIP may provide cardioprotection and to observe the role of the renal nerve in such condition. The results obtained are as follows: (1) During 45 min myocardial ischemia and subsequent 180 min reperfusion, blood pressure, heart rate and myocardial oxygen consumption decreased progressively. Epicardial electrographic ST-segment was elevated significantly in the period of ischemia and returned to the baseline gradually in the course of reperfusion. The myocardial infarct size occupied 55.80 ± 1.25% of the area at risk. (2) RIP significantly reduced the myocardial infarct size to 36.51 ± 2.80% ($P < 0.01$), indicating the cardioprotective effect of such an intervention. (3) Renal nerve section (RNS) completely abolished the cardioprotection afforded by RIP, though RNS *per se* did not affect the myocardial infarct size produced by ischemia-reperfusion. (4) During 10 min renal ischemia, the averaged multi-unit discharge rate of the renal afferent was increased from 0.14 ± 0.08 to 0.65 ± 0.12 imp/s ($P < 0.01$). (5) Pretreatment with an adenosine receptor antagonist 8-phenyltheophylline (10 mg/kg) markedly attenuated the discharge rate of the renal afferent induced by transient renal ischemia, implying that adenosine released in ischemic kidney activated the renal afferent. It is suggested that activation of renal afferents by transient renal ischemia-reperfusion plays an important role in the cardioprotection afforded by RIP.

Key words: ischemia-reperfusion; remote ischemic preconditioning; renal ischemia; renal afferent activity; heart; 8-phenyltheophylline

肾神经在肾缺血预处理对麻醉家兔心脏保护中的作用

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摘 要: 在氨基甲酸乙酯麻醉家兔上,观察肾脏缺血预处理(RIP)对缺血-再灌注心肌的影响,旨在证实 RIP 对心肌有无保护效应,并明确肾神经在其中的作用。所得结果如下:(1)在心脏 45 min 缺血和 180 min 再灌注过程中,血压、心率和心肌耗氧量呈进行性下降;心外膜电图 ST 段在缺血期明显抬高,再灌注过程中逐渐恢复到基础对照值。心肌梗塞范围占缺血心肌的 55.80 ± 1.25%。(2) RIP 时心肌梗塞范围为 36.51 ± 2.8%,较单纯心肌缺血-再灌注显著减少($P < 0.01$),表明 RIP 对心肌有保护作用。(3)肾神经切断可取消 RIP 对心肌的保护效应,但肾神经切断本身对单纯缺血-再灌注所致的心肌梗死范围无明显影响。(4)肾缺血(10 min)时,肾传入神经放电活动由 0.14 ± 0.08 增至 0.65 ± 0.12 imp/s ($P < 0.01$)。(5) 预先应用腺苷受体拮抗剂 8-苯茶碱可明显减弱肾缺血所激活的肾传入神经活动,提示肾传入活动的增强是由肾缺血产生的腺苷所介导。以上结果表明,肾短暂缺血-再灌注所诱发的肾神经传入活动在 RIP 心肌保护效应中起重要作用。

关键词: 缺血-再灌注;远程缺血预处理;肾缺血;肾神经传入放电活动;心脏;8-苯茶碱
学科分类号: Q463;R331.3

Our previous studies have demonstrated that myocardial ischemic preconditioning (IP) reduces myocardial infarct size and apoptosis during subsequent prolonged ischemia-reperfusion (IR), and it has been shown that car-

Received 2000-06-13 Accepted 2000-09-01
This research was supported by the National Natural Science Foundation of China (No.30070282).
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dioprotection provided by myocardial IP occurs through activation of potassium sensitive channels and relevant gene protein expression of apoptosis, without involvement of L-arginine — nitric oxide pathway[1~3]. It was demonstrated in the anesthetized open-chest dog that brief episodes of ischemia in the bed of the circumflex branch of coronary artery protect myocardial regions supplied by the left anterior descending coronary artery from a subsequently sustained ischemia[4]. Furthermore, a number of studies provided evidence that transient IR of kidney, small intestine or limb also protect myocardium against injury caused by subsequent sustained coronary occlusion, a phenomenon called "remote" or "interorgan" preconditioning[5~9]. The mechanism underlying the protection provided by remote IP remains to be elucidated, although several hypotheses have been suggested. Cardioprotection by renal ischemic preconditioning (RIP) may be triggered by adenosine produced in ischemic kidney since pretreatment with a nonselective adenosine antagonist 8-(p-sulfophenyl) theophylline (8-SPT) before RIP can abolish such a protective effect[7]. It is well established that renal ischemia may result in an increase of renal afferent activity[10~12]. Therefore, a question arises as to whether activation of neural afferent by adenosine released in ischemic kidney may play an important role in the initiation of cardioprotection by RIP. The purpose of the present study was to ascertain the protective effect on myocardium provided by RIP and to characterize the role of renal nerve in the effect of RIP.

1 MATERIALS AND METHODS

1.1 *General operation* The experiments were performed on 62 male rabbits weighing between 2.5 and 3.5 kg. The animals were anesthetized with urethane (1.0 g/kg, iv) and intubated by tracheotomy. The right carotid artery was catheterized for monitoring blood pressure (BP) with a pressure transducer (MPU-0.5). Heart rate (HR) was measured with a HR counter (AT-600G) triggered by arterial pressure pulse. Arterial pressure and heart rate were recorded on a polygraph (RM-6000G, Nihon Kohden). The body temperature of animals was maintained at 38~39℃.

1.2 *Renal and myocardial IR* The left renal artery and nerves were dissected via retroperitoneal approach[13]. A reversible snare occluder consisting of 3-0 sutures was placed around the renal artery. The snare was tightened to occlude the artery and was loosened to reperfuse. The method of myocardial ischemia-reperfusion (MIR) has been previously described in detail[1~3]. Epicardial electrocardiograph was monitored. Myocardial ischemia was produced by pulling the snare and confirmed by ST segment elevation of epicardial electrocardiogram as well as the presence of regional cyanosis over the myocardial surface. Reperfusion was produced by releasing the snare and confirmed by resultant reactive hyperemia. Myocardial oxygen consumption was expressed with pressure-rate index (mean arterial BP × HR/1000).

1.3 *Determination of myocardial infarct size* At the end of MIR, the heart was quickly excised and perfused retrogradely via the aorta with 37℃ saline to wash out blood. After the coronary artery was reoccluded, the heart was perfused with 3 ml of Evans blue to delineate myocardium at risk. The atria, right ventricle and great vessels were removed before the left ventricle was sectioned into 3 mm slices from the apex to the base. The slices were incubated in 1% triphenyl tetrazolium chloride (pH 7.4) for 10 min, which stains vital tissues to deep red but leaves infarct tissue unstained. Finally, the different areas of the left ventricle were weighed separately. Myocardial ischemic size was defined as the ratio of the area at risk to the left ventricle. Infarct size was expressed as the percentage of the infarct area to the area at risk.

1.4 *Recording of afferent renal nerve activity (ARNA)* After the left postganglionic sympathetic renal nerve was approached retroperitoneally, a branch of left renal nerve was found at the angle between the abdominal aorta and the left renal artery and dissected under a dissecting microscope. The exposed nerve was cut centrally to prevent efferent activity and immersed in warm (38℃) liquid paraffin. Afferent activity was picked from the distal end of nerve with an amplifier (AVB-11, band-pass width: 50 Hz~1 kHz, Nihon Kohden) and displayed on a thermal array recorder (WS-682G, band-pass width: 0~2.8 kHz, Nihon Kohden). The discharge rate of renal afferents was expressed by impulses/second (imp/s)[13].

1.5 *Experimental protocol* Thirty-six rabbits were conducted with MIR and divided into 4 groups. Animals were allowed at least 30 min to reach a steady state after surgical preparation. (1) MIR group (n = 8). The animals were subjected to a 45-min period of coronary artery

occlusion followed by 180 min of reperfusion. BP, HR and epicardial electrography were recorded during MIR. The myocardial ischemic size and infarct size were determined *vide supra*; (2) RIP + MIR group ($n = 12$). The rabbits underwent a 10-min renal ischemia and a 10-min reperfusion, and was then followed by the procedures of MIR to verify the effect of RIP; (3) Renal nerve section (RNS) + MIR group ($n = 8$). The left renal nerves were sectioned before MIR to observe the effect of renal nerve on myocardial injury caused by MIR; (4) RNS + RIP + MIR group ($n = 8$). The left renal nerves were sectioned before RIP + MIR. The cardioprotection of RIP was examined after RNS to explore the possible role of renal nerve in the effect of IP.

To characterize the effect of renal transient IR on ARNA, multi-unit discharges of ARNA were examined during renal 10-min ischemia and 10-min reperfusion in 20 rabbits after a stable recording was obtained. In the other 6 rabbits, the adenosine receptor antagonist 8-phenyltheophylline (8-PT, 10 mg/kg) was administrated intravenously and renal IR was conducted 10 min later.

1.6 *Data analysis* All data are expressed as mean ± SE. Statistical significance was performed by analysis of variance (ANOVA) followed by the paired t test for within-group comparisons and the unpaired t test for between-group comparisons. Statistical significance was accepted when $P < 0.05$.

2 RESULTS

2.1 BP, HR and myocardial oxygen consumption during MIR

During 45 min of myocardial ischemia and 180 min of reperfusion, BP, HR and myocardial oxygen consumption decreased steadily in all the four groups and there are no significant differences among them (Table 1).

2.2 Epicardial electrographic ST-segment during MIR

During myocardial ischemia, mean elevation of epicardial electrographic ST-segment (MST) and numbers of point with ST-segment elevation beyond 2 mV (NST) were increased significantly in the four groups ($P < 0.001$), but there were no significant differences among them. MST and NST returned to the baseline progressively in the course of reperfusion (Fig.1).

2.3 Myocardial area at risk and infarct size

The percentage of the myocardial ischemic size showed no difference among the four groups. MIR elicited myocardial infarct with a size of 55.8 ± 1.25%, while RIP significantly reduced the infarct size to 36.5 ± 2.80%

Table 1. Changes in heart rate, mean arterial pressure and myocardial oxygen consumption during myocardial ischemic-reperfusion in anesthetized rabbits

Group	n	Before ischemia	Ischemia (45 min)	Reperfusion (180 min)
			Heart rate (bpm)	
MIR	8	288 ± 8	278 ± 7*	267 ± 7**
RIP + MIR	12	284 ± 9	270 ± 9*	262 ± 11**
RNR + MIR	8	292 ± 8	274 ± 8*	268 ± 5**
RNR + RIP + MIR	8	289 ± 10	271 ± 8*	266 ± 7**
			Mean arterial pressure (kPa)	
MIR	8	13.07 ± 0.26	10.86 ± 0.64**	8.81 ± 0.83***
RIP + MIR	12	12.48 ± 0.57	10.40 ± 0.50**	9.28 ± 0.54***
RNS + MIR	8	13.25 ± 0.60	10.47 ± 0.80**	7.54 ± 0.45***
RNS + RIP + MIR	8	12.99 ± 0.75	10.27 ± 0.75**	8.46 ± 0.78***
			Pressure-rate index (kPa/min·1000^{-1})	
MIR	8	3.77 ± 0.13	3.02 ± 0.19**	2.32 ± 0.23***
RIP + MIR	12	3.53 ± 0.22	2.81 ± 0.19**	2.44 ± 0.19***
RNS + MIR	8	3.86 ± 0.17	2.90 ± 0.28**	2.02 ± 0.12***
RNS + RIP + MIR	8	3.75 ± 0.26	2.76 ± 0.22**	2.25 ± 0.20***

MIR, myocardial ischemia-reperfusion; RIP, renal ischemic preconditioning; RNS, renal nerve section. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with before ischemia.

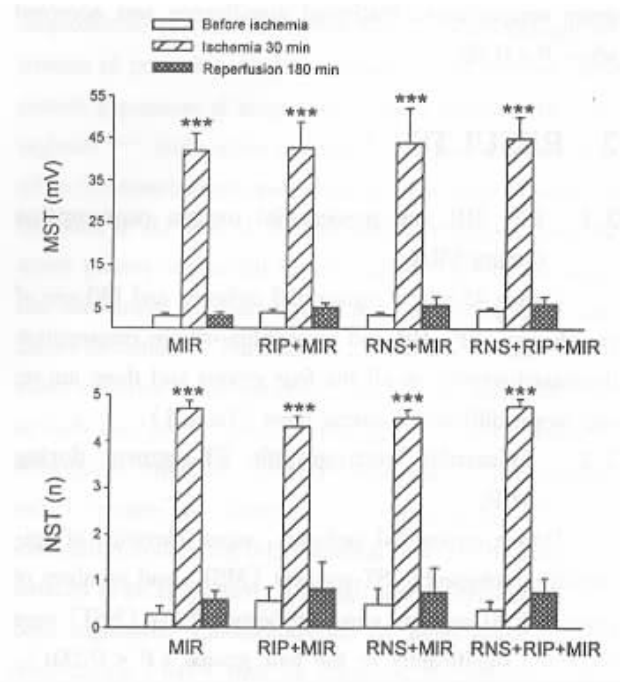

Fig.1. Changes in the epicardial electrographic ST-segment during myocardial ischemia-reperfusion in anesthetized rabbits. MST, mean elevation of epicardial electrographic ST-segment; NST, numbers of point with ST-segment elevation beyond 2 mV. $^{***}P < 0.01$ compared with before ischemia.

($P < 0.01$). Such a cardioprotective effect of RIP was abolished by RNS. However, RNS *per se* showed no effect on infarct size induced by MIR. Myocardial infarct size of RIP + MIR group was smaller than those of RNS + MIR and RNS + RIP + MIR groups ($P < 0.01$). There were no significant differences in infarct size among MIR, RNS + MIR, and RNS + RIP + MIR groups ($P > 0.05$) (Fig.2).

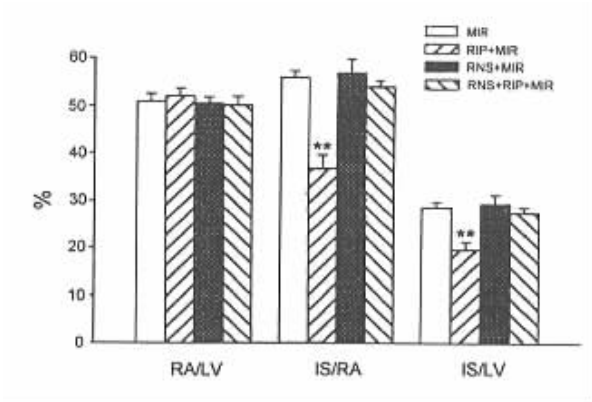

Fig.2. Histograms showing the myocardial risk area (RA)/left ventricle (LV), infarct size (IS)/RA, and IS/LV in the four groups. $^{**}P < 0.01$ compared with MIR, RNS + MIR, and RNS + RIP + MIR groups.

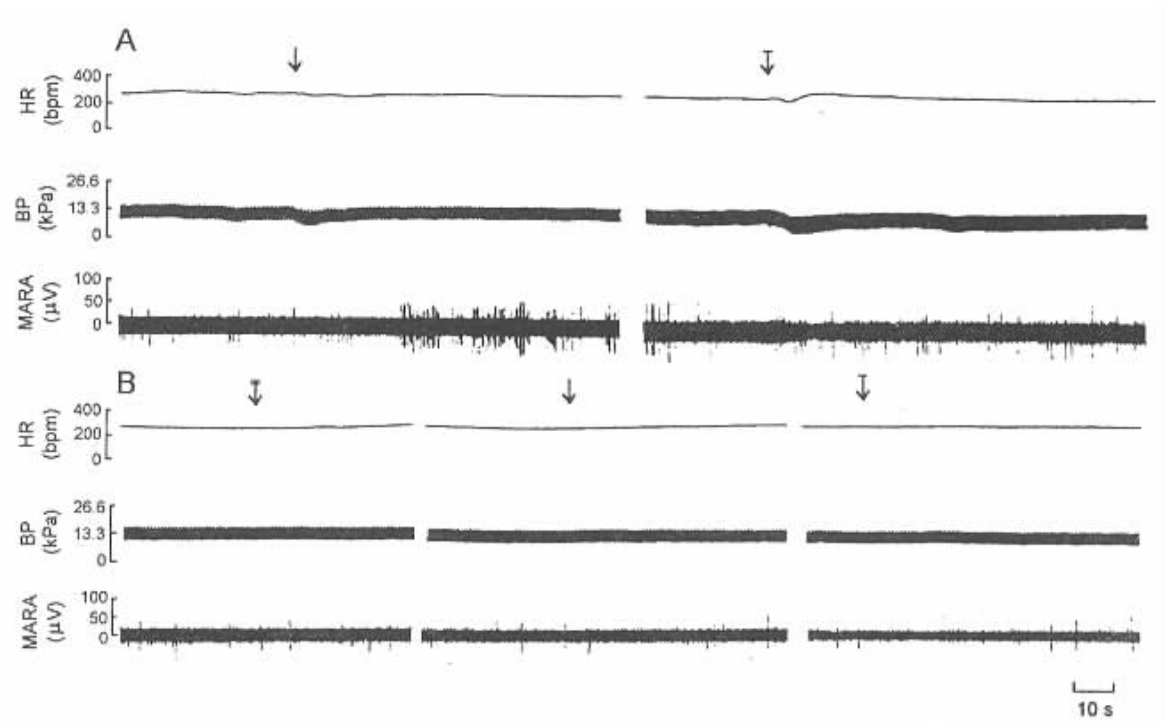

Fig.3. Tracing showing the changes in heart rate (HR), blood pressure (BP) and multi-unit activity of renal afferents (MARA). *A*. RIR. *B*. 8-phenyltheophylline (8-PT) + RIR. ↓, ischemia; ↧, reperfusion; ⇩, 8-PT.

Table 2. Changes in heart rate, mean arterial pressure and multi-unit discharge rate of renal afferent during renal ischemia-reperfusion (RIR)

Group	*n*	Baseline	Before ischemia	Ischemia	Reperfusion
			Heart rate (bpm)		
RIR	20	289 ± 9	289 ± 9	288 ± 10	287 ± 9
8-PT + RIR	6	292 ± 9	294 ± 10	293 ± 11	292 ± 8
			Mean arterial pressure (kPa)		
RIR	20	13.26 ± 0.52	13.28 ± 0.56	13.28 ± 0.54	13.26 ± 0.49
8-PT + RIR	6	13.35 ± 0.63	13.40 ± 0.80	13.50 ± 0.63	13.34 ± 0.71
			Discharge rate of renal afferent (imp/s)		
RIR	20	0.14 ± 0.08	0.14 ± 0.08	0.65 ± 0.12**	0.16 ± 0.10
8-PT + RIR	6	0.15 ± 0.72	0.16 ± 0.80	0.36 ± 0.14*#	0.18 ± 0.09

8-PT, 8-phenyltheophylline. *$P < 0.05$, **$P < 0.01$ compared with baseline, before ischemia; #$P < 0.05$ compared with RIR.

2.4 Effect of renal IR on ARNA

During 10 min of renal ischemia, the irregular and intermittent discharge of ARNA was initiated and the aveaged discharge rate was increased from 0.14 ± 0.08 to 0.65 ± 0.12 imp/s ($P < 0.01$). In the course of renal reperfusion, the discharge rate of ARNA returned to the baseline gradually. Intravenous administration with 8-PT (10 mg/kg) *per se* did not affect the baseline ARNA, but significantly reduced the discharge rate of ARNA induced by renal ischemia to 0.36 ± 0.14 imp/s ($P < 0.05$). The results are shown in Fig.3 and Table 2.

3 DISCUSSION

The present study demonstrated that RIP could reduce myocardial infarct size produced by 45 min of ischemia and 180 min of reperfusion. Such a result is in accordance with the results of the other authors[6~8]. However, it is important to note that under our experiment condition the cardioprotection provided by RIP was abolished when the renal nerve was sectioned before renal IR. During IR of myocardium, BP, HR and myocardial oxygen consumption decreased progressively in all the four groups, but there are no significant differences in these parameters among them, while myocardial infarct size (generally regarded as a golden standard for evaluating myocardial ischemic injury) showed significant differences among them (Fig.2). There is no correlation between hemodynamic parameters and infarct size observed in our study. Such a result conforms to that observed in MIR models by other workers[6~8]. In addition, MST and NST as criteria of acute myocardial ischemia were significantly elevated in the four groups to the same extent ($P > 0.05$ among groups) in the course of myocardial ischemia.

Since ischemic preconditioning was first described by Murry *et al*. in canine heart[14], it has been observed not only in the myocardium but also in a variety of organs, such as brain, liver and skeletal muscle[15~17]. Przyklenk *et al*. found that regional ischemic preconditioning protects remote virgin myocardium from subsequent sustained coronary occlusion[4]. This observation leads to a speculation that IP of one organ may confer protection on a remote organ. Recently, Liauw *et al*. demonstrated that sequential IR of gracilis muscle results in salvage of contralateral gracilis muscle[18]. Furthermore, transient IR of small intestine[6] or limb[9] also reduces myocardial ischemic injury. Our present study as well as other reports[5~8] further confirm the cardioprotection provided by RIP.

The precise mechanism(s) underlying the remote preconditioning is still unclear. Remote IP can improve tissue energy metabolism during MIR[8]. Stress reaction is involved in the protective effects of remote IP[9,18]. It is also suggested that some substances generated within an organ during IP may serve as triggers or mediators for protection to remote organs. In the studies of Pell *et al*.[7] and Takaoka *et al*.[8] pretreatment with a nonselective adenosine antagonist 8-SPT abolishes the cardioprotection provided by RIP. Accordingly, adenosine released in ischemic kidney may be the trigger or mediator. However, the amount of adenosine generated during transient renal ischemia is insufficient to offer the cardioprotection direct-

ly[7]. The report of Gho *et al*.[6] suggests that mesenteric artery occlusion may afford cardioprotection via neural ganglia in rats. In addition, the antiarrhythmic effect provided by limb ischemic preconditioning is abolished by prior depletion of catecholamine stores with reserpine[9]. These findings strongly imply that neurogenic pathway may be involved in remote preconditioning. In our present experiments, cardioprotection provided by RIP was significantly abolished by RNS, indicating the participation of renal nerve in the protective effect. With regard to the role of renal nerve in RIP, our study demonstrates that renal transient ischemia evokes an increase of ARNA which is attenuated by prior administration of 8-PT. Thus, it is conceivable that some active agents (mainly adenosine) are released during renal IR, which consequently leads to an activation of the renal afferents.

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