Effect of Ischemic Preconditioning and Mitochondrial K_{ATP} Channel Openers on Chronic Left Ventricular Remodeling in the Ischemic-Reperfused Rat Heart

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The influence of ischemic preconditioning (IP) and mitochondrial ATP-sensitive potassium (mito-K_{ATP}) channel openers on chronic left ventricular (LV) remodeling remains unknown, so the effect of IP and mito-K_{ATP} channel openers on the LV pressure–volume curve was assessed in rats subjected to 30 min ischemia followed by a 3-week reperfusion. Infarct size was histologically determined at 3 weeks after reperfusion. The LV pressure–volume curve was significantly shifted left by IP, diazoxide and nicorandil compared with the controls. These effects were blocked by the selective mito-K_{ATP} channel blocker 5-hydroxydecanoate. The LV remodeling and the infarct size at 3 weeks after reperfusion correlated well, indicating that the reduction of LV remodeling in the ischemic–reperfused model was strongly influenced by attenuation of the ischemic injury. LV remodeling in the chronic phase is attenuated by IP and mito-K_{ATP} channel openers with concomitant reduction of infarct size. (*Circ J* 2002; 66: 411–415)

Key Words: Diazoxide; Ischemic preconditioning; Nicorandil; Pressure–volume curve

A brief episode of ischemia–reperfusion reduces the size of the infarct caused by a subsequent longer ischemia–reperfusion insult. This phenomenon was first reported by Murry et al¹ and termed ischemic preconditioning (IP). The underlying mechanism of this phenomenon has been intensively investigated and one of the crucial mechanisms is the opening of the mitochondrial ATP-sensitive potassium (mito-K_{ATP}) channels^{2–4}. In order to mimic IP, the effect of mito-K_{ATP} channel openers has been investigated and these agents result in reduction of infarct size.^{5–12} However, the effect of IP and mito-K_{ATP} channel openers on chronic left ventricular (LV) remodeling has not been clarified yet. LV remodeling is a major determinant of prognosis after myocardial infarction¹³ and it may be determined by the infarct size and could be modified by drugs such as angiotensin-converting enzyme inhibitors.¹⁴ Thus, assessing LV remodeling is an important evaluation of whether or not interventions such as IP or mito-K_{ATP} channel openers are really effective. Furthermore, the reperfusion conditions, such as the no reflow phenomenon, may modify the infarct size and thus the LV remodeling in the chronic state. Therefore, the purpose of the present study was to investigate the effect of IP and mito-K_{ATP} channel openers on the extent of LV remodeling as assessed by the pressure–volume relationship, and to assess the correlation between infarct size and LV remodel-

ing during the chronic stage.

Methods

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23) and were approved by the Animal Research Committee of Yamaguchi University School of Medicine.

Surgical Preparation

Male Sprague-Dawley rats (280–330 g) were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg). Additional anesthesia was given during the experiment as required. After tracheal intubation, the rats were ventilated with a mixture of room air and 100% oxygen (respiratory rate, 65–70 breaths/min). After a thoracotomy in the fourth intercostal space, the heart was exposed and a thread was passed around the left anterior descending coronary artery (LAD) to occlude it. After 3 weeks of reperfusion, LV pressure (LVP) was measured by a 2F catheter-tip micromanometer (Model SPC-320, Millar Instruments, USA) inserted via the right carotid artery. The peak of the first derivative of LVP (+dP/dt) and the time constant of isovolumetric LVP decay (Tau) were calculated by an online data acquisition system (CODAS, DATAQ Instruments, Achron, OH, USA). The body temperature was maintained at 37±0.3°C by a heating pad.

Reagents

Diazoxide (Funakoshi, Osaka, Japan) was dissolved in dimethylsulfoxide (DMSO), the final concentration of which was less than 0.1%. Nicorandil (Chugai, Tokyo, Japan) was dissolved in saline at a concentration of 1.0

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Fig 1. Rats were subjected to a 30-min coronary occlusion followed by 3 weeks of reperfusion in all groups ($n=6$, each). Ischemic preconditioning (IP) was performed by 3 cycles of 3 min of ischemia followed by 3 min of reperfusion. Diazoxide (3.5 mg/kg IV) and nicorandil (1.75 mg/kg IV) were administered 10 min before the 30-min ischemia (arrow). 5-hydroxydecanoate (5.0 mg/kg IV) was administered 10 min before IP or the infusion of diazoxide or nicorandil (arrowheads). C, controls; IP, ischemic preconditioning; D, diazoxide; N, nicorandil; 5HD, 5-hydroxydecanoate.

mg/ml. A selective mito- K_{ATP} channel inhibitor, 5-hydroxydecanoate (HD) (Sigma)^{2,9,15,16} was dissolved in saline.

Experimental Protocols

The experimental protocols are shown in Fig 1. Seven groups of rats ($n=6$, each) were subjected to 30 min ischemia created by occluding the LAD, followed by reperfusion for 3 weeks. IP was performed by 3 cycles of 3 min of ischemia followed by 3 min of reperfusion. Diazoxide (3.5 mg/kg IV) and nicorandil (1.75 mg/kg IV) were injected 10 min before the 30-min ischemia. 5-HD (5.0 mg/kg IV) was administered 10 min before either IP or diazoxide or nicorandil.

Quantitation of Infarct Size

Three weeks after the coronary reperfusion, the LAD was re-occluded and trypan blue dye (1.5%, 1 ml, Sigma Chemical, Co, St Louis, MO, US) was injected into the ascending aorta to delineate the risk and non-risk area in the hearts. The hearts were then excised and sliced into sections approximately 1 mm in width. Each slice was imaged by a color CCD camera (FV-10, Fuji, Japan), and the images were analyzed by image analyzing software (NIH image). The area unstained by the blue dye is the area at risk. The heart was fixed in 10% phosphate-buffered formalin, processed and embedded in paraplast. Transverse sections (4 μ m) were stained by the modified azan staining. The area of infarction was identified histologically and the infarct

size expressed as a percentage of the area at risk.

Pressure–Volume Relation

According to the method of Pfeffer et al,¹⁷ the in vitro LV pressure–volume curve was measured. In brief, the heart was arrested by an infusion of KCl and then quickly excised. A double-lumen catheter was inserted into the left ventricle, which was isolated by ligating the atrioventricular groove. The right ventricle was incised to eliminate the compressive effect. The left ventricle was emptied by evacuation through the catheter with manual compression. Reproducible pressure–volume curves were generated over a pressure range of 10–30 mmHg by infusing saline at a speed of 0.6 ml/min. These procedures were performed within 10 min of cardiac arrest and before the onset of rigor mortis. The volume index (volume/body weight) of the respective LVP at 10, 20 and 30 mmHg was assessed.

Statistical Analysis

All values are expressed as the mean $\pm$ SE. Differences in hemodynamic parameters among the groups were analyzed by 2-way ANOVA. Fisher's test was used when a significant F value was obtained. Inter-group differences in area at risk, infarct size and left ventricular volumes of pressure–volume curves were analyzed by 1-way ANOVA followed by Fisher's test. Correlation between infarct size and the extent of LV remodeling was expressed as LV volume index (volume/body weight) at LVP of 20 mmHg was assessed by linear regression analysis with comparison of the 2 regression parameters of slope and intercept. Differences were considered significant at $p<0.05$.

Results

Hemodynamic Responses

Hemodynamic data at 3 weeks after reperfusion are presented in Table 1. Heart rate, peak LVP, end-diastolic LVP, (+)dP/dt, and Tau were not significantly different among the groups.

Infarct Size

In the acute phase, 15% of animals died of technical problems such as pneumothorax and bleeding, but no death from heart failure occurred. In the chronic phase, no deaths occurred. Risk area was not statistically different among the groups (Fig 2A). As shown in Fig 2B, infarct size was significantly reduced by IP ($16\pm1\%$, $p<0.01$ vs controls), compared with the controls ($72\pm4\%$), and by both diazoxide ($40\pm4\%$, $p<0.05$ vs controls) and nicorandil ($28\pm2\%$, $p<0.01$ vs controls). The effect of IP, diazoxide and nico-

Table 1 Hemodynamics After 3-Weeks of Reperfusion

	HR (beats/min)	Peak LVP (mmHg)	LVEDP (mmHg)	(+)dP/dt (mmHg/s)	Tau (ms)
C	395 $\pm$ 17	113 $\pm$ 1	5 $\pm$ 4	7,721 $\pm$ 768	12 $\pm$ 1
IP	410 $\pm$ 5	115 $\pm$ 7	7 $\pm$ 2	6,215 $\pm$ 1,207	14 $\pm$ 1
D	440 $\pm$ 5	97 $\pm$ 1	4 $\pm$ 1	7,643 $\pm$ 377	10 $\pm$ 1
N	395 $\pm$ 11	98 $\pm$ 3	4 $\pm$ 1	8,228 $\pm$ 524	10 $\pm$ 1
5HD+IP	378 $\pm$ 5	90 $\pm$ 2	4 $\pm$ 2	7,015 $\pm$ 196	12 $\pm$ 1
5HD+D	419 $\pm$ 10	96 $\pm$ 7	9 $\pm$ 3	7,270 $\pm$ 731	17 $\pm$ 3
5HD+N	430 $\pm$ 11	98 $\pm$ 6	5 $\pm$ 1	8,158 $\pm$ 834	11 $\pm$ 1

HR, heart rate; LVP, left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; (+)dP/dt, maximum rate of LVP generation; Time constant, time constant of the LVP fall during isovolumic relaxation period. Other abbreviations are the same as in Fig 1. Values are mean $\pm$ SE. $N=6$, each.

Fig 2. (A) The risk area was identical in all groups. (B) Infarct size was reduced by IP, D, and N compared with C. The effect of IP, D and N was abolished by 5-HD. * $p < 0.05$ vs C, ** $p < 0.01$ vs C, † $p < 0.05$ vs IP, ‡ $p < 0.05$ vs D, § $p < 0.05$ vs N. All abbreviations as in Fig 1.

randil on infarct size was inhibited by 5-HD ($61 \pm 2\%$ $p < 0.05$ vs IP, $72 \pm 3\%$ $p < 0.05$ vs diazoxide, $60 \pm 2\%$ $p < 0.05$ vs nicorandil).

In Vitro LV Pressure–Volume Relation

The in vitro LV pressure–volume curve shifted left with IP compared with the controls (Fig 3). With diazoxide or nicorandil, the pressure–volume curve also significantly shifted left compared with the controls. Indeed, the left shift of the LV pressure–volume curve by IP, diazoxide or nicorandil was abolished by 5-HD.

Correlation Between the Infarct Size and LV Remodeling

Linear regression analysis revealed a good correlation between the infarct size (% of area at risk) and the extent of LV remodeling expressed as the LV volume index (volume/body weight) at a LVP of 20 mmHg. The slope of the correlation was 0.014 ($r = 0.78$, $p < 0.001$) (Fig 4).

Discussion

One of the major findings of the present study is that IP attenuates LV remodeling concomitant with a reduction in infarct size in the chronic stage. Although the reduction of infarct size by IP has been demonstrated in the acute phase (ie, 4–6 h after the reperfusion determined by TTC staining),^{2,18–23} it has not been clear whether IP attenuates LV remodeling. Our study is the first to demonstrate that LV remodeling was attenuated by IP according to the reduction of the size of the histologically determined infarct. Cohen et al showed an improvement in the function of the ischemic myocardium 24 h after reperfusion in a rabbit model of ischemia–reperfusion.²⁴ However, they focused only on the ischemic area, and the change in the remote area was not determined. Our study clearly showed that using pressure–volume relation the whole LV chamber remodeling was estimated.

Fig 3. The left ventricular pressure–volume curve was significantly shifted leftward by IP, D, and N compared with C. The shift in the curve caused by IP, D and N was abolished by 5HD ($n = 6$ each). Data are shown as mean $\pm$ SE. * $p < 0.05$ vs C, † $p < 0.05$ vs IP, ‡ $p < 0.05$ vs 5HD+D, § $p < 0.05$ vs the 5HD+N. All abbreviations as in Fig 1.

Fig 4. Linear regression analysis revealed a good correlation between the infarct size (% of area at risk) and the extent of remodeling expressed as LV volume (ml/kg) at LV pressure of 20 mmHg. The slope of the correlation is 0.014 ($r = 0.78$, $p < 0.001$). All abbreviations as in Fig 1.

The infarct size determined by TTC staining correlates with the histologically determined infarct in the permanent coronary occlusion model.²⁵ However, in the ischemia–reperfusion model, the infarct size in the chronic stage may be influenced by the perfusion conditions; the no-reflow phenomenon increases the infarct size in the chronic stage in large animals and humans,^{26–28} and Shimizu et al demonstrated that no-reflow occurs in the isolated rat heart.²⁹ Furthermore, no-reflow is demonstrated in the skeletal muscle in rat and that is attenuated by IP.³⁰ So, the effect of IP or drugs on infarct size may differ between the acute and chronic stages. The infarct size may be determined within 6 h of reperfusion, but it should be taken into consideration that the infarct size determined by TTC after 6 h of reperfusion was smaller than that determined histologically at 3 weeks in the control group ($60 \pm 2\%$ vs $72 \pm 4\%$), and in the IP group the size of the infarct at 3 weeks was smaller than that after 6 h of reperfusion ($16 \pm 1\%$ vs $26 \pm 2\%$).³¹ This suggests that infarct size may be influenced by the reflow conditions.

It has been shown that attenuation of LV remodeling is a major determinant of survival after acute myocardial infarction although its underlying mechanism is not well understood.^{13,32} Pfeffer et al showed that captopril attenuated LV dilatation after myocardial infarction in a permanent coronary occlusion model.^{33,34} In their study, infarct

size was identical in the control and captopril groups, so extension of the non-ischemic myocardium was inhibited by captopril. In the present study, the infarct size was diminished by IP, thus the reduction in infarct size strongly influenced the extent of LV remodeling. Fig 4 shows the correlation between the extent of LV remodeling, which was expressed as the LV volume index of LVP at 20 mmHg, and the infarct size. Because we used IP and KATP openers to reduce infarct size, we cannot totally exclude that possibility that it is not only the infarct size–LV volume index relationship shown in Fig 4. However, IP and injection of KATP openers were performed only once before sustained ischemia and it has been shown that the topography of myocardial salvage does not differ between earlier reperfusion, IP and KATP openers. Therefore, it is likely that the relationship in Fig 4 is applicable to infarct size limitation by the earlier reperfusion. Nevertheless, a good correlation between the infarct size and LV volume index indicates that the infarct size is the most important determinant of LV remodeling in the ischemic–reperfused heart.

In the present study, the hemodynamic measurement at 3 weeks after reperfusion demonstrated that the LV end diastolic pressure was not elevated in all groups, in spite of the difference in infarct size. This result indicates that the compensation of the LV to the loss of myocardium was fully exerted. The LV remodeling may be a compensated process in this model and the lesser remodeling indicates attenuation of myocardial damage. It is important to consider where the myocardium is remodeled. In the ischemic–reperfused model, there are 3 types of LV myocardium: the non-ischemic, ischemic but viable, and infarcted regions. As has been shown, the infarcted region becomes a scar and usually shrink in 3 weeks. Thus, it is the ischemic but viable myocardium and the non-ischemic myocardium that play an important role in the remodeling, but how much each region affects the remodeling process could not be determined from the data in the present study. This should be further investigated.

The second of the major findings is that the mito-KATP channel openers, diazoxide and nicorandil, can mimic the effect of IP in terms of ventricular remodeling as well as reduction of infarct size. Because the selective blockade of the mito-KATP channels by 5-HD abolished the effect of diazoxide and nicorandil on both infarct size and LV remodeling, the opening of the mito-KATP channels is a crucial mechanism in the reduction of infarct size and attenuation of LV remodeling. The major mechanism of these drugs is the reduction in infarct size as demonstrated by the correlation in Fig 4.

Diazoxide has more effect on the mito-KATP channels than the sarcolemmal KATP channels³⁵ and it also acts strongly on the arterial smooth muscle KATP channels, which dilate the arterioles and cause systemic hypotension.³⁶ Recent evidence shows that the vascular smooth muscle KATP channels are composed of Kir6.1 or 6.2 with SUR2B subunits and that the cardiomyocyte sarcolemmal KATP channels consist of Kir6.2 with SUR2A subunits.^{37,38} Diazoxide is a potent K⁺ channel-opening drug for constructed SUR1/Kir6.2 and SUR2B/Kir6.2 channels, but not for the SUR2A/Kir6.2 channel.³⁹ The dose of diazoxide we used was almost the maximum that could be used in an in vivo study.

Nicorandil acts on both sarcolemmal and mito-KATP channels,⁴⁰ and also has a nitrate effect.⁴¹ The effect of diazoxide was completely blocked by 5-HD, but its effect

on nicorandil was not complete, as shown by Fig 4. This may be attributed to its combined effect, although it was small in the present study. However, Sanada et al showed that in a dog model, the sarcolemmal and mito-KATP channels were equally important in reducing infarct size.¹⁹ The species difference should be noted and we should be careful in extrapolating the data to humans. Recently it has been shown that nitric oxide accelerates the mito-KATP channel opening⁴² and it may be the nitric oxide effect of nicorandil that accelerates the mito-KATP channel opening. It remains to be determined how important this effect of nicorandil is on the opening of mito-KATP channels.

We conclude that chronic LV remodeling after an ischemia–reperfusion insult was suppressed by IP and by mito-KATP channel openers, with a concomitant reduction of the size of the histologically determined infarct after 3 weeks of reperfusion.

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