

Late preconditioning enhances recovery of myocardial function after infarction in conscious rabbits

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Received 28 July 1999; accepted in final form 5 June 2000

Takano, Hitoshi, Xian-Liang Tang, Eitaro Kodani, and Roberto Bolli. Late preconditioning enhances recovery of myocardial function after infarction in conscious rabbits. *Am J Physiol Heart Circ Physiol* 279: H2372–H2381, 2000.—It is unknown whether late preconditioning (PC) enhances the recovery of left ventricular (LV) function after a myocardial infarction. Thus 25 conscious rabbits were subjected to a 30-min coronary occlusion followed by 28 days of reperfusion after PC 24 h earlier with either ischemia or nitric oxide donor administration [*S*-nitroso-*N*-acetylpenicillamine (SNAP)]. The recovery of wall thickening (WTh) after reperfusion was significantly improved in the ischemic PC and SNAP PC groups compared with controls, both at rest and during dobutamine stress. Interestingly, neither ischemia- nor SNAP-induced late PC attenuated myocardial stunning from *day 1* through *day 14*. Infarct size was smaller in the ischemic PC and SNAP PC groups compared with controls. In all groups, WTh at 28 days was positively and linearly related to the percentage of viable tissue in the region underlying the ultrasonic crystal ($r = 0.90$), indicating that the improvement in LV function after both ischemia-induced and NO donor-induced late PC can be fully explained by the reduction in infarct size; a separate effect of late PC on LV remodeling or LV contractility need not be invoked. In conclusion, in conscious rabbits late PC, induced either by ischemia or pharmacologically, not only limits infarct size but also enhances the recovery of LV function after myocardial infarction. This finding has important clinical implications and provides triphenyltetrazolium chloride-independent evidence that late PC limits myocellular death after sustained ischemia.

ischemia-reperfusion injury; myocardial infarction, myocardial stunning, *S*-nitroso-*N*-acetylpenicillamine

THE LATE PHASE OF ISCHEMIC PRECONDITIONING (PC) is the phenomenon whereby a brief ischemic stress induces a delayed cardioprotective adaptation that becomes apparent 12–24 h later (16, 19). Several studies have reported that the late phase of ischemic PC limits infarct size in dogs (16), rabbits (1, 12, 19, 29, 30, 36), rats (35), and mice (8). However, other studies (13, 23, 25, 31) have failed to detect a protective effect. This apparent discrepancy has led some investigators to question whether late PC limits infarction and, in general, has promoted lingering doubts regarding the

very existence of a second (or late) phase of PC that protects against cell death. The reason(s) for the differences among the aforementioned studies (1, 8, 12, 13, 16, 19, 23, 25, 29–31, 35, 36) is unknown. All of these studies (1, 8, 12, 13, 16, 19, 23, 25, 29–31, 35, 36) have relied on histochemistry with triphenyltetrazolium chloride (TTC) staining to identify infarcted tissue. However, in the setting of transient ischemia followed by reperfusion, TTC staining has certain limitations in the accuracy with which it can resolve cell death because the reperfused myocardium consists of a complex admixture of necrotic and viable myocytes, often with clusters of surviving cells alternating with areas of inflammation or necrosis. Thus a method that is independent of TTC staining would be helpful to verify whether the late phase of ischemic PC does protect against lethal ischemic injury.

Accordingly, in the present study, we assessed the cardioprotective effects of late PC by using a different end point: the recovery of regional contractile function. Clinical studies have demonstrated that the residual left ventricular (LV) function is the most important prognostic factor in patients with acute myocardial infarction (26, 27, 32). Because the purpose of investigating ischemic PC in the experimental laboratory is ultimately to translate this knowledge into clinical therapies, it is important to determine the effects of late PC not only on infarct size but also on LV function. If late PC improves residual LV function after myocardial infarction, this would establish the significance of its infarct-sparing effect. If not, then the infarct-sparing effect would have little significance.

Very little is currently known regarding the impact of the late phase of PC on LV performance. Cohen et al. (5) have recently shown that early PC improves functional recovery at 3 days after infarction. With regard to the late phase of ischemic PC, previous studies (29, 30, 34) in conscious rabbits have found that the infarct-sparing effect of ischemia-induced or nitric oxide (NO) donor-induced late PC is associated with a modest improvement in the recovery of LV systolic wall thickening (WTh) in the early period of reperfusion after a 30-min occlusion. These studies (29, 30, 34), however,

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were limited because they examined WTh only during the first 3 days of reperfusion. This time interval is not sufficient to evaluate the full recovery of myocardial function, because myocardial stunning is known to persist for weeks after reperfusion after a sustained, partly irreversible ischemic injury (4, 17).

Whether or not reducing infarct size during the late phase of PC will translate into a significant functional improvement is unclear. Because of the complex transmural distribution of necrosis observed after reperfusion (with peninsulas and islands of necrotic tissue interdigitated with viable tissue) as well as the presence of potentially confounding factors such as LV remodeling (21), scarring of necrotic tissue, etc., it is possible that a reduction in infarct size may not necessarily result in an appreciable improvement in WTh or segment shortening in the reperfused myocardium. Furthermore, the induction of inducible NO synthase (iNOS) associated with late PC (7, 14) raises the possibility that ongoing iNOS-induced apoptosis of surviving myocytes (28) in the days after infarction might negate the infarct-sparing effects observed in the early postinfarction period. In addition, the response of the salvaged myocardium to inotropic challenges after late PC is unknown. Although baseline function may be improved by late PC, the coexistence of surviving tissue with a scar may make it difficult for inotropic stimuli to elicit enhanced contractility, because the salvaged myocardium is "tethered" to fibrotic tissue and viable and fibrotic tissue are intermixed in a complex mosaic. Thus it is conceivable that the inotropic reserve of the salvaged myocardium after late PC may be severely reduced or even nil. To date, no study has examined the effect of late PC on the final recovery of LV function after a myocardial infarction, either under resting conditions or during stress. This issue is crucial to put late PC into a proper prospective in terms of its clinical relevance. Furthermore, establishing whether late PC salvages LV function will help to establish whether late PC does or does not limit lethal ischemia-reperfusion injury, using an end point that is independent of either TTC or histology.

The goal of the present study was to thoroughly evaluate the effects of two different forms of late PC (ischemia-induced and NO donor-induced late PC) on the recovery of LV function after a myocardial infarction, both at rest and during inotropic stress. To this end, we extended the reperfusion period from 3 to 28 days, which enabled us to measure the final extent of contractile recovery, after stunning had resolved. Measurements of systolic WTh were obtained daily throughout the 28-day reperfusion phase. At 28 days, WTh was also analyzed during all inotropic challenge with dobutamine. We used conscious rabbits to avoid the potentially confounding influence of factors associated with open-chest preparations, including anesthesia, cytokine release, exaggerated reactive oxygen species generation, fluctuations in temperature, excessive adrenergic tone, variable loading conditions, and up-regulation of various enzymes such as manganese superoxide dismutase (10) and iNOS (11). The results

demonstrate, for the first time, that late PC (induced either by ischemia or by NO donor administration) results in a permanent improvement of LV function after infarction.

METHODS

Experimental Preparation

The experimental preparation has been described in detail previously (2, 3, 6, 22, 24, 29, 30, 34). Briefly, New Zealand White male rabbits (weight 2.0–2.5 kg, age 3–4 mo) were instrumented under sterile conditions with a balloon occluder around a major branch of the left coronary artery, a 10-MHz pulsed Doppler ultrasonic crystal in the center of the region to be rendered ischemic, and bipolar electrocardiogram leads on the chest wall. The animals were allowed to recover for a minimum of 10 days after surgery.

Experimental Protocol

Throughout the experiments, rabbits were kept in a cage in a quiet, dimly lit room. LV systolic WTh, range gate depth, and the electrocardiogram were recorded throughout the experiments on a thermal array chart recorder (Gould TA6000, Valley View, OH).

All rabbits (except those in the nonischemic control group) were subjected to a 30-min coronary artery occlusion followed by 28 days of reperfusion. Diazepam was administered 20 min before the onset of ischemia (4 mg/kg ip) to relieve the stress caused by the coronary occlusion. No antiarrhythmic agents were given at any time. Rabbits undergoing a 30-min

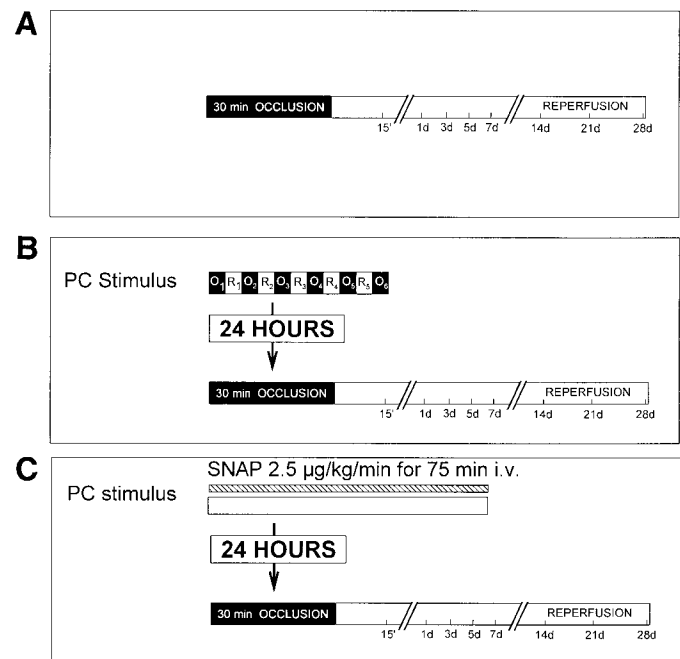


Fig. 1. Experimental protocol. Rabbits in the control group (A) underwent a 30-min occlusion followed by 28 days of reperfusion. Rabbits in the ischemic preconditioning (PC) group (B) underwent a sequence of 6 cycles of 4-min occlusion (O)/4-min reperfusion (R) as a PC stimulus and 24 h later were subjected to a 30-min occlusion followed by 28 days of reperfusion. Rabbits in the *S*-nitroso-*N*-acetylpenicillamine (SNAP) PC group (C) received an intravenous infusion of SNAP at a rate of $2.5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 75 min and 24 h later were subjected to a 30-min occlusion followed by 28 days of reperfusion.

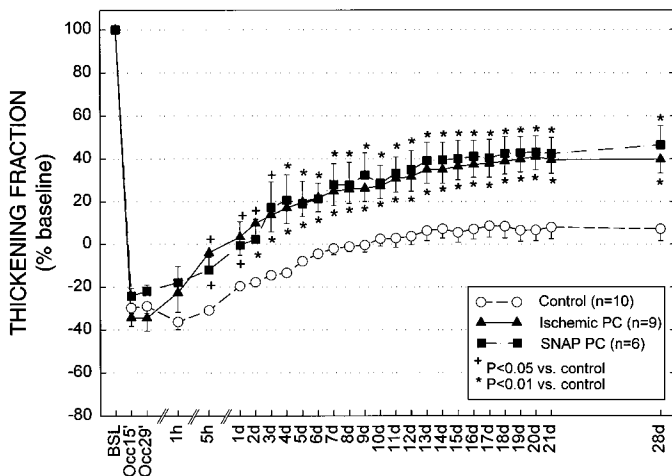


Fig. 2. Systolic thickening fraction in the ischemic-reperfused region in rabbits in the control, ischemic PC, and SNAP PC groups 5 min before the 30-min occlusion [baseline (BSL)], 15 and 29 min into the 30-min coronary occlusion (Occ), and at selected times during the 28-day reperfusion period. Thickening fraction is expressed as a percentage of baseline values. Because of Doppler probe malfunction, complete measurements of thickening fraction could not be obtained in 1 rabbit in the control group after *day 14* of reperfusion; thus data for 9 rabbits in the control group are illustrated from *day 15* to *day 28* of reperfusion. Data are means \pm SE.

occlusion were assigned to three groups (Fig. 1). *Group I* (control group) underwent the 30-min occlusion without PC. *Group II* (ischemic PC group) was preconditioned with a sequence of six 4-min coronary occlusion/4-min reperfusion cycles 24 h before the 30-min coronary occlusion. This is the same protocol that we (2, 3, 6, 22, 24, 29, 30, 34) have used previously to study the late phase of ischemic PC in conscious rabbits. *Group III* [S-nitroso-N-acetylpenicillamine (SNAP) PC group] received an intravenous infusion of SNAP at a rate of $2.5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 75 min 24 h before the 30-min coronary occlusion. SNAP (Sigma Chemical, St. Louis, MO) was dissolved in normal saline (total volume infused ~ 11 ml), and the solution was filtered through a $0.2\text{-}\mu\text{m}$ Millipore filter to ensure sterility. This dose of SNAP induces a robust late PC effect against both myocardial infarction and stunning (29). An additional group of rabbits that did not undergo coronary occlusion (nonischemic control group) was studied only during the dobutamine stress test.

Measurement of Regional Left Ventricular Function

Regional LV function was assessed as systolic WTh using the pulsed Doppler probe, as previously described (2, 3, 6, 22, 24, 29, 30, 34). The measurements were taken at baseline, during the 30-min coronary occlusion, at 1 and 5 h after reperfusion on the day of the occlusion, and daily thereafter until 28 days after reperfusion (Fig. 2). In all animals, measurements from at least 10 beats were averaged at all time points. On *day 28*, hemodynamic and WTh measurements were obtained under resting conditions and during inotropic stimulation with dobutamine. Arterial pressure was measured by cannulating the ear dorsal artery with a 22-gauge angiocatheter under local anesthesia (benzocaine), as previously described (2, 3, 22, 24, 30). A graded intravenous infusion of dobutamine was given at doses of 5, 10, 25, and 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Measurements were obtained after a 5-min infusion at each dose.

Postmortem Analysis

At the conclusion of the study, the rabbits were given heparin (1,000 U iv), after which they were anesthetized with pentobarbital sodium (50 mg/kg iv) and euthanized with KCl. The heart was excised, and the size of the ischemic-reperfused region (region at risk) was determined by tying the coronary artery at the site of the previous occlusion and by perfusing the aortic root for 2 min with a 5% solution of phthalo blue dye in normal saline at a pressure of 70 mmHg using a Langendorff apparatus, as previously described (2, 3, 6, 22, 24, 29, 30, 34). To measure total infarct size, the heart was cut into six to seven transverse slices. Because the Doppler ultrasonic probe senses WTh in a circumscribed area underlying the crystal (~ 5 mm wide; see Ref. 37), we also measured the proportion of viable myocardium in the tissue directly underlying the probe so that measurements of WTh could be correlated with the extent of infarction in the same site. To this end, a square block of tissue ($\sim 5 \times 5$ mm in diameter) underlying the Doppler probe was separated from the slices. This block consisted of two halves located in two adjacent slices (Fig. 3). All LV slices and the two halves of the tissue block were incubated for 10 min at 37°C in a 1% solution of TTC in phosphate buffer (pH 7.4). All atrial and right ventricular tissues were then excised. The slices and the two halves of the tissue block were weighed, fixed in a 10% neutral-buffered formaldehyde solution, and photographed (Nikon AF N6006). Transparencies were projected onto a paper screen at a 10-fold magnification, and the borders of the infarcted (TTC-negative) zone and noninfarcted (TTC-positive) zone within the ischemic-reperfused (blue dye-negative) region and nonischemic (blue dye-positive) zone were traced. The corresponding areas were measured by computerized planimetry (Adobe Photoshop, version

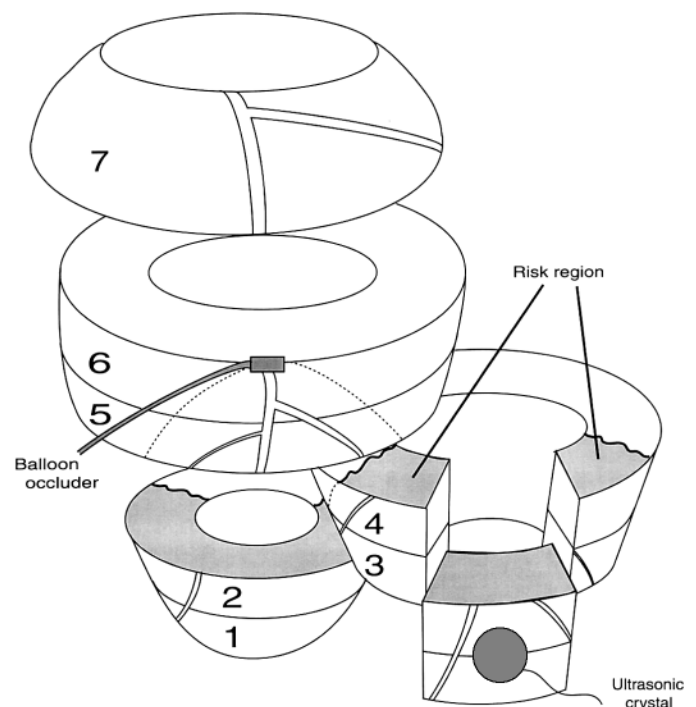


Fig. 3. Technique used to separate the tissue block underlying the Doppler crystal. The left ventricle was cut into 6–7 transverse slices. A square block of tissue ($\sim 5 \times 5$ mm in diameter) underlying the Doppler crystal was separated from 2 adjacent slices. This block consisted of 2 halves, each located in one of the two slices.

4.0). The total infarct size was calculated from the measurements obtained in all of the slices and in the tissue block. The percentage of viable tissue in the tissue block underlying the Doppler probe was calculated from the measurements obtained in the two halves of the block.

Statistical Analysis

Data are reported as means \pm SE. For intragroup comparisons, hemodynamic variables and WTh were analyzed by a one-way ANOVA followed by Student's *t*-tests with the Bonferroni correction (33). For intergroup comparisons, data were analyzed by either a one-way ANOVA or a two-way repeated measures (time and group) ANOVA followed by unpaired Student's *t*-tests with the Bonferroni correction, as appropriate (33). The relationship between the thickening fraction and the percentage of viable tissue at the site of the Doppler probe was assessed by linear regression analysis using the least-squares method. Statistical analyses were performed using SigmaStat for Windows, version 2.0.

RESULTS

Exclusions, Arrhythmias, and Mortality

Twelve rabbits were assigned to the control group, ten to the ischemic PC group, and eight to the SNAP PC group. In the control group, 2 of 12 rabbits (17%) developed ventricular fibrillation and 1 of 12 rabbits (8%) developed ventricular tachycardia during the 30-min occlusion, whereas 2 of 10 rabbits (20%) developed ventricular tachycardia after reperfusion. One control rabbit died within 24 h after reperfusion. In the ischemic PC group, none of the rabbits developed ventricular tachycardia or fibrillation during the 30-min occlusion; one rabbit died of ventricular fibrillation within 60 min after reperfusion. In the SNAP PC group, none of the rabbits developed ventricular tachycardia or fibrillation during the 30-min occlusion or after reperfusion. Overall, 3 of 12 rabbits (25%) died in the control group, 1 rabbit (10%) died in the ischemic PC group, and no rabbits died in the SNAP PC group. The differences in the incidence of severe ventricular arrhythmias (ventricular fibrillation or tachycardia) or mortality between two groups were not statistically significant. After the postmortem analysis was completed, two rabbits in the SNAP PC group were excluded because the ischemic region was found to be $<10\%$ of the LV weight.

Hemodynamics and Regional LV Function

Hemodynamics. There were no significant differences in heart rate among the three groups during the

30-min coronary occlusion and the 28 days reperfusion (Table 1). In the SNAP PC group, heart rate and arterial pressure were measured during the administration of SNAP. Consistent with our previous study (30), an intravenous infusion of SNAP at a rate of $2.5 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 75 min did not cause any hemodynamic changes (heart rate was 255 ± 6 beats/min immediately before SNAP infusion and 265 ± 5 beats/min at the end of the infusion; arterial pressure was 84 ± 3 and 82 ± 2 mmHg, respectively).

Recovery of systolic WTh. WTh measurements were obtained in all rabbits until *day 14* of reperfusion (10 control, 9 ischemic PC, and 6 SNAP PC rabbits). In one rabbit in the control group, the WTh signal was lost on *day 15* because of Doppler probe malfunction; consequently, data from 9 control rabbits, 9 ischemic PC rabbits, and 6 SNAP PC rabbits were analyzed after *day 15* of reperfusion. The baseline WTh fraction, taken before the 30-min occlusion, was similar in the control, ischemic PC, and SNAP PC groups (31.8 ± 1.6 , 30.7 ± 2.3 , $35.1 \pm 2.6\%$, respectively). Figure 2 illustrates the serial measurements of thickening fraction, expressed as a percentage of baseline values, throughout the experimental protocol. During the 30-min coronary occlusion, all three groups exhibited paradoxical systolic wall thinning of comparable severity (Fig. 2). After reperfusion, control rabbits displayed essentially no recovery of WTh throughout the first 5 h of reperfusion. A delayed, slow recovery began 24 h after reperfusion and continued for the ensuing week, after which systolic WTh did not change significantly (Fig. 2). On average, control rabbits exhibited akinesia from *day 7* to *day 28* (Fig. 2).

In the ischemic PC and SNAP PC groups, however, the recovery of systolic WTh was significantly enhanced compared with the control group. This improvement was already statistically significant at 5 h after reperfusion, with the thickening fraction averaging $-4.1 \pm 9.1\%$ in the ischemic PC group, $-12.0 \pm 5.9\%$ in the SNAP PC group, and $-30.9 \pm 1.3\%$ in the control group ($P < 0.05$) (Fig. 2). Over the next 4 wk, the differences between the two preconditioned groups and controls continued to widen; on *day 28*, the thickening fraction averaged $39.8 \pm 6.4\%$ in rabbits preconditioned with ischemia, $46.5 \pm 9.0\%$ in rabbits preconditioned with SNAP, and $7.2 \pm 5.6\%$ in controls ($P < 0.01$) (Fig. 2). The measurements of thickening fraction were significantly greater in both the ischemic PC and SNAP PC groups versus the control group at all time

Table 1. Heart rate during coronary occlusion and reperfusion

	<i>n</i>	Baseline	Occlusion	Reperfusion					
				1 h	1 day	7 days	14 days	21 days	28 days
Control group	10	260 \pm 7	262 \pm 10	270 \pm 9	247 \pm 8	238 \pm 3	241 \pm 6	239 \pm 15	240 \pm 14
Ischemic PC group	9	261 \pm 9	259 \pm 13	272 \pm 8	255 \pm 9	247 \pm 5	232 \pm 11	250 \pm 6	240 \pm 13
SNAP PC group	6	266 \pm 6	274 \pm 6	279 \pm 5	259 \pm 6	254 \pm 7	244 \pm 5	253 \pm 8	248 \pm 8

Data are means \pm SE; *n* represents the number of rabbits in each group. All rabbits underwent a 30-min coronary occlusion followed by 28 days of reperfusion. Heart rate was measured 5 min before occlusion (baseline), at 15 min into the 30-min coronary occlusion, and at selected times after reperfusion. PC, preconditioning; SNAP, *S*-nitroso-*N*-acetylpenicillamine.

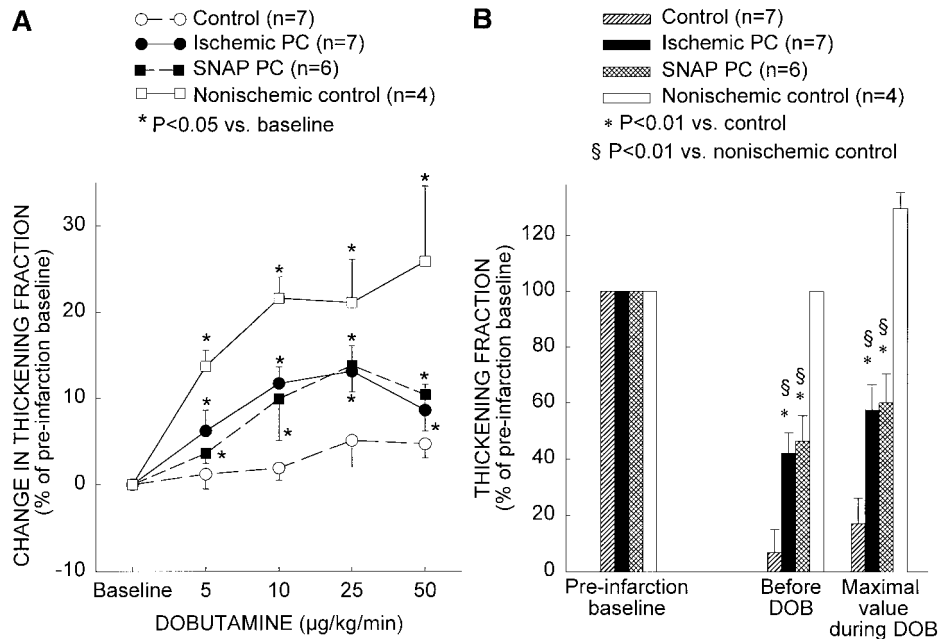


Fig. 4. **A**: increase in thickening fraction during incremental infusion of dobutamine at 5, 10, 25, and 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in rabbits subjected to a 30-min coronary occlusion without any intervention (control group), in rabbits subjected to the 30-min occlusion 24 h after PC with ischemia or SNAP (ischemic PC and SNAP PC groups, respectively), and in rabbits not subjected to coronary occlusion/reperfusion (nonischemic control group). In the control, ischemic PC, and SNAP PC groups, the response to dobutamine was evaluated at 28 days after the 30-min occlusion; the increase in thickening fraction is expressed as a percentage of the preinfarction baseline values (values measured before the 30-min coronary occlusion). Measurements were obtained after a 5-min infusion at each dose of dobutamine. Data are means \pm SE. **B**: thickening fraction measured 5 min before the 30-min coronary occlusion (preinfarction baseline), at 28 days after reperfusion, before dobutamine (DOB), and during the infusion of dobutamine. The maximal value of thickening fraction recorded at any time during the infusion of 4 incremental doses of dobutamine is illustrated. Thickening fraction is expressed as a percentage of preinfarction baseline. Data are means \pm SE.

points from 5 h through 28 days of reperfusion. The time course of recovery was similar in all three groups, with a plateau of WTh noted at \sim 2 wk (Fig. 2). Thus ischemic PC and NO donor-induced PC resulted in a greater final recovery of regional function but did not shorten the time necessary for the recovery to be complete.

Response to inotropic stimulation. At 28 days after reperfusion, when the recovery of WTh was complete, seven rabbits in the control group, seven rabbits in the ischemic PC group, and six rabbits in the SNAP PC group were subjected to an inotropic challenge with dobutamine to measure contractile reserve. (Because 3 rabbits in the control group and 2 in the ischemic PC group did not undergo dobutamine challenge, WTh at the *day 28* time point in Fig. 2 differs from that measured at the predobutamine time point in Fig. 4.) Four additional rabbits not subjected to coronary occlusion/reperfusion (nonischemic control group) were included for comparison. The infusion of dobutamine at four incremental doses (5, 10, 25, and 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was associated with a dose-dependent increase in heart rate in all three groups, which became statistically significant at 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in the control group and 25 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in the PC groups (Table 2). Mean arterial pressure decreased significantly at the two higher doses in all three groups (Table 2). There

were no significant differences in heart rate or arterial pressure among the three groups with any dose of dobutamine.

Because the predobutamine baseline thickening fraction was vastly different in the three groups with infarction (39.8 ± 6.4 , 45.0 ± 8.2 , and 7.2 ± 5.6 of preinfarction baseline in the ischemic PC, SNAP PC, and control groups, respectively), it was not possible to express the changes in thickening fraction as a percentage of predobutamine baseline values, since relatively small changes in the control group would have resulted in large percentage changes. Consequently, the changes in the thickening fraction were expressed either as absolute measurements (Table 2) or as percentage of the preinfarction baseline (Fig. 4). This analysis is more appropriate also because the goal of the inotropic challenge was not to examine the percentage changes in WTh from predobutamine levels, but rather to determine whether the maximal recruitable levels of WTh during stress were affected by ischemia-induced and NO donor-induced PC. The preinfarction baseline values of thickening fraction averaged $31.2 \pm 1.6\%$ in the control group, $30.7 \pm 2.3\%$ in the ischemic PC group, and $35.1 \pm 2.6\%$ in the SNAP PC group; the corresponding absolute values of systolic WTh were 0.93 ± 0.09 , 0.87 ± 0.10 , and 0.95 ± 0.06 mm, respectively. As shown in Fig. 4A, the three lower doses of

Table 2. Hemodynamic changes during infusion of dobutamine

	Predobutamine Baseline	Dobutamine, $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$			
		5	10	25	50
HR					
Control	236 \pm 7	241 \pm 17	251 \pm 7*	277 \pm 4*	302 \pm 4*
Ischemic PC	234 \pm 7	234 \pm 9	238 \pm 13	272 \pm 16*	309 \pm 12*
SNAP PC	248 \pm 8	246 \pm 6	255 \pm 11	280 \pm 12*	297 \pm 17*
Nonischemic control	269 \pm 16	271 \pm 18	275 \pm 19	287 \pm 17	290 \pm 17
MAP					
Control	72 \pm 2	75 \pm 1	70 \pm 4	67 \pm 6*	62 \pm 6*
Ischemic PC	76 \pm 2	74 \pm 2	76 \pm 4	70 \pm 4*	64 \pm 5*
SNAP PC	78 \pm 2	77 \pm 3	71 \pm 3	69 \pm 4*	67 \pm 5*
Nonischemic control	75 \pm 3	68 \pm 4*	64 \pm 5*	63 \pm 6	64 \pm 7
Thickening fraction					
Control	3.0 \pm 2.4	3.2 \pm 2.5	3.5 \pm 2.2	4.5 \pm 1.9	4.4 \pm 2.1
Ischemic PC	12.2 \pm 2.1	13.9 \pm 2.0	15.6 \pm 2.2*	16.1 \pm 2.9*	14.8 \pm 2.6*
SNAP PC	16.2 \pm 3.4	17.5 \pm 3.3	19.5 \pm 4.0*	20.9 \pm 3.7*	19.9 \pm 10.7*
Nonischemic control	28.2 \pm 4.2	32.1 \pm 2.1*	34.3 \pm 2.1*	34.2 \pm 2.3*	35.5 \pm 3.0

Data are means \pm SE. A graded intravenous infusion of dobutamine was given at doses of 5, 10, 25, and 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ to rabbits subjected 28 days earlier to a 30-min coronary occlusion without any intervention (control group) and to rabbits subjected to the 30-min occlusion 24 h after PC with ischemia or SNAP (ischemic PC and SNAP PC groups, respectively). The heart rate (HR; beats/min), mean arterial pressure (MAP; mmHg), and thickening fraction were recorded before dobutamine (baseline) and after a 5-min infusion at each dose. * $P < 0.05$ versus baseline values.

dobutamine (5, 10, and 25 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) elicited a dose-dependent increase in thickening fraction in the ischemic PC and SNAP PC groups but little or no response in the control group. No further increase in the thickening fraction was observed at the highest dose (50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) in any group (Fig. 4A). In the ischemic PC and SNAP PC groups, the thickening fraction was statistically different from baseline during each of the four doses of dobutamine ($P < 0.05$). In contrast, the thickening fraction during dobutamine did not differ significantly from baseline ($P =$ not significant) in the control group, even at the higher doses (Fig. 4A). Importantly, the maximal levels of the thickening fraction attained during the infusion of dobutamine were significantly greater in the ischemic PC and SNAP PC groups compared with the control group (Fig. 4B). As expected, the response to dobutamine in the ischemic PC and SNAP PC groups, albeit greater than that in the control group, was significantly less than that in the nonischemic control group (which did not undergo coronary occlusion/reperfusion; Fig. 4).

Postmortem Analysis

In all rabbits included in the final analysis, the Doppler crystal was found to be in the center of the ischemic-reperfused region, at least 3 mm from the borders of the nonischemic region. There were no significant differences among the control, ischemic PC, and SNAP PC groups with respect to the weight of the region at risk [0.63 \pm 0.05 g (15.5 \pm 1.07% of LV weight), 0.72 \pm 0.08 g (16.8 \pm 1.8% of LV weight), and 0.60 \pm 0.02 g (14.6 \pm 0.8% of LV weight), respectively]. The average total infarct size was 50% smaller in the ischemic PC and SNAP PC groups compared with the control group (28.0 \pm 4.1, 27.7 \pm 6.2, and 55.2 \pm 4.6 of the region at risk, respectively, $P < 0.05$; Fig. 5A), confirming the infarct-sparing effect of late PC. Con-

sistent with this, the extent of infarction in the tissue block underlying the Doppler probe was 50–60% less in the ischemic PC and SNAP PC groups compared with the control group (32.8 \pm 6.5%, 34.3 \pm 10.7% and 71.8 \pm 5.5% of the tissue block, respectively, $P < 0.05$; Fig. 5B). Interestingly, the extent of infarction in the tissue block underlying the Doppler probe correlated closely with infarct size in the entire risk region ($r = 0.90$ for the control, ischemic PC, and SNAP PC groups combined; Fig. 5C). It should be noted that, in control rabbits, the average infarct size measured at 28 days in this study (55% of the risk region) did not differ appreciably from that previously measured at 3 days in this same conscious rabbit model ($\sim 57\%$ of the risk region; see Refs. 22, 29, and 34). The reasons for this probably include the relatively small size of the region at risk ($\sim 16\%$ of LV) and the nontransmural nature of these reperfused infarcts, both of which would be expected to limit LV remodeling and infarct shrinking due to scar development.

The levels of WTh measured at the end of the follow-up period (day 28) were linearly and positively related both to the percentage of viable tissue in the block underlying the Doppler probe ($r = 0.90$ for the control, ischemic PC, and SNAP PC groups combined; Fig. 6A) and to the total percentage of viable tissue in the entire risk region ($r = 0.84$ for the three groups combined; Fig. 6B). The individual measurements obtained in control and preconditioned rabbits appeared to lie along the same regression line (Fig. 6), demonstrating that the differences in the recovery of WTh among the ischemic PC and SNAP PC groups and the control group can adequately be accounted for by differences in infarct size. This indicates that the salutary action of both ischemia-induced and NO donor-induced late PC on the recovery of WTh is a consequence of an

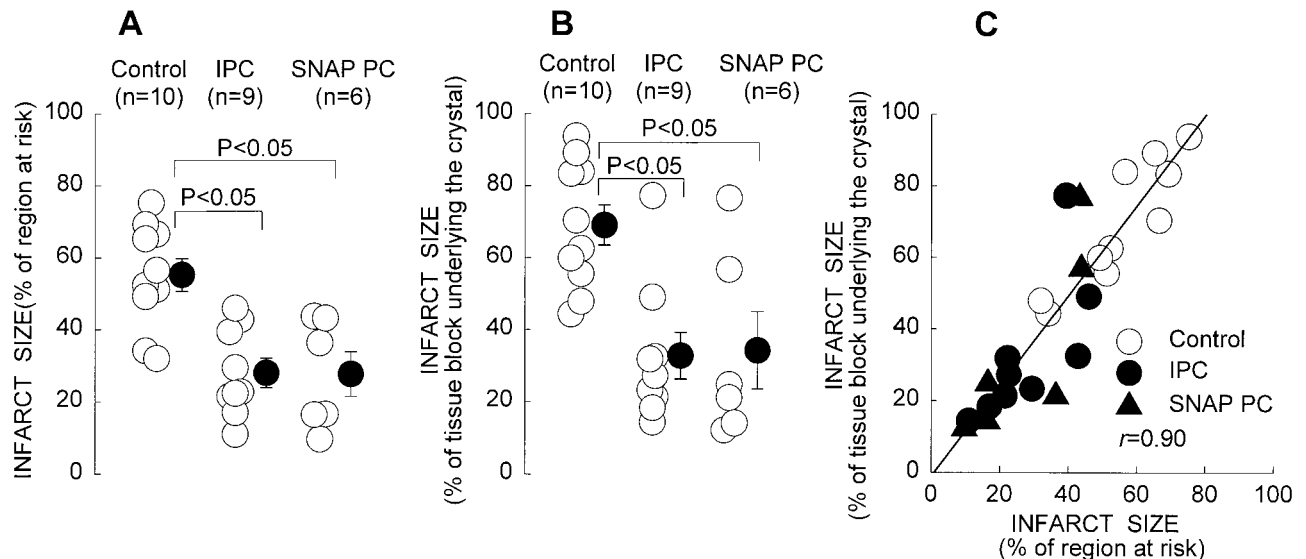


Fig. 5. *A*: myocardial infarct size (expressed as a percentage of the region at risk) in the control and PC groups. *B*: infarct size (expressed as a percentage of the tissue block underlying the Doppler crystal) in the 3 groups. In both *A* and *B*, open circles represent individual rabbits, whereas solid circles represent means \pm SE. *C*: relationship between infarct size in the tissue block underlying the Doppler crystal and infarct size in the entire risk region. The regression line was obtained by linear regression analysis from all values in all 3 groups. Infarct size in the tissue block underlying the crystal was positively and linearly related to that in the entire region at risk. The regression equation was $y = 1.26x - 1.11$ ($r = 0.90$). IPC, ischemic PC; SNAP PC, SNAP-induced PC.

infarct-sparing effect rather than of a specific effect of late PC on myocardial function.

DISCUSSION

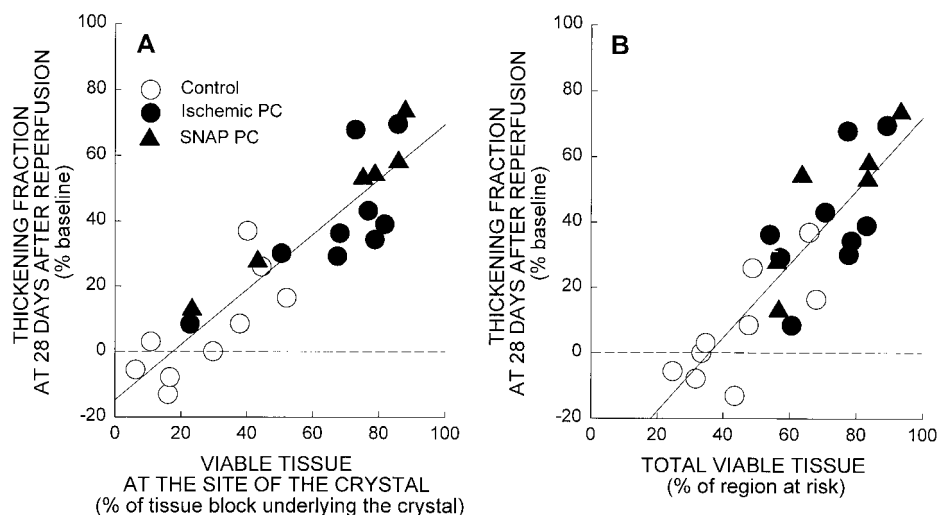
This study reveals a beneficial effect of the late phase of ischemia-induced PC and NO donor-induced PC that had not been demonstrated heretofore, that is, a significant and sustained improvement in the recovery of regional myocardial function after infarction, both at rest and during stress. Thus, in addition to limiting tissue destruction, late PC also salvages contractile function. This concept has not been documented before. Whereas previous investigations (5, 22, 29, 30) have shown a salutary influence of ischemia-induced early or late PC on regional contractile performance soon

after myocardial infarction (first few hours or days), to our knowledge, this is the first study to demonstrate that late PC improves the final recovery of LV function after 4 wk, a reperfusion interval sufficient for myocardial stunning to resolve. Furthermore, this is the first report that the salutary effects of ischemia-induced late PC on LV function can be reproduced pharmacologically by the administration of NO donors, a class of clinically-relevant PC mimetics.

Controversy Regarding Late PC

Although many investigators (16, 19, 22, 29, 30, 34–36) have found that the late phase of ischemic PC is associated with a decrease in infarct size, others (13, 23, 25, 31) have not. With the use of open-chest rabbits,

Fig. 6. *A*: relationship between thickening fraction at 28 days after reperfusion and the percentage of viable tissue in the block underlying the Doppler crystal. *B*: relationship between thickening fraction at 28 days after reperfusion and the percentage of viable tissue in the entire region at risk. The regression lines were obtained by linear regression analysis from the pooled individual values in all 3 groups. Thickening fraction was positively and linearly related to the percentage of viable tissue, both in the block underlying the Doppler crystal and in the entire region at risk. The linear regression equations were $y = 0.84x - 14.8$ ($r = 0.91$) and $y = 1.12x - 40.3$ ($r = 0.86$), respectively.



Marber et al. (19) described delayed protection when the animals were preconditioned with four cycles of 5-min occlusion/10-min reperfusion 24–72 h before the 30-min occlusion. Downey and co-workers (36) were the first to report that this protection was observed with the same PC protocol (4 cycles of 5-min occlusion/10-min reperfusion) in a conscious rabbit model, which is believed to be a more stable preparation to study ischemic PC. On the other hand, Tanaka et al. (31) failed to detect any infarct-limiting effect of PC when rabbits were subjected to the same protocol used in the two aforementioned studies (19, 36). Jagasia et al. (13) and Qian et al. (23) failed to detect late PC in rats, and Qiu et al. (25) did not observe a significant reduction in infarct size in pigs that were preconditioned 24 h earlier with an ischemic protocol that was effective in eliciting robust protection during the early phase of PC (25). Miki et al. (20) recently found reduction in infarct size at 3 h (using TTC) but not at 3 days (using histology). As a consequence of these conflicting reports, concerns have been raised that the infarct-sparing effects of late PC may be dependent upon the technique used to delineate infarcted myocardium (macrohistochemistry with TTC staining). The present study addresses these concerns by furnishing TTC-independent evidence of protection. Our finding that late PC salvages basal and recruitable myocardial function supports the concept that the reduction in infarct size observed in previous studies (16, 19, 22, 29, 30, 34–36) represents true salvage of tissue rather than an artifact of TTC staining.

Effect of Late PC on Recovery of Function

Cohen et al. (5) have recently shown that the early phase of ischemic PC is associated with enhanced segment shortening at 3 days after infarction. No information is available regarding the long-term effects of late PC on LV function. Therefore, it is unknown whether the salvage of tissue afforded by late PC is sufficient to enhance contractile performance or contractile reserve. We (22, 29, 30, 34) have previously shown that late PC not only reduces infarct size but also produces a modest improvement in the recovery of WTh during the first 3 days after a 30-min coronary occlusion. However, the limitation of those studies (22, 29, 30) was that a 3-day reperfusion period was not long enough to evaluate myocardial function after infarction because myocardial stunning persists for longer than 3 days in this setting (4, 17). Therefore, those results cannot distinguish between an antistunning effect and an infarct-sparing effect of late PC. A robust antistunning effect of late PC has been demonstrated 24–72 h after brief, reversible ischemia (6 cycles of 4-min occlusion/reperfusion; see Refs. 2, 3, 22, 24, 30, and 34), and it is entirely possible that the improvement in WTh observed at 3 days after infarction (22, 29, 30) simply represents an antistunning action of late PC, in which case it would be short-lived (i.e., the differences in WTh would disappear after a longer period of reperfusion). If this were the case, the

improvement in WTh would have limited significance. Our finding that the differences in WTh after reperfusion were sustained and actually continued to widen for 15 days after reperfusion (Fig. 2) clearly demonstrates that late PC does not merely afford a transient enhancement of contractile performance secondary to mitigation of myocardial stunning but instead produces a long-lasting augmentation of the final levels of regional myocardial function in the salvaged tissue.

As illustrated in Fig. 2, we found that WTh in control rabbits continued to improve gradually for 2 wk after reperfusion and then plateaued, indicating that myocardial stunning lasts ~2 wk in this model. This is similar to the duration of stunning observed after a partly irreversible ischemic insult in conscious dogs (4, 17) and baboons (9). Interestingly, the interval required to attain the maximal levels of WTh after reperfusion was not shorter in the PC groups compared with the control group (Fig. 2), indicating that late PC failed to protect against myocardial stunning.

Relationship Between Regional Function and Infarct Size

We analyzed the relationship between WTh and viable tissue at 28 days of reperfusion (Fig. 6) to determine whether the former is linearly related to the latter in this ischemia-reperfusion model and whether the improved WTh is a consequence of the infarct-sparing action of late PC or of other factors [e.g., alterations of LV remodeling (21) or an independent effect of late PC on contractility]. In the latter case, one would expect that, for any given amount of viable tissue, PC rabbits would exhibit a higher level of WTh compared with control rabbits. Instead, the data displayed in Fig. 6 demonstrate that control and preconditioned rabbits lie along the same linear regression line, both when the amount of viable tissue was measured as a percentage of the block underlying the crystal (Fig. 6A) and as a percentage of the region at risk (Fig. 6B). The close correlation ($r = 0.88$ and 0.84 , respectively) indicates that the variance in the amount of viable tissue accounted for 77 and 71%, respectively, of the total variance in WTh. Thus, for a given infarct size, the level of functional recovery appeared to be similar irrespective of whether or not rabbits were preconditioned (Fig. 6). The regression lines calculated from our data indicate that WTh was still depressed (~20–30% below baseline) with very small infarcts (Fig. 6). This is likely the result of the tethering of viable myocytes by adjacent fibrotic tissue. We recognize that measurements of infarct size taken at 28 days may not accurately reflect the amount of myocardium initially salvaged, owing to changes in the geometry of both the risk region and the scar (15, 18, 21). Nevertheless, measurements taken at 28 days do reflect the amount of scarred and viable tissue present at that time. We conclude that the enhancement of regional myocardial function afforded by late PC can be fully accounted for by the salvage of myocardium; thus a separate effect of LV remodeling or a specific action of late PC on LV

function need not be invoked. The results of this analysis also predict that changes in the amount of salvaged myocardium will result in quantitatively similar changes in regional function.

Response to Inotropic Challenge

Having found that late PC preserves resting LV function, we next determined whether it also enhances recruitable function during stress. We found that infusion of dobutamine at 28 days after infarction resulted in a significant, dose-dependent increase in WTh in the ischemic PC and SNAP PC groups but not in the control group (Fig. 4B). The relatively small additional improvement observed in the PC group with 25 versus 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, and the deterioration observed with 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (Fig. 4B), probably reflect the marked increase in heart rate elicited by these high doses of dobutamine (Table 2), because WTh is known to be highly dependent on the duration of the diastolic filling period. Importantly, the maximal levels of thickening fraction attained during dobutamine stress were considerably higher in preconditioned versus control rabbits (Fig. 4B). Thus both ischemia-induced and NO donor-induced late PC improve not only the resting levels of myocardial function but also the inotropic reserve of the salvaged myocardium.

In conclusion, the present observations add a new dimension to the phenomenon of late PC. The results presented herein demonstrate that either ischemic or pharmacological PC 24 h before myocardial infarction enhances the final recovery of LV function in conscious rabbits and that this salutary effect is associated with augmented inotropic reserve during dobutamine infusion. These findings extend previous acute or short-term studies (5, 22, 29, 30) by indicating that the cardioprotective effects of late PC are maintained for 4 wk and thus can be regarded as permanent. Because residual LV function is the most powerful predictor of mortality in patients surviving myocardial infarction (5, 26, 32), these results have potential implications with respect to the clinical significance of late PC, particularly in view of the fact that an improvement in LV function essentially equivalent to that afforded by ischemic PC can be attained with NO donors, a class of compounds that are available for use in patients. In addition, this study suggests that the mechanism for the improved recovery of myocardial function after PC is the reduction in infarct size per se rather than a hypothetical separate effect of late PC on LV remodeling or on LV contractility. Finally, the present results provide TTC-independent evidence that late PC limits myocellular death after sustained ischemia and thus should help to resolve the existence of an infarct-sparing effect of late PC.

We gratefully acknowledge Gregg Shirk, Wen-Jian Wu, and Dr. Zhongtuo Tan for expert technical assistance and Trudy Keith for expert secretarial assistance.

This study was supported in part by National Heart, Lung, and Blood Institute Grants R01 HL-43151 and HL-55757 (to R. Bolli), by American Heart Association Ohio Valley Affiliate Grant 9951533V (to X.-L.Tang), by the American Heart Association Ohio Valley Af-

iliate Fellowship Award 9804558 (to H. Takano), and by the Medical Research Grant Program of the Jewish Hospital Foundation, Louisville, Kentucky. H. Takano was an International Research Fellow from Nippon Medical School, Tokyo, Japan.

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