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Preconditioning in Intact and Previously Diseased Myocardium: Laboratory or Clinical Dilemma?

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ABSTRACT

We studied the effects of various cycles of preconditioning (PC) (one cycle, 1×PC; two cycles, 2×PC; three cycles, 3×PC; and four cycles, 4×PC) on cardiac function, infarct size, and the incidence of reperfusion-induced arrhythmias in isolated hearts obtained from rabbits with hypercholesterolemia. After 8 weeks of hypercholesterolemia, hearts were subjected to 30 min of ischemia followed by 120 min of reperfusion. Various cycles of PC resulted in a "cycle-dependent" reduction in infarct size in the age-matched nonhypercholesterolemic group. In the 8-week hypercholesterolemic group, increasing cycles of PC resulted in a significant increase in infarct size from their nonpreconditioned ischemic/reperfused control value of 44 ± 5% to 45 ± 6%, 49 ± 5%, 59 ± 6% ($p < 0.05$), and 58 ± 5% ($p < 0.05$), respectively. PC increased the vulnerability of the myocardium to reperfusion-induced arrhythmias in hypercholesterolemics indicating that PC may be an "intact heart" phenomenon. The effects of PC appear currently to be a dilemma in laboratories and clinics. The solution to the problem of PC in intact and diseased myocardium requires further data from two different sources: (a) previously "diseased" animals, and (b) diseased human myocardium from clinics. Once these data are available, then the effects under which PC will be beneficial rather than harmful could be established and the dilemma solved. *Antioxid. Redox Signal.* 6, 325–333.

INTRODUCTION

SUDDEN CARDIAC DEATH most frequently results from chronic heart failure and malignant ventricular arrhythmias related to the short- and long-term effects of ischemic heart disease. These pathophysiological changes result from electrophysiological and metabolic alterations due to acute ischemia, as well as structural alterations related to previous infarction. Since the discovery by Murry *et al.* (43), cardiac preconditioning (PC) of the myocardium has come to be emphasized as one of the most consistently powerful and reproducible interventions of delaying the development of ischemic injury. The cardiac protection associated with PC can be seen immediately, the so-called "first window of protection," but disappears rapidly. A new concept has been later discovered, introduced, and termed as the "second window of cardiac protection" (39). Since the discovery of the first and second window of cardiac protection, a considerable body of evidence has been accumulated indicating that PC indeed offers a substantial cardiac protection against ischemia-induced damage in intact myocardium. Concerning the underlying mechanisms, the PC has been suggested to promote the formation and release of various endogenous protective substances, such as nitric oxide, bradykinin, prostacyclin, and adenosine, that are supposed to mediate the antiischemic and antiarrhythmic effects (7, 29, 33) in ischemic/reperfused myocardium. However, PC and the great majority of animal studies, as well as the proposed PC mechanisms, have been done, without any preliminary coronary or myocardial diseases, in intact rabbit, rat, dog, and pig myocardium. Thus, the results obtained are of limited significant pathophysiological and clinical relevance, leading to a laboratory and clinical dilemma.

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Hyperlipidemia and atherosclerosis and their major clinical sequel, the ischemic heart disease, constitute the leading cause of death in modern and industrialized society. The myocardium of hyperlipidemic and atherosclerotic patients is hardly capable of adapting to physical exercise and various kinds of stress. The present study was designed to examine whether an impaired capability of the myocardium to adapt to repeated ischemic insults, the PC, was involved in the mechanisms responsible for the increased severity of ischemic attacks in rabbit hearts with hypercholesterolemia (HC) induced by exposure to a cholesterol-enriched diet. Herein, the presented data suggest that the protective effects of various cycles of PC are lost with the development of HC, in previously diseased myocardium, in hearts subjected to a prolonged period of myocardial ischemia. Thus, the PC must be an "intact heart" phenomenon, and the adaptation of PC and its mechanisms to an actual clinical situation should be carefully reevaluated.

MATERIALS AND METHODS

Animals and induction of HC

The experiments were carried out with adult male New Zealand white rabbits weighing 2.2–2.6 kg. Animals received humane care in compliance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research prepared by the National Academy of Sciences (publication no. 86-23, revised 1985). Age-matched control rabbits were fed ordinary laboratory chow, whereas in the hypercholesterolemic groups, rabbits received laboratory chow enriched with 1.0% cholesterol for 8 weeks *ad libitum* (51). Serum cholesterol levels were measured after 0, 2, 4, 6, and 8 weeks of the cholesterol-enriched diet by an automatic analyzer using Boehringer cholesterol kits (Ingelheim, Germany).

Isolated working rabbit hearts

At the end of the cholesterol-enricheddiet period, intravenous heparin (1,000 IU/kg) and ketamine/xylazine (40/5 mg/kg) were intravenously injected. Following the thoracotomy, the heart was excised and placed in ice-cold perfusion buffer. Immediately after preparation, the aorta was cannulated, and the heart was perfused according to the Langendorf method for a 5-min washout period at a constant perfusion pressure equivalent to 100 cm of water (10 kPa). The perfusion medium consisted of a modified Krebs–Henseleit bicarbonate buffer: 118 m*M* NaCl, 4.7 m*M* KCl, 1.7 m*M* $CaCl_2$, 25 m*M* $NaHCO_3$, 0.36 m*M* KH_2PO_4, 1.2 m*M* $MgSO_4$, and 10 m*M* glucose. The left atrium was cannulated, and the Langendorff system was adapted and switched to the isolated working rabbit heart, as initially described by Neely *et al.* (45) in rat heart, with a left atrial filling pressure of 17 cm of buffer (1.7 kPa) and aortic afterload pressure of 100 cm (10 kPa) of buffer. Aortic flow (AF) was measured by a calibrated flow meter (Gilmont Instruments, Barrington, IL, U.S.A.), and coronary flow (CF) rate was measured by a timed collection of the coronary perfusate that dripped from the heart.

Induction of global ischemia and reperfusion

After a 10-min aerobic perfusion of the heart, the atrial inflow and aortic outflow lines were clamped at a point close to the origin of the aortic cannula, and the peristaltic pump (Masterflex) was stopped. Reperfusion was initiated by unclamping the atrial inflow and aortic outflow lines. To eliminate the incidence of arrhythmias, the initial 10 min of reperfusion was carried out in Langendorff mode. To prevent the myocardium from drying out during normothermic global ischemia, the thermostated glassware (in which hearts were suspended) was covered and the vapor content was kept at a constant level.

Ischemia and reperfusion in preconditioned hearts

In noncholesterolemic age-matched controls, and 8-week hypercholesterolemic groups ($n = 6$ in each group), one, two, three, and four cycles of PC (1×PC, 2×PC, 3×PC, and 4×PC, respectively), each consisting of 5 min of global ischemia followed by 5 min of reperfusion, were carried out before the induction of 30 min of normothermic global ischemia followed by 120 min of reperfusion. One to four cycles of ischemic PC were selected for our studies in the isolated working rabbit heart, because generally one to four PC cycles protected the ischemic myocardium, in various degree, against the incidence of reperfusion-induced damage in the models of PC in intact myocardium (10, 20, 26, 29, 41, 42, 55, 57, 58, 72). PC studies have been carried out after 8 weeks of cholesterol diet, and comparisons were made with the age-matched noncholesterolemic groups. Cardiac function was registered before the induction of normothermic global ischemia, and after 60 and 120 min of reperfusion.

Indices measured

Blood samples were obtained intravenously from all rabbits, and serum cholesterol was measured. An epicardial electrocardiogram (ECG) was recorded by a polygraph throughout the experimental period by two silver electrodes attached directly to the heart. The ECGs were analyzed to determine the incidence of reperfusion-induced ventricular fibrillation (VF) and ventricular tachycardia (VT). The heart was considered to be in VF if an irregular undulating baseline was apparent on the ECG. VT was defined as four or more consecutive premature ventricular complexes. The heart was considered to be in sinus rhythm if normal sinus complexes occurring in a regular rhythm were apparent on the ECG. Before ischemia and during reperfusion, heart rate (HR), CF, and AF rates were registered. Left ventricular developed pressure (LVDP), which was defined as the difference between left ventricular systolic and end-diastolic pressure, was also recorded by the insertion of a Millar catheter into the left ventricle via the left atria and mitral valve.

Determination of infarct size

Hearts for infarct size measurement were perfused, at the end of each experiment, with 100 ml of 1% triphenyltetrazolium (TTC) solution in phosphate buffer (88 m*M* Na_2HPO_4, 1.8 m*M* NaH_2PO_4) via the side arm of the aortic cannula and then stored at −70°C for later analysis. Frozen hearts were sliced transversely (56) in a plane perpendicular to the apico-basal axis into 2–3 mm thick sections, weighed, blotted dry, placed between microscope slides, and scanned on a Hewlett–Packard Scanjet 5p single-pass flat-bed scanner (Hewlett–Packard, Palo

Alto, CA, U.S.A.). Using the NIH Image 1.61 image processing software, each digitized image was subjected to equivalent degrees of background subtraction, brightness and contrast enhancement for improved clarity, and distinctness. Infarct zones of each slice were traced, and the respective areas were calculated in terms of pixels (15). The areas were measured by computerized planimetry software; these areas were multiplied by the weight of each slice, and then the results summed up to obtain the weight of the risk zone (total weight of the left ventricle, in grams) and the infarct zone (in grams). Infarct size was expressed as the ratio, in percent, of the infarct zone to the risk zone (weight of the left ventricle).

Statistical analysis

Results are expressed as means ± SEM ($n = 6$ in each group). One-way analysis of variance was first carried out to test any differences between the mean values of different groups. If differences were established, the results between two groups were compared by Dunnett's test. Because the incidence of reperfusion-induced VT and VF followed a nonparametric distribution, therefore, a χ^2 test was used for the statistical analysis of reperfusion-induced VT and VF. Results were considered to be significant if $p < 0.05$.

RESULTS

Serum cholesterol (Fig. 1) was monitored and significantly increased (about sixfold) after 2 weeks of the cholesterol diet, and reached a constant level (~12-fold) after 4-, 6-, and 8-week periods in all groups. Table 1 shows the absolute values for preischemic cardiac function in all nonhypercholesterdemic and hypercholesterolemic groups. Thus, statistically significant differences were found in cardiac function between the nonhypercholesterolemic age-matched controls and rabbits subjected to 8-week cholesterol diet (hypercholesterolemic groups). Under aerobic conditions, all preischemic values in HR, CF, AF, and LVDP were significantly reduced (Table 1, $^{\dagger}p < 0.05$) in the 8-week hypercholesterolemic groups in comparison with the corresponding nonhypercholesterolemic age-matched control groups, indicating the development of HC-induced cardiac failure (Table 1).

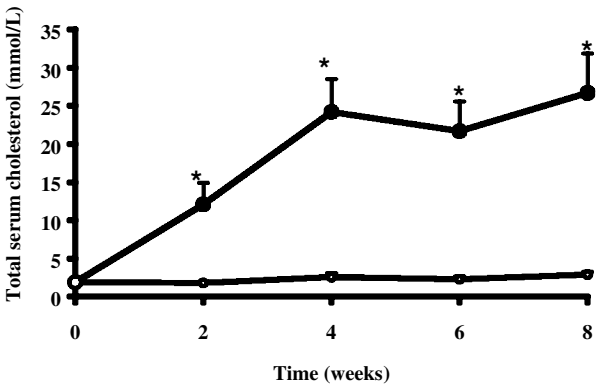

FIG. 1. Serum cholesterol during the 8-week cholesterol diet. Data are means ± SEM ($n = 6$ in each group). $*p < 0.05$ compared with the age-matched nonhypercholesterolemic control values at each time point. ○, nonhypercholesterolemic controls; ●, hypercholesterolemic groups.

Table 1 shows the postischemic recovery of cardiac function after 30 min of ischemia followed by 60 min and 120 min of reperfusion, without PC, in nonhypercholesterolemics and in rabbits subjected to 8-week cholesterol diet. Thus, 8-week HC reduced the recovery of myocardial function upon reperfusion compared with the 8-week age-matched nonhypercholesterolemics showing a reduced resistance to ischemia/reperfusion injury in the previously diseased myocardium.

A further question was whether 1×PC, 2×PC, 3×PC, and 4×PC could protect the ischemic/reperfused myocardium obtained from previously diseased (HC) animals in comparison with the well known protection afforded by PC in intact myocardium. Hearts from age-matched nonhypercholesterolemic controls and 8-week (Table 1) hypercholesterolemics were subjected to one, two, three, and four cycles of PC before the induction of 30 min of ischemia followed by 120 min of reperfusion, and cardiac function was monitored. Table 1 shows, in agreement with many previous studies (5, 6, 15, 25, 30, 42, 57), that PC, independent of the numbers of PC cycles, does attenuate the ischemia/reperfusion-induceddamage during reperfusion in the hearts obtained from intact animals. In our studies, the data show (Table 1) and confirm the results of many "preconditioning investigators" that 3×PC and 4×PC significantly improved ($*p < 0.05$) the recovery of CF, AF, and LVDP in isolated hearts obtained from intact animals and subjected to ischemia/reperfusion. However, in our studies, PC-induced cardiac protection was not detected during reperfusion in hearts obtained from intact rabbits and subjected to 1×PC or 2×PC. Furthermore, in contrast with the positive results obtained from previously intact myocardium subjected to various cycles of PC and ischemia/reperfusion, in the 8-week hypercholesterolemic hearts, three or four cycles of PC significantly reduced ($^{\ddagger}p < 0.05$), instead of improved, the recovery of postischemic cardiac function (AF and LVDP) in comparison with the nonhypercholesterolemic or hypercholesterolemic age-matched preconditioned (3×PC and 4×PC) myocardium (Table 1). Thus, our results clearly show that 1×PC and 2×PC did not result in any cardiac protection, whereas 3×PC and 4×PC increased the extent of ischemia/reperfusion-induced injury in the hypercholesterolemic rabbit myocardium.

The experimental condition defined for PC studies, 30 min of ischemia followed by 120 min of reperfusion, for infarct size measurement in both nonhypercholesterolemic and hypercholesterolemic hearts was selected. The results demonstrate (Fig. 2A) that in hearts subjected to 1×PC, 2×PC, 3×PC, and 4×PC followed by 30 min of ischemia and 120 min of reperfusion, the infarct size was "cycle-dependently" reduced from its nonhypercholesterolemic control value (Fig. 2A) of 39 ± 6% to 37 ± 5%, 25 ± 5%, 21 ± 7% ($p < 0.05$), and 20.0 ± 6% ($p < 0.05$), respectively. With HC (after 8 weeks), this PC-induced cardiac protection in infarct size was abolished, and a significant "cycle-dependent" increase in infarct size was observed in comparison with the nonpreconditioned hypercholesterolemic control group (Fig. 2B). Thus, it is reasonable to assume that the lack of PC-induced protective effect in the 8-week hypercholesterolemic ischemic/reperfused groups is dependent on the state of myocardium (previously diseased or not) and serum cholesterol levels.

TABLE 1. CARDIAC FUNCTION IN CONTROL, HYPERCHOLESTEROLEMIC, AND PRECONDITIONED ISCHEMIC/REPERFUSED WORKING RABBIT HEARTS

	Preischemic values				*After 60 min RE*				*After 120 min RE*			
Groups	*HR*	*CF*	*AF*	*LVDP*	*HR*	*CF*	*AF*	*LVDP*	*HR*	*CF*	*AF*	*LVDP*
Time-matched nonhypercholesterolemic control, no IS/RE	220 ± 8	74 ± 6	91 ± 6	117 ± 6	214 ± 7	73 ± 6	85 ± 8	114 ± 9	209 ± 9	71 ± 5	80 ± 6	106 ± 10
Nonhypercholesterolemia, IS/RE	216 ± 6	74 ± 7	88 ± 8	120 ± 7	199 ± 8	52 ± 5	19 ± 3	54 ± 4	195 ± 7	51 ± 5	17 ± 3	50 ± 4
Nonhypercholesterolemia, 1×PC, IS/RE	212 ± 6	68 ± 6	93 ± 6	109 ± 8	192 ± 9	53 ± 6	21 ± 3	55 ± 6	200 ± 8	52 ± 6	16 ± 4	48 ± 5
Nonhypercholesterolemia, 2×PC, IS/RE	212 ± 7	71 ± 6	87 ± 7	114 ± 9	200 ± 8	60 ± 5	23 ± 4	61 ± 5	193 ± 6	58 ± 5	23 ± 5	59 ± 6
Nonhypercholesterolemia, 3×PC, IS/RE	218 ± 8	67 ± 7	88 ± 9	121 ± 8	205 ± 6	67 ± 5*	41 ± 6*	80 ± 7*	202 ± 8	67 ± 6*	47 ± 7*	78 ± 6*
Nonhypercholesterolemia, 4×PC, IS/RE	211 ± 6	77 ± 7	91 ± 6	112 ± 7	198 ± 7	70 ± 6*	55 ± 5*	90 ± 6*	196 ± 9	69 ± 6*	53 ± 6*	86 ± 7*
Time-matched hypercholesterolemic control, no IS/RE	195 ± 6†	59 ± 6†	72 ± 5†	90 ± 6†	191 ± 6	61 ± 5	74 ± 5	82 ± 7	192 ± 5	58 ± 5	68 ± 5	80 ± 8
Hypercholesterolemia, IS/RE	200 ± 5†	63 ± 5†	75 ± 6†	87 ± 8†	188 ± 7	40 ± 5	12 ± 2	38 ± 5	185 ± 8	40 ± 6	11 ± 2	36 ± 4
Hypercholesterolemia, 1×PC, IS/RE	201 ± 5†	58 ± 4†	69 ± 6†	93 ± 7†	191 ± 8	44 ± 6	14 ± 3	42 ± 6	184 ± 7	39 ± 7	15 ± 3	43 ± 5
Hypercholesterolemia, 2×PC, IS/RE	198 ± 6†	61 ± 5†	74 ± 6†	85 ± 7†	180 ± 7	45 ± 4	10 ± 2	35 ± 4	182 ± 8	41 ± 5	9 ± 2	34 ± 5
Hypercholesterolemia, 3×PC, IS/RE	202 ± 5†	57 ± 5†	68 ± 5†	88 ± 8†	190 ± 9	39 ± 5	7 ± 1‡	25 ± 3‡	191 ± 7	43 ± 6	6 ± 1‡	21 ± 3‡
Hypercholesterolemia, 4×PC, IS/RE	194 ± 8†	62 ± 4†	71 ± 5†	91 ± 6†	191 ± 8	44 ± 6	5 ± 1‡	19 ± 2‡	181 ± 9	38 ± 5	4 ± 1‡	17 ± 2‡

Data are the means ± SEM (n = 6 in each group). IS, ischemia; RE, reperfusion; HR, heart rate (beats/min); CF, coronary flow (ml/min); AF, aortic flow (ml/min); LVDP, left ventricular developed pressure (mm Hg).

*$p < 0.05$ compared with the values of nonhypercholesterolemic IS/RE group.

†$p < 0.05$ compared with the corresponding values of nonhypercholesterolemic groups.

‡$p < 0.05$ compared with the values of hypercholesterolemic IS/RE group.

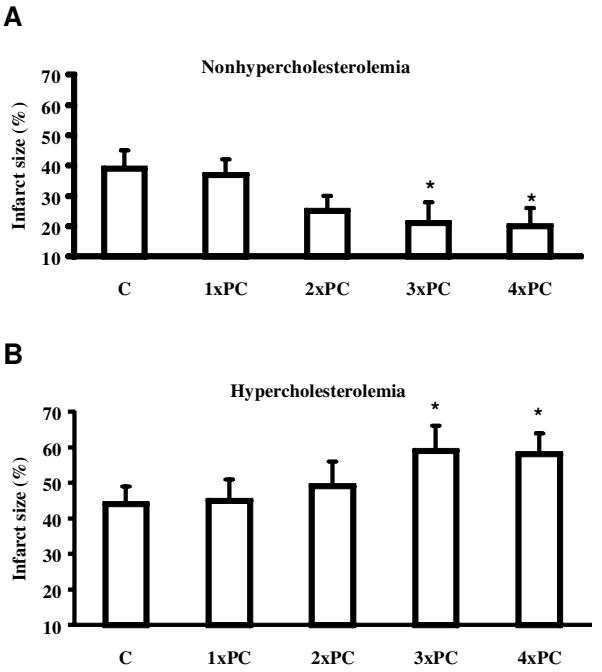

FIG. 2. Effects of PC on infarct size (%) in age-matched nonhypercholesterolemic and 8-week hypercholesterolemic rabbits. Hearts ($n = 6$ in each group) were subjected to 30 min of ischemia followed by 120 min of reperfusion. Various cycles of ischemic PC (1×PC, 2×PC, 3×PC, and 4×PC) "cycle-dependently" reduced the extent of infarct size in the age-matched nonhypercholesterolemic groups (**A**). Using the same cycles of PC failed to reduce the infarct size in the 8-week hypercholesterolemic group (**B**), and a "cycle-dependent" significant increase in the extent of infarct size was measured. Comparisons were made with the corresponding control (C) values of nonpreconditioned hearts in the nonhypercholesterolemic (A) and 8-week hypercholesterolemic (B) groups. $*p < 0.05$.

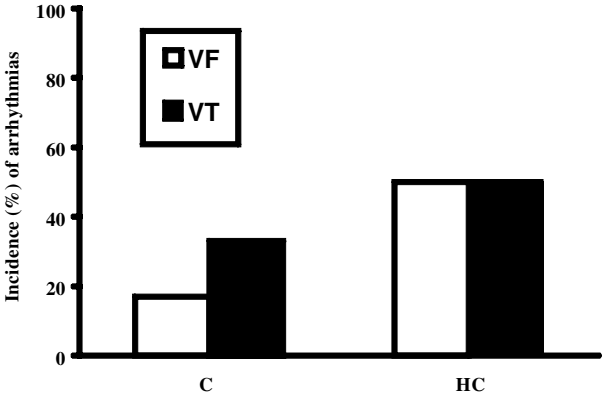

FIG. 3. Effects of HC on the incidence (%) of reperfusion-induced arrhythmias in isolated working rabbit hearts. A nonsignificant increase was observed in the incidence of VT and VF in the HC group in comparison with the nonhypercholesterolemic control (C) values. $n = 6$ in each group.

Figure 3 shows the incidence of arrhythmias, including VT and VF, after 30 min of ischemia followed by 120 min of reperfusion in nonhypercholesterolemic and hypercholesterolemic rabbit hearts. A very low incidence of VF (17%) and VT (33%) was observed in control nonhypercholesterolemic and nonpreconditioned hearts in our model, respectively. In hypercholesterolemic and nonpreconditioned myocardium, the incidence of VT (50%) and VF (50%) was increased (Fig. 3), indicating a higher vulnerability of the myocardium to arrhythmias in hypercholesterolemic subjects. The lack of statistical significance between the nonhypercholesterolemic and hypercholesterolemic values can be explained, because of the nonparametric distribution, by the low numbers of animals ($n = 6$) used in each group. In the 4×PC nonhypercholesterolemic group, the incidence of VT (17%) and VF (8%) was at a low level, whereas in the 4×PC hypercholesterolemic group, the incidence of reperfusion-induced VT (67%) and VF (67%) was at a relatively high level.

DISCUSSION

About two decades after the discovery and intensive investigation of the "first" (43), and after a decade of the observation of the "second window protection" (4–6, 62, 75) of PC in intact myocardium, relative little attention has been paid to the PC phenomenon in previously diseased hearts. Indeed, thousands of studies are now available and indicate the powerful protective effect of PC against sustained myocardial ischemia- and reperfusion-induced damage in various models of intact myocardium (21, 50, 64, 68). Thus, as a consequence, many pathways have been investigated and suggested as a potential mechanism responsible for the adaptation of myocardium to anaerobe conditions in healthy myocardium. However, it is highly probable that a number of interacting mechanisms, rather than a single mechanism, combine to determine the outcome of PC to ischemia/reperfusion-induced damage, and a variety of such triggers have been postulated, including α-adrenoceptor (3), protein tyrosine kinase signaling related to nitric oxide production (40, 47, 64), stress-activated protein kinases (22), p38 mitogen-activated protein kinase (44), heat shock protein 27 kinases (28), phospholipase D (11), protein kinase C (5, 13, 48), $G_{i/o}$ proteins (24, 58), NF-κB (42, 53), signal transducer activator of transcription (73, 74), bradykinin (12, 30), opioid receptors (15, 59), free radicals (34, 46), prostanoids (37, 68, 73), and angiotensin receptors (17).

Probably, the two most important mechanisms involved in PC are ATP-sensitive K^+ (K_{ATP}) channels and adenosine or adenosine receptors. However, the roles and functions of K_{ATP} channels and adenosine in PC are somehow controversial. Thus, it has been suggested and stressed in many studies that K_{ATP} channels may or may not play a central role in the mechanism of PC-induced cardiac protection (2, 19, 34, 36, 46, 54, 56, 57, 60, 70). The same conclusion was reached with the studies of adenosine and adenosine receptors in intact preconditioned myocardium. Thus, it has been initially proposed that adenosine and adenosine receptors could play a significant role in PC (8, 36). However, later it was stressed by the same investigators that adenosine and adenosine receptors do not play an important role in this phenomenon (9), and finally it was found that the adenosine pathway is critical depending on the time of initiation (65) of an adenosine antagonist. Recently, it has also been suggested that adenosine does or does not trigger PC

through phosphatidylinositol3-kinase and tyrosine kinase mechanisms (31, 49) in intact hearts. The same conclusions were stressed regarding the importance or unimportance of adenosine in both additional animal and human studies.

An important point could be that all aforementioned PC studies have been done in various models of ischemia/reperfusion in intact and healthy myocardium instead of a diseased heart. However, a previously diseased and preconditioned heart may respond to ischemia/reperfusion in a completely different way in comparison with the intact myocardium. Thus, the clinical implication of PC from results obtained in previously diseased and preconditioned myocardium could be much more important in comparison with the data obtained in intact hearts. Therefore, in the present study, we investigated whether PC could protect the myocardium in previously diseased, hypercholesterolemic hearts. It was not the aim of our experiments to study and determine what could be the most important PC mechanism(s), if there is any, in hypercholesterolemic myocardium, but we studied the final outcome of PC including cardiac function (HR, CF, AF, and LVDP), infarct size, and arrhythmias as functional end points.

Although the effects and mechanisms of PC on cardiac performance, in various aspects, have been extensively investigated in intact myocardium, relatively little information is available concerning this phenomenon in diseased hearts, *e.g.,* diabetic, aging, arteriosclerotic, hypertrophic, and failing ischemic/reperfused hearts. For instance, the prognosis following infarction appears to be worse in diabetic patients, who exhibit a higher incidence of congestive heart failure and death compared with nondiabetics (61). It has been found that the progress of diabetes increases myocardial infarct size and abolishes PC-induced cardiac protection in both rats and dogs (27, 52, 66, 67). It is of interest to note the findings of Ghosh *et al.* (18) in diabetic human myocardium, showing that failure to precondition the diabetic heart is due to the dysfunction of the K_{ATP} channels, and this malfunction in K_{ATP} channels of diabetic hearts lies in elements of the signal transduction pathway different from K_{ATP} channels. It has been also emphasized that ischemic PC has detrimental effects in failing and hypertrophied myocardium because it advances the moment of irreversible ischemic damage (14).

Another important factor in PC could be aging because the pharmacotherapy and dosages of various drugs are different in elderly patients in comparison with the young and middle age subjects. Therefore, it is also reasonable to investigate the PC phenomenon in senescent patients. It has been found and assessed by clinical, ECG, and metabolic evidence that ischemic PC failed to provide any protection in elderly patients (35, 38). It has been also found that the protective effect of ischemic PC is diminished in aged patients undergoing coronary bypass surgery (71) and in senescent subjects suffering from angina (1).

To determine how the HC affects PC-induced protection to reperfusion-induced injury, we have compared heart function, infarct size, and the incidence of reperfusion-induced VT and VF in isolated hearts obtained from hypercholesterolemic and nonhypercholesterolemic age-matched control rabbits. The results of our study show that HC resulted in a low recovery in postischemic cardiac function and increased infarct size in the isolated rabbit heart. With progressive HC (after 8 weeks), the development of cardiac failure was observed before the induction of PC and ischemia/reperfusion in our model. Although the incidence of reperfusion-induced VF and VT was very low in our isolated rabbit heart studies, HC increased the incidence of VF and VT from their nonhypercholesterolemic control values of 17% and 33% to 50% and 50%, respectively. In the 4×PC hypercholesterolemic ischemic/reperfused group, the incidence of reperfusion-induced arrhythmias was further increased. The effects of HC on cardiac performance have been extensively investigated, but relatively little effort has been made to study effects on infarct size and cardiac function of PC in hypercholesterolemic ischemic and reperfused myocardium. For instance, Szilvassy *et al.* (63) have observed that the cardioprotection conferred by ischemic PC against myocardial stunning and electrophysiologicalchanges was lost when rabbits developed HC and atherosclerosis after 8 weeks of exposure to the cholesterol diet. The loss of first window protection in ischemic PC was subsequently confirmed in hearts obtained from rats exposed to a cholesterol diet without development of atherosclerosis (16). The loss of PC-induced protection in hyperlipidemia has been confirmed in humans undergoing repeated balloon inflations during coronary angioplasty (32). The mechanism by which hyperlipidemia could influence the outcome of cardiac ischemia and PC is currently not known; however, the accumulation and redistribution of tissue/membrane cholesterol and the resulting changes in mitochondrial and sarcolemmal membrane microviscosity, rather than a direct effect of high serum lipoprotein levels and coronary atherosclerosis, may account for this phenomena (23, 69).

Thus, with progressive HC, the PC-induced protection is abolished and cardiac failure with reduced function of the heart due to ischemia/reperfusion was observed. Furthermore, our results clearly show that the protective effect of ischemic PC cannot be observed in hypercholesterolemic hearts, indicating that the PC might be an "intact heart" phenomenon.

In summary, the mechanisms of PC, even the first and second window protection and their implication for an actual clinical situation, were studied mainly in intact myocardium. Despite this, the effects of PC or its mechanisms appear currently to be controversial and a dilemma especially in previously diseased myocardium. The solution to the problem of PC in intact and diseased myocardium requires further data from two different sources: (a) in diseased animals from laboratories, and (b) in diseased or aging human myocardium from the clinic. Once the data are available in previously diseased human or animal subjects, then the conditions and mechanisms under which PC will be beneficial rather than harmful could be established and the dilemma could be solved.

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ABBREVIATIONS

AF, aortic flow; CF, coronary flow; ECG, electrocardiogram; HC, hypercholesterolemia; HR, heart rate; K_{ATP} chan-

nel, ATP-sensitive K^+-sensitive channel; LVDP, left ventricular developed pressure; PC, preconditioning; VF, ventricular fibrillation; VT, ventricular tachycardia; 1×PC, 2×PC, 3×PC, and 4×PC, one, two, three, and four cycles of preconditioning, respectively.

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