ISCHEMIC PRECONDITIONING WITH OPENING OF MITOCHONDRIAL ADENOSINE TRIPHOSPHATE-SENSITIVE POTASSIUM CHANNELS OR Na^+/H^+ EXCHANGE INHIBITION: WHICH IS THE BEST PROTECTIVE STRATEGY FOR HEART TRANSPLANTS?

Egidijus Kevelaitis, MD, PhD^d
Abdeslam Oubénaissa, MD, PhD^b
Christian Mouas^b
Jacqueline Peynet, MD^c
Philippe Menasché, MD, PhD^{a,b}

Objective: This study was designed to compare ischemic preconditioning with opening of mitochondrial adenosine triphosphate-sensitive potassium channels and Na^+/H^+ exchange inhibition in an isolated heart model of cold storage, simulating the situation of cardiac allografts.

Methods: Sixty-seven isolated isovolumic buffer-perfused rat hearts were arrested with and stored in Celsior solution (Imtix-Sangstat) at 4°C for 4 hours before a 2-hour reperfusion. Group I hearts served as controls and were arrested with and stored in Celsior solution. In group II, hearts were preconditioned by two 5-minute episodes of global ischemia, each separated by 5 minutes of reperfusion before arrest with Celsior solution. Group III hearts were arrested with and stored in Celsior solution supplemented with 100 $\mu\text{mol/L}$ of the mitochondrial adenosine triphosphate-sensitive potassium channel opener diazoxide. In group IV, hearts received an infusion of diazoxide (30 $\mu\text{mol/L}$) during the first 15 minutes of reperfusion. Group V hearts underwent a protocol combining both interventions used in groups III and IV. In group VI, hearts were arrested with and stored in Celsior solution supplemented with 1 $\mu\text{mol/L}$ of the Na^+/H^+ exchange inhibitor cariporide. Group VII hearts received an infusion of cariporide (1 $\mu\text{mol/L}$) during the first 15 minutes of reperfusion. In group VIII, hearts underwent a protocol combining both interventions used in groups VI and VII. Group IX hearts were ischemically preconditioned as in group II, and sustained Na^+/H^+ exchange inhibition during both storage and early reperfusion was used as in group VIII.

Results: On the basis of comparisons of postischemic left ventricular contractility and diastolic function, coronary flow, total creatine kinase leakage, and myocardial water content, values indicative of improved protection were obtained by combining ischemic preconditioning with Na^+/H^+ exchange inhibition by cariporide given during storage and initial reperfusion. The endothelium-dependent vasodilatory postischemic responses to 5-hydroxytryptamine or acetylcholine and endothelium-independent responses to papaverine were not affected by these interventions.

Conclusions: These data suggest that cardioprotection conferred by the Na^+/H^+ exchange inhibitor cariporide is additive to that of ischemic preconditioning and might effectively contribute to improve donor heart preservation during cardiac transplantation. (*J Thorac Cardiovasc Surg* 2001;121:155-62)

From the Department of Cardiovascular Surgery, Hôpital Bichat,^a INSERM U-127,^b the Department of Biochemistry,^c Hôpital Lariboisière, Paris, France, and the Department of Physiology, Kaunas University of Medicine, Kaunas, Lithuania.^d

Received for publication March 23, 2000; revisions requested May 15, 2000; revisions received Sept 1, 2000; accepted for publication Sept 8, 2000.

Address for reprints: Philippe Menasché, MD, PhD, Department of Cardiovascular Surgery, Hôpital Bichat, 46, rue Henri Huchard, 75877 Paris Cedex 18, France.

Copyright © 2001 by The American Association for Thoracic Surgery

0022-5223/2001 \$35.00 + 0 12/1/111417

doi:10.1067/mtc.2001.111417

Ischemic preconditioning has been identified as a successful strategy for improving the preservation of cardiac allografts.^{1,2} However, concerns about the implementation of an ischemic-type stimulus³ have led to the search for preconditioning-mimetic pharmacologic agents. Because the end effectors of the ischemic preconditioning pathway are likely to be the adenosine triphosphate-sensitive potassium channels (K_{ATP}) and, more specifically, those of mitochondria,⁴ selective openers of these channels, such as diazoxide, have emerged as potential cardioprotective agents^{5,6} that can closely dupli-

cate the salutary effects of classical ischemic preconditioning.^{7,8} Although the mechanism of this protection has not been completely elucidated, there is strong evidence that it involves a reduction of calcium overload.⁴

During the past years, the reduction of calcium overload and related tissue injury has also been the target of another very effective strategy on the basis of Na^+/H^+ exchange (NHE) inhibition. This antiport allows the extrusion of intracellular protons in exchange for sodium ions. However, in the setting of ischemia-reperfusion, the depletion of energy stores leads to a defective efflux of sodium ions through the ATP-driven Na^+/K^+ ATPase. The subsequent increase in intracellular sodium results in calcium overload because of increased calcium influx through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. The above hypothesis is now validated by a number of experimental studies that have convincingly demonstrated the efficacy of NHE inhibitors to blunt the ischemia-reperfusion injury.⁹ From a surgical standpoint, this approach is particularly appealing because of the clinical availability of cariporide, a highly selective inhibitor of the predominant cardiac isoform of the exchanger (NHE-1)¹⁰ that has achieved a satisfactory safety record in the GUARDIAN trial.¹¹

The present study was therefore designed to compare ischemic preconditioning, diazoxide, and cariporide in a rat model of prolonged cold storage simulating the situation of cardiac allografts.

Material and methods

Experimental preparation. Male Wistar rats weighing 270 to 330 g were injected intravenously with 0.2 mL (200 UI) heparin and intraperitoneally anesthetized with pentobarbital sodium (60 mg/kg). Their hearts were excised and rapidly mounted on a nonrecirculating Langendorff perfusion column. All animals were cared for in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Retrograde aortic perfusion of isolated hearts was instituted at a constant pressure of 75 mm Hg with ultrafiltered (5- μm pore filter), oxygenated (95% oxygen and 5% carbon dioxide), and normothermic (37°C) Krebs-Henseleit solution. The solution had a pH of 7.3 to 7.4 when gassed. The pulmonary outflow tract was incised to allow drainage of the coronary effluent. A catheter was placed through the apex of the left ventricle to drain the thebesian flow. The left atrium was opened, and a latex balloon was inserted into the left ventricle. Left ventricular pressure was continuously recorded online with a pressure transducer (TSD104, BIOPAC Systems, Inc) connected through the universal interface module (UIM100A) to the actual data acquisition unit (MP100A). Data were processed with a Power Macintosh 6100/66 computer (Apple Computer) using the software AcqKnowledge III for the MP100WS. Left ventricular developed pressure

(LVDP) was measured as the difference between peak systolic pressure and left ventricular end-diastolic pressure (LVEDP). The first derivative (dP/dt) was calculated with the software. Coronary flow was measured by means of timed collection of the coronary venous effluent. Left ventricular pacing was established at a constant rate of 320 beats/min throughout the control and reperfusion periods.

Experimental time course. The hearts were initially allowed to equilibrate for 15 minutes after being instrumented, and stable recordings were established. The left ventricular balloon was inflated to the volume that gave an LVEDP of 8 mm Hg. After stabilization, systolic and diastolic function was measured under these isovolumic conditions. In addition, the left ventricular balloon was inflated in 0.02-mL increments to construct pressure-volume curves. Two sets of pressure-volume measurements were generated, the first of which was discarded because of potential small balloon shifts. The zero volume was defined at the point at which LVEDP was zero.

At the end of the 1-hour control preischemic period, all hearts were arrested by using 50 mL of Celsior (Imtix-Sangstat) heart preservation solution delivered through a sidearm on the aortic cannula at 4°C under a pressure of 45 mm Hg. Hearts were then removed from the Langendorff column and placed in plastic containers (50 mL) filled with the same solution and surrounded by crushed ice. They were subsequently stored for 4 hours.

On completion of the storage interval, hearts were transferred back to the Langendorff column, and the balloon catheter was reinserted into the left ventricle. The balloon volume was set to the value that had given a preischemic LVEDP of 8 mm Hg. Reperfusion was started with normothermic (37°C) Krebs-Henseleit solution at 45 mm Hg pressure during the first 15 minutes, and the perfusion pressure was increased thereafter to 75 mm Hg. Left ventricular pacing was started at a constant rate of 320 beats/min once a regular spontaneous heart rhythm had resumed. The coronary effluent was collected for measurements of total creatine kinase release over the first 15 minutes of reperfusion. Total creatine kinase activity was assessed enzymatically with an automatic analyzer (Olympus). The results are expressed as international units per gram of dry weight. After 60 and 120 minutes of reperfusion, pressure-volume curves were generated by incrementally inflating the left ventricular balloon in 0.02-mL aliquots, and the first set of each measurement was discarded.

At the end of reperfusion, the ventricles were weighed. Wet weights were measured after both ventricles were incised and the excess fluid was blotted. Dry weights were measured after drying for 24 hours at 80°C. Water content was computed from the following formula:

$$100 \times (\text{wet weight} - \text{dry weight}) / \text{wet weight}$$

Experimental groups. The hearts were divided into 9 groups. Group I hearts ($n = 12$) served as controls and were only arrested with and stored in Celsior solution. In group II, hearts ($n = 8$) were preconditioned by two 5-minute episodes of total (no flow) global ischemia, each separated by 5 minutes of reperfusion before arrest with Celsior solution. Group III hearts ($n = 8$) were arrested with and stored in Celsior

Table I. Diastolic and systolic function

Group	LVEDP (mm Hg)		LVDP (mm Hg)		LV dP/dt max (mm Hg/s)	
	Baseline	Reperfusion	Baseline	Reperfusion	Baseline	Reperfusion
Control	8.6 ± 1.0	25.4 ± 6.3	121.4 ± 11.5	72.5 ± 27.3	3346 ± 400	2041 ± 659
Ischemic PC	8.2 ± 0.8	13.2 ± 3.7*	125.9 ± 12.5	81.0 ± 22.1	3487 ± 436	2322 ± 527
Diazoxide (A/S)	8.4 ± 0.9	15.6 ± 8.6	131.9 ± 13.5	82.0 ± 12.9	3510 ± 398	2252 ± 412
Diazoxide (R)	8.7 ± 1.1	25.4 ± 9.2	125.1 ± 17.8	60.1 ± 14.5	3549 ± 530	1761 ± 501
Diazoxide (C)	8.4 ± 0.6	17.8 ± 9.1	129.7 ± 9.2	89.9 ± 15.5	3681 ± 640	2424 ± 470
Cariporide (A/S)	7.8 ± 1.1	16.4 ± 11.6	129.0 ± 5.6	75.3 ± 22.5	3687 ± 301	2348 ± 719
Cariporide (R)	8.2 ± 1.6	25.4 ± 12.7	123.5 ± 12.6	72.4 ± 10.5	3522 ± 565	2183 ± 334
Cariporide (C)	8.5 ± 0.9	12.1 ± 5.9*	127.3 ± 9.7	88.8 ± 17.5	3674 ± 268	2697 ± 606
Ischemic PC + cariporide (C)	8.7 ± 0.9	9.5 ± 1.1†	122.9 ± 9.1	101.6 ± 12.2*	3204 ± 489	2830 ± 486*

Reperfusion values represent the average of values measured at 60 minutes of reperfusion. All values are means ± SD. PC, Preconditioning; A/S, arrest-storage; R, reperfusion; C, combined.

* $P < .05$ versus control, diazoxide (reperfusion), and cariporide (reperfusion).

† $P < .01$ versus control, diazoxide (reperfusion), and cariporide (reperfusion).

solution supplemented with 100 $\mu\text{mol/L}$ of the mitochondrial K_{ATP} opener diazoxide. In group IV, hearts ($n = 6$) received an infusion of diazoxide (30 $\mu\text{mol/L}$) during the first 15 minutes of reperfusion. Group V hearts ($n = 6$) underwent a protocol combining the 2 interventions used in groups III and IV (ie, diazoxide during both arrest-storage and early reperfusion). In group VI, hearts ($n = 7$) were arrested with and stored in Celsior solution supplemented with 1 $\mu\text{mol/L}$ of the NHE inhibitor cariporide. Group VII hearts ($n = 6$) received an infusion of cariporide (1 $\mu\text{mol/L}$) during the first 15 minutes of reperfusion. In group VIII, hearts ($n = 6$) underwent a protocol combining the 2 interventions used in groups VI and VII. Group IX hearts ($n = 8$) were ischemically preconditioned as in group II, and combined NHE inhibition was used as in group VIII. This combined treatment regimen was selected on completion of the preceding experiments showing that groups II and VIII were those that had separately yielded the best functional outcome (Table I).

Studies of coronary vasodilatory responses. In groups I, II, and IX, during the preischemic period and after 60 minutes of reperfusion, the endothelium-dependent coronary flow response was tested by a 5-minute perfusion with 5-hydroxytryptamine (5-HT; 10^{-7} mol/L). Coronary flow was measured during the last 4 minutes of 5-HT administration. This was followed by a 12-minute washout perfusion with drug-free Krebs-Henseleit solution to reestablish baseline coronary flow. The hearts were subsequently perfused with the endothelium-independent vasodilator papaverine (5×10^{-6} mol/L) for 5 minutes, and the coronary flow was again measured over the last 4 minutes of this perfusion. The preischemic application of vasodilatory agonists had no significant influence on the postischemic functional recovery of hearts in control group I, and therefore they were also used in groups II and IX.

After 100 minutes of reperfusion, the constant-pressure heart model was converted to a constant-flow model by using a calibrated roller pump (Minipuls 2, Gilson). The arterial pressure was continuously measured with a pressure transducer (TSD104A) connected through fluid-filled polyethylene tubing to the aortic cannula. The coronary resistance was calculated as arterial pressure over coronary flow. After base-

line measurements at constant flow, the coronary bed was precontracted by continuous perfusion with prostaglandin $\text{F}_{2\alpha}$ (10^{-5} mol/L), and the endothelium-dependent coronary relaxation to acetylcholine (10^{-6} mol/L) was tested.

Solutions and drugs. The Krebs-Henseleit buffer contained the following: NaCl, 118 mmol/L; KCl, 4.7 mmol/L; MgSO_4 , 1.2 mmol/L; NaHCO_3 , 25 mmol/L; KH_2PO_4 , 1.2 mmol/L; CaCl_2 , 2.5 mmol/L; and glucose, 11 mmol/L. The Celsior solution was provided by Imtix-Sangstat and had the following composition: potassium, 15 mmol/L; sodium, 100 mmol/L; magnesium, 13 mmol/L; calcium, 0.26 mmol/L; chloride, 41.5 mmol/L; histidine, 30 mmol/L; glutamate, 20 mmol/L; lactobionate, 80 mmol/L; mannitol, 60 mmol/L; and reduced glutathione, 3 mmol/L. 5-HT, papaverine hydrochloride, prostaglandin $\text{F}_{2\alpha}$ tris salt, and acetylcholine hydrochloride were purchased from Sigma Chemical Co. Diazoxide (Hyperstat) was obtained from Schering-Plough. Cariporide mesilate (HOE 642) was obtained from Hoechst Marion Roussel and dissolved in dimethyl sulfoxide. All drugs were added to Krebs-Henseleit buffer immediately before use.

Statistical analysis. Functional data were compared by using 2-factor analysis of variance with repeated measures, taking treatment as one factor and time as the second factor. Differences among the various experimental groups at the same reperfusion time points were tested by using 1-way factorial analysis of variance. Intergroup differences were specified by using the Student-Newman-Keuls test for multiple comparisons. Left ventricular compliance curves were assessed by using linear regression analysis of LVEDP data to calculate a slope. Preischemic and postischemic coronary flow responses to 5-HT and papaverine within the same group were compared by using paired 2-tailed t tests. Data are reported as the mean ± SD.

Results

Left ventricular diastolic function. Baseline diastolic data were not significantly different among the 9 groups (Table I). After 60 minutes of reperfusion, the value of

Fig 1. Left ventricular stiffness. The pressure-volume curves were obtained at baseline (before cardiac arrest) and after 60 minutes of reperfusion. Baseline data are expressed as a pooled group average. All values are means $\pm$ SEM. PC, Preconditioning. $*P = .024$ ischemic preconditioning versus control; $**P < .001$ ischemic preconditioning and cariporide (combined) versus control.

Fig 2. Total creatine kinase leakage during the initial 15 minutes of reperfusion. All values are means $\pm$ SD. PC, Preconditioning; A/S, arrest-storage; R, reperfusion; C, combined. $*P = .029$ versus control; $**P = .004$ versus control.

Fig 3. Endothelium-dependent coronary flow response to 5-HT (10^{-7} mol/L). All values are means $\pm$ SD. CF, Coronary flow; PC, preconditioning. * $P < .001$ versus preischemia and baseline; ** $P < .01$ versus reperfusion and baseline (paired 2-tailed t tests); # $P = .034$ versus control group, reperfusion, and baseline (Student-Newman-Keuls test).

Table II. Endothelium-dependent coronary relaxation to acetylcholine (10^{-6} mol/L)

Group	Coronary flow ($\text{mL} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ dry weight)	Coronary vascular resistance ($\text{mm Hg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ dry weight)			Relaxation to ACh (%)
		Baseline	After $\text{PGF}_{2\alpha}$	After ACh	
Control (n = 7)	59.6 ± 4.2	0.96 ± 0.16	1.80 ± 0.63	1.52 ± 0.58	39.6 ± 23.5
Ischemic PC (n = 8)	62.3 ± 6.5	0.94 ± 0.11	1.48 ± 0.17	1.27 ± 0.14	39.9 ± 15.6
Ischemic PC + cariporide (C, n = 7)	65.4 ± 4.5	0.87 ± 0.11	1.40 ± 0.16	1.17 ± 0.16	43.3 ± 10.6

All experiments were performed after 100 minutes of reperfusion under conditions of constant flow. All values are means $\pm$ SD. ACh, Acetylcholine; $\text{PGF}_{2\alpha}$, prostaglandin $\text{F}_{2\alpha}$ (10^{-5} mol/L); PC, preconditioning; C, combined.

LVEDP was significantly lower in ischemically preconditioned hearts and hearts treated with cariporide during storage and early reperfusion compared with control hearts. The combination of these 2 strategies led to an additional improvement of left ventricular diastolic function (Table I). The recovery of left ventricular diastolic function tended to be improved in hearts treated with diazoxide during arrest-storage; however, the difference with control hearts failed to reach the level of statistical significance ($P = .086$). Similar patterns were seen when LVEDP was analyzed in relation to balloon volume and pressure-volume curves were constructed. The upward shift of reperfusion LVEDP-volume curves was significantly less pronounced in hearts preconditioned with ischemia or treated with cariporide than in control hearts (Fig 1). Thus the slopes of pressure-volume curves were

increased from a baseline value of 402 ± 138 mm Hg/mL to a reperfusion value of 649 ± 202 mm Hg/mL in control hearts, from 390 ± 93 mm Hg/mL to 420 ± 82 mm Hg/mL in ischemically preconditioned hearts ($P = .024$ vs control reperfusion), and from 389 ± 164 mm Hg/mL to 436 ± 156 mm Hg/mL in hearts treated with cariporide during arrest-storage and early reperfusion ($P = .079$ vs control reperfusion). The coadministration of ischemic preconditioning and cariporide further reduced the postischemic slope of pressure-volume curves to 363 ± 37 mm Hg/mL ($P < .001$ vs control reperfusion), which was not significantly different from the baseline value of 355 ± 59 mm Hg/mL.

Left ventricular systolic function. Although LVDP decreased significantly ($P < .001$) after storage and reperfusion in all hearts, its highest values were achieved

with the combination of ischemic preconditioning with cariporide given during arrest-storage and initial reperfusion (Table I). An additive effect of ischemic preconditioning and cariporide was apparent in this group, with LVDP recovery being significantly ($P = .016$) improved compared with that of the control group. Treatment-related effects on postischemic maximum dP/dt grossly paralleled those on LVDP in all groups (Table I).

Creatine kinase leakage. Total creatine kinase release during the initial 15 minutes of reperfusion was significantly ($P = .029$) lower in ischemically preconditioned hearts than in control hearts (Fig 2). In keeping with functional data, combination of ischemic preconditioning and treatment with cariporide during storage and initial reperfusion presumably resulted in additional infarct limitation because creatine kinase leakage was significantly ($P = .004$) reduced in this group compared with that in the control group (Fig 2).

Myocardial water content. The percentage of tissue water was significantly ($P = .042$) smaller in ischemically preconditioned hearts ($79.95\% \pm 0.55\%$) than in control hearts ($81.1\% \pm 1.02\%$). Administration of cariporide during storage and early reperfusion significantly reduced myocardial edema compared with that found in the control group, regardless of whether the drug was given alone ($79.28\% \pm 0.78\%$, $P = .009$) or in combination with ischemic preconditioning ($79.30\% \pm 0.71\%$, $P = .006$).

Coronary vascular responsiveness. Before storage, administration of 5-HT significantly ($P < .001$) increased coronary flow above baseline values (Fig 3). During reperfusion, 5-HT still elicited a significant increase in coronary flow, but this endothelium-dependent response was smaller than that obtained before storage ($P < .01$ vs reperfusion baseline values). The endothelium-independent relaxation to papaverine featured similar patterns (data not shown). In the constant-flow experiments the endothelium-dependent vasodilatory response to acetylcholine was not significantly different among the groups (Table II).

Discussion

The salient findings of this study are as follows: (1) ischemic preconditioning improves preservation of cold-stored rat hearts; (2) there was a trend for this protection to be closely duplicated by pharmacologic interventions targeted at opening mitochondrial K_{ATP} channels (diazoxide), inhibiting NHE (cariporide), or both; and (3) the highest values of efficacy end points were found when ischemic preconditioning was combined with NHE inhibition.

Ischemic preconditioning resulted in a better recovery of function than that found in control hearts. This

improvement was primarily manifested as a decrease in reperfusion end-diastolic pressures and creatine kinase leakage, thereby supporting the idea that the salutary effects of preconditioning on functional outcome are primarily mediated by a reduction in cell necrosis.^{1,8} Ischemic preconditioning is thought to cause a protein kinase C-mediated activation of mitochondrial K_{ATP} channels.¹² It is postulated that the entry of K^+ through these open channels dissipates the membrane potential normally established across the inner mitochondrial membrane by the proton pump. This in turn should decrease the driving force for mitochondrial calcium uptake,⁴ which is a major determinant of postischemic functional recovery.¹³ This hypothesis is consistent with the ability of preconditioning to reduce calcium overload¹⁴ and the related contracture, as shown in the present study. An additional contributor to this preservation of diastolic function could be the limitation of myocardial edema yielded by our group of preconditioned hearts. A likely mechanism is the reduction of intracellular acidosis, which is a major metabolic consequence of ischemic preconditioning¹⁴ and is expected to slow NHE activity.¹⁵ This hypothesis is supported by the finding that preconditioning reduces the intracellular accumulation of Na^+ ,¹⁴ which in turn should limit water influx. It is also possible that the better preservation of high-energy phosphates resulting from preconditioning may allow a more effective functioning of the ATP-driven Na^+-K^+ ATPase,¹⁶ but such a mechanism is expected to be transient because preconditioning has been shown to decrease the rate of ATP depletion only during the early phase of ischemia.¹⁴ Finally, our findings that endothelium-dependent coronary responses were unaffected by preconditioning are consistent with previous observations that the protection afforded by preconditioning to myocytes does not extend to endothelial cells.^{8,17}

The addition of diazoxide to the storage solution closely duplicated the beneficial effects of preconditioning on postischemic function. The drug was used at a concentration that has been reported to confer maximal cardioprotection.⁵ Because this dose is yet 8-fold lower than that required for opening sarcolemmal K_{ATP} channels,¹⁸ it is reasonable to attribute the effects of diazoxide to a selective opening of the mitochondrial K_{ATP} channels, which supports the role of these channels as mediators of the cardioprotective effects of ischemic preconditioning.⁴ That the protection conferred by diazoxide involved a reduction of calcium overload subsequent to channel opening is supported by a recent study¹⁹ showing that exposure of cold-stored rat hearts to nicorandil, a drug that shares with diazoxide the ability to activate mitochondrial K_{ATP} channels,²⁰ markedly

reduces intracellular calcium accumulation during reperfusion. Basically, the patterns of improvement yielded by supplementation of diazoxide to the storage solution parallel those reported in our previous study,⁸ in which diazoxide was given before storage according to a preconditioning protocol. This observation is at variance with the recent study of Baines and colleagues⁷ in which diazoxide administered after the onset of coronary artery occlusion failed to reduce infarct size. This discrepancy, however, can be explained by several differences in the experimental design, including dosing, route and duration of drug administration, and selected end point. That diazoxide can remain effective when given into the ischemic period is consistent with its direct effect on mitochondrial K_{ATP} channels, which allow bypass of the upstream protein kinase C-mediated signaling pathway triggered by classical ischemic preconditioning.¹² In addition, this observation bears some clinical relevance because this drug exerts marked hypotensive effects⁷ so that its prearrest administration could conceivably worsen the hemodynamic instability that is common at the time of donor heart harvest. This issue is obviously addressed by direct drug supplementation to the arrest-storage medium. However, in contrast to the good results yielded by this treatment protocol, administration of diazoxide during the sole period of early reperfusion resulted, in the present study, in a loss of protection. In addition to a reduction of calcium overload, another effect of mitochondrial K_{ATP} channel opening is matrix swelling and reduced ATP synthesis.⁴ Although such an effect can be protective during ischemia because it limits energetic waste, it may conceivably become undesirable at the time of reperfusion when an effective workload has to be reestablished and could consequently have accounted for the failure of postischemic diazoxide treatment to improve recovery over that of control hearts.

The administration of cariporide during either arrest-storage or early reperfusion yielded results equivalent to those of diazoxide, and only a combined administration protocol provided better cardioprotection. In fact, for 2 major end points (left ventricular diastolic function and myocardial edema), the effects of cariporide added to the arrest-storage solution and the initial reperfusate matched those of ischemic preconditioning. A previous study by Tritto and colleagues²¹ has documented similar patterns of improvement in cardioplegically arrested hearts exposed to cariporide and further demonstrated a significant correlation between the gain in myocardial water content and the functional outcome. Thus these data and ours support the view that NHE is operative during ischemia, as well as under hypothermic conditions.²² However, the activity of the

exchanger is expected to become still more operative at the time of reperfusion, when its potential blockade by extracellular acidosis is suddenly relieved. In addition to limiting sodium-driven calcium overload, NHE inhibition prevents the intracellular alkaline overshoot occurring during early reperfusion²³ and the related hypercontracture of myofilaments,²⁴ which should contribute to preservation of postischemic function. It is noteworthy that the initial decrease in intracellular pH is temporally linked to the limitation of reperfusion contracture. Taken together, these data provide a strong rationale for blocking NHE activity during both ischemia and reperfusion. The salutary effects of maximizing the duration of exposure of myocardial tissue to NHE inhibitors have been previously demonstrated under normothermic conditions²¹ and are further supported by our data showing superior cardioprotection when cariporide was added to both the arrest-storage solution and the initial reperfusate. It should finally be noted that although these results were obtained in an isolated heart model, their clinical relevance is strengthened by the findings that NHE inhibitors exert similar cardioprotective effects in large animal models of orthotopic transplantation.²⁵

The combination of ischemic preconditioning with cariporide given during both arrest-storage and early reperfusion resulted in the greatest degree of cardioprotection among all treatment regimens, even though differences did not consistently achieve statistical significance, possibly because of the large number (9) of experimental groups and the use of adjustment for multiple comparison analysis. In particular, these hearts demonstrated a lesser degree of reperfusion contracture and creatine kinase release than those receiving either intervention alone; enzyme leakage, for example, was reduced by 52% and 71% compared with preconditioning alone and cariporide alone, respectively. These hearts also had significantly higher postischemic contractile indices and basal coronary flows than control hearts. These data support previous studies showing that ischemic preconditioning and NHE inhibition exert additive protective effects, particularly after prolonged ischemic injury,²² regardless of the type of inhibitor (an amiloride derivative in the study of Bugge and colleagues²⁶ or a benzoylguanidine-based drug like cariporide in the study of Shipoli and colleagues²²) and the end point of injury (infarct size²⁶ or left ventricular function²²). Taken together, these data suggest that ischemic preconditioning and NHE inhibition elicit cardioprotection through different mechanisms. Although the present data do not allow further dissection of the mechanism by which each intervention elicited cardioprotection, it is noteworthy that the

reduction of postischemic myocardial edema in cariporide-treated hearts was similar irrespective of whether preconditioning was used, whereas only ischemically preconditioned hearts featured a significant reduction of postischemic creatine kinase release, regardless of whether they had been previously exposed to cariporide. This supports the view that preconditioning primarily exerts infarct-limiting effects, whereas NHE inhibition may also act on the stunning component of postischemic cardiac dysfunction.²⁶ The combination of anti-infarct and antistunning effects would then account for our finding that hearts exposed to preconditioning and cariporide were the only ones to yield a significant improvement of contractile indices compared with control hearts. We acknowledge, however, that these assumptions are based on data obtained in a buffer-perfused isolated rat heart model and, consequently, require validation in a more physiologically relevant, blood-perfused, large animal preparation.

In conclusion, our data show that maximal cardioprotection can be conferred to cold-stored rat hearts by combining ischemic preconditioning with NHE inhibition by cariporide given in a sustained fashion throughout the period of ischemia and during early reperfusion. Future studies are warranted to assess whether and how the additional component of protection provided by ischemic preconditioning can be duplicated by a clinically relevant pharmacologic intervention.

REFERENCES

- Karck M, Rahmani P, Haverich A. Ischemic preconditioning enhances donor heart preservation. *Transplantation* 1996;62:17-22.
- Landymore RW, Bayes AJ, Murphy JT, Fris JH. Preconditioning prevents myocardial stunning after cardiac transplantation. *Ann Thorac Surg* 1998;66:1953-7.
- Perrault LP, Menasché P, Bel A, Chaumaray T, Peynet J, Mondry A, et al. Ischemic preconditioning in cardiac surgery: a word of caution. *J Thorac Cardiovasc Surg* 1996;112:1378-86.
- Gross GJ, Fryer RM. Sarcolemmal versus mitochondrial ATP-sensitive K⁺ channels and myocardial preconditioning. *Circ Res* 1999;84:973-9.
- Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, D'Alonzo AJ, et al. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K⁺ channels: possible mechanism of cardioprotection. *Circ Res* 1997;81:1072-82.
- Liu Y, Sato T, O'Rourke B, Marbán E. Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? *Circulation* 1998;97:2463-70.
- Baines CP, Liu GS, Birincioglu M, Critz SD, Cohen MV, Downey JM. Ischemic preconditioning depends on interaction between mitochondrial K_{ATP} channels and actin cytoskeleton. *Am J Physiol* 1999;276:H1361-8.
- Kevelaitis E, Oubénaissa A, Peynet J, Mouas C, Menasché P. Preconditioning by mitochondrial ATP-sensitive potassium channel openers: an effective approach for improving the preservation of heart transplants. *Circulation* 1999;100(Suppl):II345-50.
- Karmazyn M. Sodium-hydrogen exchange inhibition - a superior cardioprotective strategy. *J Thorac Cardiovasc Surg* 1996;112:776-7.
- Myers ML, Farhangkhoei P, Karmazyn M. Hydrogen peroxide induced impairment of post-ischemic ventricular function is prevented by the sodium-hydrogen exchange inhibitor HOE 642 (cariporide). *Cardiovasc Res* 1998;40:290-6.
- Erhardt LRW. GUARD during ischemia against necrosis (GUARDIAN) trial in acute coronary syndromes. *Am J Cardiol* 1999;83:G23-5.
- Sato T, O'Rourke B, Marbán E. Modulation of mitochondrial ATP-dependent K⁺ channels by protein kinase C. *Circ Res* 1998;83:110-4.
- Miyamae M, Camacho SA, Weiner MW, Figueredo VM. Attenuation of postischemic reperfusion injury is related to prevention of Ca²⁺_m overload in rat hearts. *Am J Physiol* 1996;271:H2145-53.
- Steenbergen C, Perlman ME, London RE, Murphy E. Mechanism of preconditioning: ionic alterations. *Circ Res* 1993;72:112-25.
- Lazdunski M, Frelin C, Vigne P. The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH. *J Mol Cell Cardiol* 1985;17:1029-42.
- Nawada R, Murakami T, Iwase T, Nagai K, Morita Y, Kouchi I, et al. Inhibition of sarcolemmal Na⁺, K⁺-ATPase activity reduces the infarct size-limiting effect of preconditioning in rabbit hearts. *Circulation* 1997;96:599-604.
- Shirai T, Rao V, Weisel RD. Preconditioning human cardiomyocytes and endothelial cells. *J Thorac Cardiovasc Surg* 1998;115:210-9.
- Garlid KD, Paucek P, Yarov-Yarovoy V, Sun X, Schindler PA. The mitochondrial K_{ATP} channel as a receptor for potassium channel openers. *J Biol Chem* 1996;271:8796-9.
- Hachida M, Lu H, Ohkado A, Gu H, Zhang X-I, Furukawa H, et al. Effect of ATP-potassium channel opener nicorandil on long-term cardiac preservation. *CVE* 1999;4:18-22.
- Sato T, Sasaki N, O'Rourke B, Marbán E. Nicorandil activates mitochondrial ATP-dependent potassium channels in rabbit ventricular cells. *Circulation* 1998;98(Suppl):I-343.
- Tritto F, Insette J, Garcia-Dorado D, Riuz-Meana M, Soler-Soler J. Sodium/hydrogen exchanger inhibition reduces myocardial reperfusion edema after normothermic cardioplegia. *J Thorac Cardiovasc Surg* 1998;115:709-15.
- Shipoli AR, Galíñanes M, Edmondson SJ, Hearse DJ, Avkiran M. Na⁺/H⁺ exchanger inhibitor HOE-642 improves cardioplegic myocardial preservation under both normothermic and hypothermic conditions. *Circulation* 1997;96(Suppl):II266-73.
- Hendriks M, Mubagwa K, Verdonck F, Overloop K, Van Hecke P, Vanstapel F, et al. New Na⁺-H⁺ exchange inhibitor HOE 694 improves postischemic function and high-energy phosphate resynthesis and reduces Ca²⁺ overload in isolated perfused rabbit heart. *Circulation* 1994;89:2787-98.
- Bond JM, Chason E, Herman B, Lemasters JJ. Intracellular pH and Ca²⁺ homeostasis in the pH paradox of reperfusion injury to neonatal rat cardiac myocytes. *Am J Physiol* 1993;265:C129-37.
- Kim Y-IL, Herijgers P, Laycock S, Van Lommel A, Verbeken E, Flameng WJ. Na⁺/H⁺ exchange inhibition improves long-term myocardial preservation. *Ann Thorac Surg* 1998;66:436-42.
- Bugge E, Ytrehus K. Inhibition of sodium-hydrogen exchange reduces infarct size in the isolated rat heart—a protective additive to ischemic preconditioning. *Cardiovasc Res* 1995;29:269-74.