CARDIOPULMONARY BYPASS, MYOCARDIAL MANAGEMENT, AND SUPPORT TECHNIQUES

PRECONDITIONING WITH POTASSIUM CHANNEL OPENERS

A new concept for enhancing cardioplegic protection?

Ischemic preconditioning defines an adaptive endogenous mechanism in which a brief episode of reversible ischemia renders the heart more resistant to a subsequent period of sustained ischemia. Because the cardioprotective effects of ischemic preconditioning might be mediated by an activation of adenosine triphosphate-sensitive potassium channels, this study was designed to assess whether these effects could be duplicated by the preischemic administration of a potassium channel opener. Fifty isolated isovolumic buffer-perfused rat hearts underwent 45 minutes of normothermic potassium arrest followed by 1 hour of reperfusion. They were divided into five equal groups that differed with regard to the preconditioning regimen: Group 1 hearts were left untreated and served as controls; in group 2, preconditioning was achieved with 5 minutes of total global ischemia followed by 5 minutes of buffer reperfusion before cardioplegic arrest; in group 3, the preconditioning stimulus consisted of a 5-minute infusion of the potassium channel opener nicorandil (10 $\mu\text{mol/L}$) followed by 5 minutes of drug-free buffer perfusion before arrest; group 4 hearts underwent a similar protocol except that the infusion of nicorandil was preceded by that of the potassium channel blocker glibenclamide (10 $\mu\text{mol/L}$); group 5 hearts were ischemically preconditioned like those of group 2 except that the no-flow preconditioning period was also preceded by a 5-minute infusion of glibenclamide (50 $\mu\text{mol/L}$). The results demonstrate that ischemic preconditioning significantly improved contractility and reduced contracture during reperfusion, as compared with results in control hearts. These protective effects were duplicated by pretreatment with nicorandil but were abolished when the drug was antagonized by a prior infusion of glibenclamide. Likewise, the glibenclamide-induced blockade of potassium channels largely blunted the beneficial effects of ischemic preconditioning. These data suggest that opening of adenosine triphosphate-sensitive potassium channels substantially contributes to preconditioning-induced cardiac protection in a surgically relevant model of global ischemia and, consequently, that the use of potassium channel openers like nicorandil could be an effective means of enhancing cardioplegic protection. (*J THORAC CARDIOVASC SURG* 1995;110:1606-14)

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Ischemic preconditioning defines an adaptive mechanism by which a short period of ischemic stress increases the resistance of the myocardium to

a subsequent, more prolonged period of that same stress.¹ Although the efficacy of this form of endogenous myocardial protection has now been documented by a large number of experimental studies, its mechanism is not yet fully characterized. Nevertheless, one of the currently prevailing hypotheses is that ischemic preconditioning could be mediated in

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part by endogenously released adenosine and a subsequent adenosine-induced activation of adenosine triphosphate (ATP)-sensitive potassium channels.^{2,3} The therapeutic exploitation of the preconditioning phenomenon is an appealing prospect in cardiac surgery, because the possibility of planning the onset of the ischemic period induced by aortic crossclamping should allow a timely appropriate implementation of the preconditioning stimulus. However, in a clinical perspective, a pharmacologic induction of preconditioning would have obvious advantages in terms of safety and practicality over an induction regimen of the ischemic type. Consequently, the present study was designed to assess whether the protective effects of ischemic preconditioning could be mimicked by the administration of a potassium channel opener in a surgically relevant model of cardioplegic arrest.

Methods

Experimental preparation. Fifty male Wistar rats weighing 300 gm were anesthetized intraperitoneally with pentobarbital (180 mg). All animals received human care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). The animals were given 0.2 ml heparin intravenously; the hearts were then quickly excised and mounted on a nonrecirculating Langendorff perfusion column.

Retrograde aortic perfusion was instituted at a pressure of 100 cm H₂O with filtered Krebs-Henseleit solution (in millimoles per liter: NaCl, 118; KCl, 4.7; MgSO₄, 1.2; NaHCO₃, 25; KH₂PO₄, 1.2; CaCl₂, 2.5; glucose, 11) gassed with a mixture of 95% oxygen and 5% carbon dioxide. Both the column and the heart chamber were enclosed in a water jacket so as to maintain myocardial temperature at 37° C throughout the experimental time course.

Functional measurements. A latex balloon was inserted into the left ventricle and connected through fluid-filled polyethylene tubing (inner diameter 1 mm) to a pressure transducer (P23ID, Gould, Inc., Cleveland, Ohio). Output from the pressure transducer was differentiated electronically (model 13-4615-71, Gould) to record maximum rate of rise of left ventricular pressure (dP/dt). End-diastolic pressure was set to approximately 10 mm Hg by filling the left ventricular balloon with 80 to 110 μ l of perfusate. Developed pressure was measured as the difference between peak systolic pressure and end-diastolic pressure. All pressure and dP/dt signals were displayed on a Schlumberger OM-4502 four-channel direct-writing recorder (Enertec, St. Etienne, France). Coronary flow was measured by timed collection of the coronary venous effluent. Left ventricular pacing was maintained at a rate of 320 beats/min throughout the control and reperfusion periods.

Experimental protocol. Hearts were allowed to recover for 15 minutes after being instrumented and stable hemodynamic recordings were established. All hearts were then subjected to 45 minutes of normothermic potassium arrest followed by 60 minutes of reperfusion. Initial asystole was achieved by adding concentrated potassium chloride directly to the Krebs perfusate (to a final concentration of approximately 20 mEq/L), after which hearts were maintained in a globally ischemic state for the remainder of the arrest period. The left ventricular balloon was kept inflated throughout this period at the same volume as during the control period, which allowed on-line monitoring of ischemic contracture.

The hearts were divided into five groups of 10 each, which differed only in the treatment administered before cardioplegic ischemia. Group 1 consisted of control hearts that underwent no manipulations during the preischemic period. In group 2, hearts were preconditioned with 5 minutes of global total (no flow) ischemia followed by 5 minutes of reperfusion with the Krebs buffer before the onset of cardioplegic arrest. In group 3, the preconditioning regimen consisted of a 5-minute period of administration of the potassium channel opener nicorandil (10 μ mol/L) followed by 5 minutes of perfusion with drug-free buffer before cardioplegic arrest. This concentration of nicorandil was selected after preliminary dose-response experiments had established that it exerted cardioprotective effects without significantly affecting prearrest hemodynamic variables. Group 4 hearts underwent a protocol similar to that used in group 3 except that the administration of nicorandil was preceded by that of the potassium channel blocker glibenclamide (10 μ mol/L) over a 5-minute period. In group 5, hearts were ischemically preconditioned as in group 2 but also received glibenclamide (50 μ mol/L) for 5 minutes before the 5-minute no-flow preconditioning challenge. These various protocols are depicted in Fig. 1.

Functional measurements were taken during the control period, both before and after the various ischemic and pharmacologic interventions. Measurements were repeated at 15, 30, 45, and 60 minutes of reperfusion. During arrest, maximal isovolumic diastolic pressures and the time required to achieve these peak contractures were recorded in all hearts.

Nicorandil was supplied by Rhone-Poulenc Rorer (Antony, France) and glibenclamide was purchased from Sigma Chemical Co. (St. Louis, Mo.). Both drugs were dissolved in Krebs buffer immediately before use and delivered into the aortic root at a pressure of 100 cm H₂O via a separate column.

Statistical analysis. Statistical analysis was performed with two-way analysis of variance with repeated measures and Student's *t* test, where appropriate. A *p* value of less than 0.05 was considered significant. All results are expressed as means $\pm$ the standard error of the mean.

Results

Prearrest data. Analysis of variance indicated no significant between-group differences for any pa-

Fig. 1. Experimental time course. PC, Preconditioning.

Table 1. Baseline data for the five experimental groups

Preconditioning regimen (n = 10)	Coronary flow (ml/min)	Diastolic pressure (mm Hg)	LV dP/dt (mm Hg/sec ⁻¹)
None (controls)	15.8 ± 0.4	9.6 ± 0.6	4745 ± 170
Zero-flow ischemia	16.0 ± 0.3	9.2 ± 0.7	4570 ± 167
Nicorandil	15.3 ± 0.6	9.1 ± 0.3	4767 ± 161
Glibenclamide+nicorandil	15.1 ± 0.6	11.3 ± 0.9	5002 ± 117
Glibenclamide+zero-flow ischemia	15.1 ± 0.4	10.2 ± 0.4	4770 ± 96

rameter before arrest (Table I). Therefore baseline data depicted in Figs. 2 and 3 are pooled group averages.

Ischemic preconditioning resulted in a slight, but significant, decline in contractile function, as evidenced by a decrease in dP/dt values from 4570 ± 167 mm Hg/sec⁻¹ before the 5-minute period of no-flow ischemia to 4065 ± 146 mm Hg/sec⁻¹ after the ischemic challenge ($p < 0.03$). Conversely, hemodynamic values recorded after nicorandil pretreatment were unchanged from baseline data collected before drug infusion. The greatest declines in coronary flow (shown in Table II) and left ventricular function were seen in the two glibenclamide-pretreated groups. The dP/dt fell from 5002 ± 117 mm Hg/sec⁻¹ before the preconditioning sequence to 3105 ± 216 mm Hg/sec⁻¹ after the sequence ($p < 0.0001$) in the glibenclamide-nicorandil group and from 4770 ± 96 mm Hg/sec⁻¹ to 2905 ± 152 mm Hg/sec⁻¹ ($p < 0.0001$) in the glibenclamide-zero-flow ischemia group. In none of these two groups, however, did diastolic pressure measured after pre-

conditioning significantly change compared with the baseline value.

Arrest data. The most effective limitation of ischemic contracture was achieved with nicorandil preconditioning, which yielded peak diastolic pressures significantly lower than those of the four other groups (Table III). These protective effects of potassium channel opening were mirrored by the detrimental effects of potassium channel blockade. Thus glibenclamide pretreatment before ischemic or nicorandil preconditioning resulted in a significant increase in diastolic pressures during arrest compared with each of these preconditioning regimens alone.

In keeping with these observations, both ischemic and nicorandil preconditioning lengthened the time to peak contracture, although the difference with control hearts attained statistical significance only for the drug-pretreated hearts (see Table III). Again, the detrimental effects of blocking potassium channels were demonstrated by the finding that administration of glibenclamide before ischemic (group 5) or nicorandil (group 4) preconditioning significantly shortened the time to peak contracture compared with the corresponding control values (groups 2 and 3, respectively). Indeed, the worst results were seen in the glibenclamide+ischemia preconditioning protocol, which was associated with a time to peak contracture that was even shorter than in control untreated hearts.

Postarrest data

Coronary flow. Results are summarized in Table II. The greatest postischemic coronary flows were found in hearts that had undergone ischemic pre-

Fig. 2. Effects of different preconditioning (PC) regimens on isovolumic diastolic pressures. Reperfusion values represent the average of data recorded over the 60-minute ischemic period that followed 45 minutes of normothermic potassium arrest. Data points are arithmetic means $\pm$ 1 standard error of the mean, obtained with 10 hearts per group.

conditioning (group 2). Although nicorandil pretreatment was associated with significantly lower values ($p < 0.005$ versus group 2), the contribution of potassium channel opening to preconditioning-induced cardiac protection is suggested by the observation that blockade of this opening by glibenclamide before ischemic preconditioning resulted in a significant ($p < 0.0001$) reduction in postischemic coronary flow, as compared with ischemic preconditioning alone. Likewise, hearts that were treated with glibenclamide before nicorandil had significantly ($p < 0.0001$) lower postarrest coronary flows than those in which the potassium channel opener had been given alone.

Left ventricular diastolic pressure. Pooled pre-reperfusion and postreperfusion values for diastolic pressure are depicted in Fig. 2. Both ischemic and nicorandil preconditioning resulted in an almost twofold reduction in postischemic contracture, as compared with control hearts that were not preconditioned. However, this protective effect was largely lost when either of these two preconditioning regimens was preceded by a glibenclamide-induced blockade of potassium channels. Group 4 hearts

(glibenclamide-nicorandil) were then found to incur losses of diastolic function similar to those of untreated hearts, in contrast to group 5 hearts (glibenclamide-ischemic preconditioning), which had post-arrest diastolic pressures still slightly lower than those of control hearts ($p < 0.01$ between groups 5 and 1).

Left ventricular function. As shown in Fig. 3, preconditioning, whether induced by a brief period of no-flow ischemia or a nicorandil pretreatment of similar duration, significantly improved the recovery of dP/dt over the entire reperfusion period, as compared with recovery of untreated hearts. This improvement was of similar magnitude in the two preconditioned groups. In keeping with data on diastolic function, blockade of potassium channels by glibenclamide significantly decreased the recovery of contractile function in both ischemically and pharmacologically preconditioned hearts (as it did in a separate group of experiments (data not shown), in which it was used alone, that is, without subsequent preconditioning). However, whereas glibenclamide almost completely blocked the protective effects of nicorandil pretreatment, it abol-

Fig. 3. Effects of different preconditioning (PC) regimens on isovolumic left ventricular dP/dt (LV dP/dt). Reperfusion values represent the average of data recorded over the 60-minute postischemic period that followed 45 minutes of normothermic potassium arrest. Data points are arithmetic means $\pm$ 1 standard error of the mean, obtained with 10 hearts per group.

ished those of ischemic preconditioning to a lesser extent. Thus glibenclamide pretreatment followed by ischemic preconditioning yielded a recovery that was roughly intermediate between that of control hearts and that of hearts subjected to ischemic preconditioning alone, thereby suggesting that the latter challenge involves other protective mechanisms than opening of ATP-sensitive potassium channels.

The recovery patterns of left ventricular developed pressure paralleled those of dP/dt.

Discussion

Preconditioning in the setting of cardiac surgery.

As previously mentioned, the efficacy of ischemic preconditioning as a means of attenuating myocardial injury caused by a subsequent episode of prolonged ischemia has been demonstrated in several animal studies, but most of them have actually been performed in regionally ischemic preparations and have used infarct size and arrhythmias as primary end points. It is therefore interesting to note that, more recently, evidence has been brought that the preconditioning phenomenon could also pertain to global myocardial ischemia and was then reflected by an improved recovery of function during reperfusion.⁴⁻⁶

This assumption is supported by the results of the present study. Thus hearts subjected to 5 minutes of no-flow ischemia before the sustained period of cardioplegic arrest had a better tolerance to ischemia than their nonpreconditioned counterparts, as demonstrated by a lesser degree and a delayed onset of contracture during arrest and an improved recovery of function during reperfusion.

These results support the clinical findings of Alkhulaifi and coworkers,⁷ who have shown in patients undergoing coronary artery bypass grafting that two 3-minute periods of aortic crossclamping separated by 2 minutes of reperfusion before a 10-minute period of global ischemia under hypothermic ventricular fibrillation were associated with a better preservation of myocardial ATP levels. Interestingly, the protection afforded by preconditioning in the setting of global ischemia seems to remain operative under the surgically relevant conditions of hypothermic ischemia⁴ and cold cardioplegia.⁶ Only a recent study⁸ reported that ischemic preconditioning did not improve functional recovery after 2 hours of multidose cold crystalloid cardioplegia. However, analysis of the data presented in this paper suggests that hearts preconditioned with 5 or 10 minutes of global ischemia indeed had a better tolerance for arrest than the control hearts in that

Table II. Effects of different preconditioning regimens of coronary flow

Preconditioning regimen (n = 10)	Coronary flow (ml/min)		
	Baseline		Reperfusion
	Before treatment	After treatment	
None (controls)	15.8 ± 0.4	—	13.6 ± 0.3
Zero-flow ischemia	16.0 ± 0.3	15.5 ± 0.4	15.3 ± 0.3‡
Nicorandil	15.3 ± 0.6	15.3 ± 0.6	13.9 ± 0.3
Glibenclamide+nicorandil	15.1 ± 0.6	12.2 ± 0.9*	8.8 ± 0.3§
Glibenclamide+zero-flow ischemia	15.1 ± 0.4	12.6 ± 0.8‡	10.9 ± 0.3

Baseline prearrest values were recorded before the preconditioning intervention, after stabilization of the preparation, and after the preconditioning intervention, during the 5 minutes of standard Krebs buffer perfusion that preceded the onset of cardioplegic arrest. Hearts were then maintained in an arrested ischemic state for 45 minutes at 37° C. Reperfusion values are pooled data over the 60-minute ischemic period. Values are arithmetic means ± 1 standard error of the mean.

*p < 0.003 versus the corresponding pretreatment value.

‡p < 0.009 versus the corresponding pretreatment value.

§p < 0.005 versus the nicorandil group.

§p < 0.0001 versus the nicorandil group.

||p < 0.0001 versus the zero-flow ischemia group.

they were able to achieve similar postischemic levels of contractility at the expense of twofold lower diastolic pressures.

Link between preconditioning and opening of potassium channels. Although the mechanism of preconditioning remains incompletely understood, an important role is attributed to an activation of the A₁ adenosine receptors, which would subsequently evoke a G protein-mediated translocation of protein kinase C to the membrane, where it becomes active.⁹ The ATP-sensitive potassium channel is one of the cellular components that might be phosphorylated by protein kinase C and, as such, has been proposed as a possible effector of this cellular signaling pathway.

This hypothesis is primarily based on the observation made in several species, including pig, rabbit, and dog, that the protection afforded by preconditioning can be equally achieved by potassium channel openers whereas it is abolished by potassium channel blockers.^{2, 3, 10} Our data extend these observations to the setting of global ischemia in rat hearts by demonstrating that a 5-minute infusion of nicorandil completely mimicked the protective effects of an ischemic preconditioning stimulus of similar duration on functional recovery after an extended period of normothermic cardioplegic arrest.

Previous studies have already documented the beneficial effects of potassium channel openers given as pretreatments before episodes of global

Table III. Effects of different preconditioning regimens on contracture during cardioplegic arrest (45 minutes at 37° C)

Preconditioning regimen (n = 10)	Peak contracture (mm Hg)	Time to peak contracture (sec)
None (controls)	51.8 ± 3.0	1808 ± 91
Zero-flow ischemia	50.0 ± 2.8	1978 ± 132
Nicorandil	40.4 ± 2.1*†	2068 ± 45§
Glibenclamide+nicorandil	51.1 ± 2.8	1501 ± 162
Glibenclamide+zero-flow ischemia	57.7 ± 3.0‡	1421 ± 69¶

Values are arithmetic means ± 1 standard error of the mean.

*p < 0.01 versus the control, zero-flow ischemia and glibenclamide+nicorandil groups.

†p < 0.0001 versus the glibenclamide+zero-flow ischemia group.

‡p < 0.02 versus the zero-flow ischemia group.

§p < 0.01 versus the control and glibenclamide+nicorandil groups.

||p < 0.0001 versus the glibenclamide+zero-flow ischemia group.

¶p < 0.001 versus the zero-flow ischemia group.

ischemia.^{11, 12} The present data, however, provide stronger evidence for a mechanistic link between preconditioning and opening of potassium channels and are thus consistent with the observations of Guo and coworkers,⁵ who have shown that pretreatment with the potassium channel opener aprikalim preserved left ventricular function to the same extent as did ischemic preconditioning in isolated rat hearts undergoing 30 minutes of unprotected global ischemia. In this study, the common protective mechanism shared by both ischemic preconditioning and aprikalim pretreatment was identified as an extracellular accumulation of potassium ions before the sustained episode of ischemia. Altogether, these data may appear to be at variance with those of Liu and Downey,¹³ who have reported that pharmacologic blockade of potassium channels by glibenclamide failed to abolish the protective effects of ischemic preconditioning in the rat and concluded that opening of potassium channels was therefore unlikely to mediate the phenomenon in this species. In contrast, the data of Guo and colleagues,⁵ as well as our data, show that a pharmacologic blockade of potassium channels results in a dramatic reduction of the cardioprotective effects of ischemic preconditioning. Interestingly, a similar phenomenon has been reported in patients receiving glibenclamide before coronary angioplasty.¹⁴ Actually, several differences in the experimental design may account for these discrepant results, in particular the use, in the study of Liu and Downey,¹³ of a regionally ischemic preparation of infarct size (as opposed to recovery

of function) as the primary end point and of much lower doses of glibenclamide, as compared with our study and those of Guo and associates.⁵ Furthermore, the fact that glibenclamide was quite effective in abolishing the protective effects of nicorandil preconditioning in the present study provides additional evidence that the drug-induced improvement in myocardial protection was primarily due to potassium channel opening and not to nitrovasodilatory effects.¹⁵ The direct cytoprotective effects of nicorandil are also supported by the observation that its administration failed to affect coronary flow significantly during the prearrest period. The dose of 10 $\mu\text{mol/L}$ was used in the glibenclamide-nicorandil group because both literature data¹¹ and personal dose-response studies suggest that the effects of potassium channel openers can be antagonized on an equimolar basis. In the glibenclamide-ischemic preconditioning group, the dose of 50 $\mu\text{mol/L}$ was selected to allow comparison with the results of Guo and coworkers,⁵ who have used this concentration of the potassium channel in a protocol of preconditioning similar to ours (5 minutes of ischemia and 5 minutes of reperfusion before the sustained episode of ischemia). This high concentration is consistent with the possibility that the ability of glibenclamide to block potassium channels is reduced during ischemia,¹⁵ so that such a blockade requires higher doses of this compound than those that are effective in the presence of a potassium channel opener (without superimposed ischemia, as in our group 4).

However, the finding that glibenclamide completely abolished the cardioprotective effects of nicorandil whereas it only partially blunted those of ischemic preconditioning suggests that the protective mechanisms of this latter mode of preconditioning extend beyond the sole opening of ATP-sensitive potassium channels. This is indeed consistent with the observation that, similar to what has been described in the rabbit,⁹ preconditioning in the rat also seems to be mediated by activation of protein kinase C,¹⁶ whose target proteins for phosphorylation are not restricted to those making up the potassium channel.

The mechanism whereby opening of potassium channels may provide improved myocardial protection is not yet fully elucidated. It is, however, likely that this mechanism primarily involves a reduction of the inward calcium current through voltage-operated *sarcolemmal* channels subsequent to an attenuation of membrane depolarization.¹⁵⁻¹⁷ This should result in a limitation of calcium overload and

a sparing of tissue energy stores² which, in turn, could account for the attenuation of contracture seen in our nicorandil-preconditioned hearts and also reported in globally ischemic rat^{5, 19} and rabbits hearts¹⁷ exposed to aprikalim before the ischemic interval. These mechanisms could also explain the efficacy of potassium channel openers when given either as additives to^{11, 12, 18} or even as substitutes for potassium¹⁷ in cardioplegic solutions. Whether the *mitochondrial* ATP-sensitive potassium channel²⁰ can further contribute to preserve cytosolic calcium homeostasis still must be determined. Regardless of the exact mechanism by which potassium channel openers reproduce the cardioprotective effects of ischemic preconditioning, the mechanism by which the cell keeps the "memory" of its brief exposure to nicorandil or other related drugs also remains to be explained. Sustained opening of potassium channels and decreased threshold for their activation during the ensuing period of protracted ischemia are among the possibilities that warrant further investigation.

Clinical implications. The results of this study suffer from several methodologic limitations, including the use of an isolated heart preparation, the crystalloid nature of the perfusate, maintenance of normothermia during arrest, and the potential for subendocardial injury resulting from the presence of the inflated balloon within the left ventricular cavity throughout the period of ischemia.

Despite these caveats, it is noteworthy that the protection yielded by nicorandil preconditioning was achieved according to a clinically relevant protocol. Consequently, additional experimental in vivo studies seem to be warranted. Should their results confirm those reported herein, pharmacologic preconditioning with a potassium channel opener might become an effective adjunct to current myocardial protection strategies. Beyond the presumed interest of potassium channel openers, the results of this study also emphasize the potential benefits of exploiting the heart's natural protective mechanisms for improving myocardial tolerance to surgically induced ischemic arrest.

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Discussion

Dr. Andrew S. Wechsler (Richmond, Va.). Events that trigger secondary messengers involved in preconditioning are both physical and chemical, and potassium channel openers are among the putative agents capable of simulating specific events during ischemia. In this case, the event is altered conductance of potassium that results in cell hyperpolarization and collapse of the action potential with limitation of calcium entry into the cell during the plateau phase of the action potential. Presumably this sequence of events mimics endogenous cell protective events during ischemia, fools the cell, serves as a surrogate for an ischemic episode, and triggers secondary messengers responsible for the preconditioning response.

Dr. Menasché, you have tested this hypothesis nicely, but a couple of questions remain. First, did you notice any difference in the time to electrical or mechanical arrest among the groups? Dr. Damiano and his colleagues at our institution have demonstrated persistence of depolarization potentials long into the ischemic period after treatment with aprikalim or nicorandil, although these spikes are not associated with mechanical activity.

Second, critical to your studies is an absence of any residual effect of the potassium channel opener immediately before and during the ischemic interval. What evidence do you have that the potassium channel effect is completely gone after the 5-minute washout interval? This is obviously of importance because, if the channel is still partially open, one is studying the protective effects of potassium channel openers rather than the effects of preconditioning.

Dr. Menasché. There was absolutely no difference in the time to arrest between the different groups. In Dr. Damiano's study, so far as I remember, the potassium channel openers were actually used for inducing arrest. This is a different approach from the one used in our study, in which the potassium channel opener was given before the onset of cardioplegic arrest. This cardioplegic arrest was actually induced by potassium chloride, which caused immediate asystole, within a few seconds, in all groups.

Regarding your second question: On the basis of what is known about the pharmacokinetics of nicorandil, most of the drug, if not all, would be expected to have been washed out during the 5-minute period of intervening reperfusion between the end of drug delivery and the onset of cardioplegia.

If we assume that one of the primary mechanisms by which ischemic preconditioning protects the heart is the opening of these potassium channels, I wonder if it is

important whether the channels remain open because some residual drug remained within the heart or just because they are open as a consequence of a second or third intracellular messenger, the initial release of which was caused by exposure of the heart to nicorandil. Again, however, given the pharmacokinetics of the drug, almost all of this drug is expected to have been washed out by the time cardioplegic arrest was started.

Dr. Robert M. Mentzer, Jr. (*Madison, Wis.*). Your presentation brings to our attention the potential importance of potassium channel openers in providing myocardial protection during cardiac operations. I am concerned, however, about the use of the term "preconditioning" in the clinical setting, because the role of preconditioning, if any, in protecting the human heart against ischemic damage remains to be determined. You have nicely demonstrated that ischemic preconditioning can attenuate myocardial stunning in the rat and that inhibition of the potassium channel opener obviates the salutary effect. On the other hand, ischemic preconditioning is generally recognized to protect the heart via infarct size limitation and not attenuation of stunning. Although attenuation of

stunning has been reported in the rat, this has not been confirmed in other animal species. In fact, there is considerable controversy as to whether ischemic preconditioning affects stunning in in vivo experiments. My question is this: Did you measure infarct size in these rat hearts in addition to assessing recovery of developed pressure, and what was the effect, if any, of the potassium channel opener on infarct size reduction in the rat?

Dr. Menasché. In this particular study we did not evaluate infarct size. The potassium channel hypothesis came from studies dealing with models of regional ischemia in which potassium channel openers were able to completely duplicate the protective effects of ischemic preconditioning on infarct size. Actually, this provided the starting point for us to assess whether the protection that had been demonstrated in these regionally ischemic preparations with infarct size as the main end point could be extended to the field of global ischemia with function as an end point. I acknowledge, however, that in the absence of histologic assessment, we cannot exclude that the improvement of stunning seen in this study was mediated by a reduction in infarct size.

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