Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a $K_{ATP}$-dependent mechanism: first demonstration of remote ischemic preconditioning

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Schmidt MR, Smerup M, Konstantinov IE, Shimizu M, Li J, Cheung M, White PA, Kristiansen SB, Sorensen K, Dzavik V, Redington AN, Kharbanda RK. Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a $K_{ATP}$-dependent mechanism: first demonstration of remote ischemic preconditioning. Am J Physiol Heart Circ Physiol 292: H1883–H1890, 2007. First published December 15, 2006; doi:10.1152/ajpheart.00617.2006.—Remote ischemic preconditioning reduces myocardial infarction (MI) in animal models. We tested the hypothesis that the systemic protection thus induced is effective when ischemic preconditioning is administered during ischemia (PerC) and before reperfusion and examined the role of the $K_{ATP}$-dependent ATP ($K_{ATP}$) channel. Twenty 20-kg pigs were randomized (10 in each group) to 40 min of left anterior descending coronary artery occlusion with 120 min of reperfusion. PerC consisted of four 5-min cycles of lower limb ischemia by tourniquet during left anterior descending coronary artery occlusion. Left ventricular (LV) function was assessed by a conductance catheter and extent of infarction by tetrazolium staining. The extent of MI was significantly reduced by PerC (60.4 ± 14.3 vs. 38.3 ± 15.4%, $P = 0.004$) and associated with improved functional indexes. The increase in the time constant of diastolic relaxation was significantly attenuated by PerC compared with control in ischemia and reperfusion ($P = 0.01$ and 0.04, respectively). At 120 min of reperfusion, preload-recruitable stroke work declined 38 ± 6% and 3 ± 5% in control and PerC, respectively ($P = 0.001$). The force-frequency relation was significantly depressed at 120 min of reperfusion in both groups, but optimal heart rate was significantly lower in the control group ($P = 0.04$). There were fewer malignant arrhythmias with PerC during reperfusion ($P = 0.02$). These protective effects of PerC were abolished by glibenclamide.

Remote preconditioning has therapeutic implications for modifying reperfusion injury in clinical syndromes such as acute MI, where prior preconditioning cannot be applied and where local postconditioning cannot be delivered.

In this study, we tested the hypothesis that short periods of limb ischemia administered contemporaneously, at the time of established myocardial ischemia, would reduce MI, and we propose the term “remote preconditioning” (PerC) to emphasize that the stimulus is administered during coronary ische-
mria. Furthermore, we examined the role of the ATP-dependent K\textsuperscript{+} (K\textsubscript{ATP}) channel in this form of myocardial protection.

**METHODS**

**Animals and Study Design**

All animals were treated according to the principles stated in Danish law on animal experiments and conformed to the guidelines of the American Heart Association on animal research. Furthermore, the experimental protocol was approved by the Institutional Animal Care Committee and Ethical Review Committee.

**Experiment 1: Effect of PerC on MI**

Twenty 20-kg Danish Landrace pigs were randomized to PerC (n = 10) or sham (n = 10, control) procedure.

**Experiment 2: Role of the K\textsubscript{ATP} Channel**

Ten 20-kg Danish Landrace pigs were randomized to PerC alone (n = 4) or pretreatment with glibenclamide before PerC (n = 6).

**Experimental Preparation**

Animals were preanesthetized with midazolam and pentobarbital sodium, intubated, and ventilated at 4.5 l/min with 50:50 atmospheric air-oxygen. Anesthesia was maintained with an infusion of pentobarbital sodium. Alterations to the ventilation or electrolytes to maintain physiological levels of oxygenation, electrolytes, and ventilation were guided by hourly blood gas measurements. Central temperature was monitored continuously and was kept at ~37°C by use of a heating blanket.

Heparin (50 U/kg) was administered to maintain anticoagulation. Each animal was instrumented with valved sheaths placed in the neck vessels. An eight-polar 5-Fr conductance catheter (5-cm total inter-electrode distance) with integrated micromanometer (Millar Instruments, Houston, TX) was placed, under fluoroscopic guidance, into the apex of the left ventricle (LV) via the right carotid artery and connected to a signal-processing unit (Sigma 5DF, CD Leycom), as previously described (23). A 10-Fr, 35-mm Fogerty balloon was inserted via the right internal jugular vein and positioned at the apex of the left ventricle (LV) via the right carotid artery and connected to a dedicated cardiac output-processing computer (Baxter Healthcare). Finally, a 5-Fr bipolar pacing wire was positioned in the right atrial appendage via the right external jugular vein and connected to a custom-made external pacemaker.

**Induction of PerC**

PerC was performed during myocardial ischemia and before reperfusion. The PerC stimulus was tourniquet occlusion of blood flow to one hindlimb, with four cycles of 5 min of occlusion followed by 5 min of rest. This was commenced immediately subsequent to occlusion of the LAD (see above). Circulatory arrest in the limb was confirmed by vascular Doppler. This method was developed and standardized in a previous study describing remote preconditioning (9), where it was shown to reliably and consistently occlude blood flow in the leg.

**Assessment of MI**

The right ventricular free wall was removed from the ventricular mass, and the LV was cut into 5-mm slices, perpendicular to the septum from the apex to the base. All slices were weighed, and the area at risk delineated by fluorescein was marked under a Woods lamp. Viable myocardium was stained by incubation of the slices in 1% 2,3,5-triphenyltetrazolium chloride (Sigma) at pH 7.4 at 37°C for 15 min. Area at risk and infarct size were measured as previously described (23). All analysis was performed by an investigator blinded to the treatment groups.

**Assessment of Ventricular Function**

Real-time LV pressure-volume loops generated by the conductance catheter were recorded continuously using Notocord HEM data acquisition software. With use of this method, the whole time course of I/R injury was stored as a digital file with a temporal resolution of 500 Hz. Maximal pressure developed over time (dP/dt\textsubscript{max}) and the time constant of diastolic relaxation (τ) were calculated directly from the digital pressure traces using a nonzero asymptote monoeXponential model approximating pressure decay from the time point of dP/dt\textsubscript{max} to the time point of minimal pressure. At baseline and 30, 60, and 120 min, preload was varied by transient balloon occlusion of the inferior vena cava, as previously described (23). Preload-recruitable stroke work (PRSW) was calculated using PVAN software (Millar Instruments). Measurements were made with the heart atrially paced at 150 beats/min and at held end expiration. Myocardial force-frequency relations (FFR) were also defined at baseline and 30 and 120 min by incremental pacing, increasing by 10 beats/min to the heart rate at which atrioventricular block developed. The force-frequency curves were constructed using mathematical approximation to second-degree polynomials.

**Assessment of Cardiac Rhythm**

No prophylactic antiarrhythmic drugs were administered. Ventricular arrhythmia causing sustained hemodynamic collapse was treated with direct-current cardioversion. Each episode was documented, and normal sinus rhythm for ≥60 s was used to define individual episodes for analysis.

**Investigation of the Role of the K\textsubscript{ATP} Channel**

A total of 10 pigs were studied in this protocol: 6 were randomized to receive glibenclamide (0.5 mg/kg), which was infused intravenously over 10 min at 5 min before induction of myocardial ischemia, and 4 served as a second control group. All 10 animals were subjected to remote limb ischemia during coronary occlusion with the use of the protocol described above. Blood glucose was monitored every 30 min and maintained at 4–8 mmol/l with intravenous dextrose infusion.

**Calculations and Statistics**

On the basis of our previous data using this model, we calculated that a 30% reduction in the extent of MI at a significance level of 0.05 with a power of 0.8 would require 10 animals in each group. Indexes of LV performance were expressed as absolute values, absolute
change, and percent change relative to baseline. The changes over the time course during ischemia and reperfusion were compared between groups by repeated-measures two-way ANOVA to examine any group-time effects, and direct comparison was made at time points by unpaired t-tests. The extent of MI was corrected for area at risk and expressed as a percentage. Values are means ± SE, unless otherwise stated. Parametric data were compared using Student’s t-test. In all cases, P < 0.05 was considered statistically significant.

RESULTS

Core temperature in one animal in each group fell below 37.4°C (to 36.5°C in the PerC group and 36.8°C in the control group). Both episodes lasted <15 min and occurred late in the reperfusion phase. In no animal did core temperature exceed 38.0°C. All animals completed the protocol. Epinephrine (0.2 mg iv) was administered to one pig in the PerC group and one pig in the control group.

Experiment 1: Effect of PerC on MI

Assessment of MI. There were no significant differences in LV mass [55.7 ± 2.0 and 57.6 ± 2.3 g for control and PerC, respectively, P = nonsignificant (NS)], mass of area at risk (19.1 ± 1.5 and 18.9 ± 1.6 g for control and PerC, respectively, P = NS), or their ratio between the groups. In control animals, MI occurred in 60 ± 5% of the area at risk (n = 10). However, in the PerC group, MI was significantly reduced, with 38 ± 5% of the area at risk showing infarction (n = 10, P = 0.004, unpaired t-test; Fig. 1A). When the relation between area at risk and mass of infarction was calculated, there was no difference in the slope of the two curves [slope = 0.87, r² = 0.77 (control) and slope = 0.74, r² = 0.70 (PerC)], but the intercepts were significantly different (P = 0.02), suggesting that, for any given area at risk, there was less MI in the PerC group (Fig. 1B).

Assessment of LV Developed Pressure and Volume Over the Course of I/R Injury

Figure 2 shows the changes in LV pressure and volume throughout the study protocol. During occlusion of the LAD, LV developed pressure declined and LV volume increased. At reperfusion, LV developed pressure fell abruptly and LV volume was markedly reduced, with a nadir evident in the first 5 min and gradual recovery over the next 15–30 min of reperfusion. This was followed by gradual recovery of volume and then pressure during reperfusion. The functional data are summarized in Table 1.

Changes during LAD occlusion. Two animals in each group required inotropic support (epinephrine at 10 μg/kg iv) during

![Fig. 1](http://ajpheart.physiology.org/)

![Fig. 2](http://ajpheart.physiology.org/)
and PerC groups, respectively (occurred at 185 and PerC animals over the time of ischemia (ANOVA confirmed a significant difference between control and PerC groups: 52 ± 3% and 33 ± 4% in control and PerC, respectively (P = 0.002, t-test)). By 15 min of reperfusion, the difference between the groups had resolved. The degree of reduction in LV end-diastolic volumes was significantly different between control and PerC groups: 52 ± 3% and 33 ± 4% in control and PerC, respectively (P = 0.002, t-test). Furthermore, there was a significant correlation between the degree of LV contracture and the extent of infarction in the control group (Pearson’s coefficient = 0.89, r² = 0.79, P = 0.006; Fig. 4), which was abolished in the PerC group (Pearson’s coefficient = −0.01, r² = 0.0001, P = 0.97; Fig. 4).

Late phase of reperfusion. There was a gradual reduction in calculated volumetric ejection fraction (EF) in both groups over the 2 h of reperfusion. EF declined from 63 ± 4% to 41 ± 4% at 30 min and 37 ± 4% at 120 min of reperfusion in the control group (P = 0.002 and 0.001, respectively, vs. control, paired t-test). In the PerC group, the trend was similar, although the reduction in EF from baseline to 120 min of reperfusion was greater in the control group: 40 ± 6% vs. 22 ± 5% (P = 0.03; Table 1).

PRSW decreased in both groups in the reperfusion phase: in the control group from 38 ± 2 mmHg at baseline to 22 ± 4, 25 ± 2, and 23 ± 2 mmHg at 30, 60, and 120 min of reperfusion, respectively (P = 0.01, 0.001, and 0.001, respectively, paired t-test vs. baseline). In the PerC group, there was less reduction in PRSW: from 33 ± 2 mmHg at baseline to 27 ± 4, 31 ± 2, and 32 ± 2 mmHg at 30, 60, and 120 min of reperfusion, respectively (P = 0.07, 0.03, and 0.4 respectively, paired t-test vs. baseline). At 120 min of reperfusion, PRSW declined 3 ± 5% compared with baseline in the PerC group vs. 38 ± 6% in the control group (P = 0.001, t-test). There was a significant difference between groups over the time course of reperfusion (P = 0.01, 2-way repeated-measures ANOVA). In the control group, τ (Fig. 3) was prolonged 38 ± 1% from baseline to 120 min of reperfusion compared with 13 ± 1% in the PerC group (P = 0.003, t-test).

At atrial pacing of 150/min, dP/dtmax declined in both groups over the time course of reperfusion: from 1,551 ± 139 mmHg/s at baseline to 942 ± 111, 1,117 ± 103, and 926 ± 83

LAD occlusion. In the control and PerC groups, dP/dtmax was reduced from 1,551 ± 139 mmHg/s at baseline to 1,057 ± 62 mmHg/s (P = 0.001, paired t-test) and from 1,386 ± 103 mmHg/s at baseline to 1,011 ± 125 mmHg/s (P = 0.04, paired t-test), respectively. In the control group, τ (Fig. 3) increased from 30 ± 1 ms to a peak of 61 ± 5 ms at 20 min of ischemia (P = 0.0001, paired t-test) and remained elevated. In the PerC group, the peak increase in τ occurred at 10 min of ischemia (from 32 ± 1 to 43 ± 5 ms, P = 0.05), but thereafter there was no further significant change. Two-way repeated-measures ANOVA confirmed a significant difference between control and PerC animals over the time of ischemia (P = 0.01).

Early phase of reperfusion. At reperfusion, there was a profound reduction in LV volume and pressure. The nadir occurred at 185 ± 16 and 142 ± 19 s of reperfusion in control and PerC groups, respectively (P = 0.09). The minimal LV volume at end systole was 7 ± 1 ml in the control group and 13 ± 2 ml in the PerC group (P = 0.006, t-test). This difference was also manifest in end-diastolic volumes: 34 ± 2 and 45 ± 4 ml in control and PerC, respectively (P = 0.02, t-test). By 15 min of reperfusion, the difference between the groups had resolved. The degree of reduction in LV end-diastolic volumes was significantly different between control and PerC groups: 52 ± 3% and 33 ± 4% in control and PerC, respectively (P = 0.002, t-test).

Table 1. LV functional data in experiment 1

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>30 min</th>
<th>120 min</th>
<th>%Change</th>
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<tr>
<td>EF, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>63 ± 4</td>
<td>41 ± 4</td>
<td>37 ± 4</td>
<td>40 ± 6*</td>
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<tr>
<td>PerC</td>
<td>58 ± 5</td>
<td>46 ± 5</td>
<td>44 ± 3</td>
<td>22 ± 5*</td>
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<td>PRSW, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>38 ± 2</td>
<td>22 ± 4</td>
<td>23 ± 2</td>
<td>38 ± 6b</td>
</tr>
<tr>
<td>PerC</td>
<td>33 ± 2</td>
<td>27 ± 3</td>
<td>32 ± 3</td>
<td>3 ± 5b</td>
</tr>
<tr>
<td>dP/dtmax, mmHg/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1,551 ± 111</td>
<td>942 ± 111</td>
<td>926 ± 83</td>
<td>37 ± 7c</td>
</tr>
<tr>
<td>PerC</td>
<td>1,386 ± 105</td>
<td>1,215 ± 105</td>
<td>1,122 ± 145</td>
<td>20 ± 7c</td>
</tr>
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<td>Optimal HR, beats/min</td>
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<tr>
<td>Control</td>
<td>163 ± 6</td>
<td>159 ± 10</td>
<td>151 ± 4d</td>
<td></td>
</tr>
<tr>
<td>PerC</td>
<td>164 ± 6</td>
<td>164 ± 7</td>
<td>174 ± 5d</td>
<td></td>
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<tr>
<td>τ, ms</td>
<td>30 ± 1</td>
<td>41 ± 3</td>
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<td>38 ± 1c</td>
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<tr>
<td>Control</td>
<td>32 ± 1</td>
<td>34 ± 2</td>
<td>36 ± 1</td>
<td>13 ± 5c</td>
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<tr>
<td>PerC</td>
<td></td>
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Values are means ± SE. EF, left ventricular; EF, ejection fraction; PRSW, preload-recruitable stroke work; dP/dtmax, maximal pressure developed over time; HR, heart rate; τ, time constant of diastolic relaxation; PerC, remote perconditioning. %Change = baseline − 120-min reperfusion. *P = 0.03; †P = 0.001; ‡P = 0.07; ¶P = 0.04; ‡‡P = 0.003.

Fig. 3. LV time constant of diastolic relaxation (τ) during ischemia and reperfusion in control (●) and PerC (○) groups. *P = 0.01 (2-way repeated-measures ANOVA).

Fig. 4. Relation between change in end-diastolic volume (EDV) from baseline to nadir during early reperfusion and extent of myocardial infarction in control (●), PerC (○), and PerC + glibenclamide (□) groups. AAR, area at risk.
mmHg/s at 30, 60, and 120 min of reperfusion, respectively, in the control group ($P = 0.003, 0.01,$ and $0.001$, respectively, paired $t$-test vs. baseline) and from 1,386 ± 103 mmHg/s at baseline to 1,215 ± 105, 1,205 ± 110, and 1,122 ± 145 mmHg/s at 30, 60, and 120 min of reperfusion, respectively, in the PerC group ($P = 0.01, 0.02,$ and $0.02$, respectively, paired $t$-test vs. baseline). At 120 min of reperfusion there was a $37 \pm 7\%$ reduction in the control group compared with baseline vs. a $20 \pm 7\%$ reduction in the PerC group ($P = 0.07, t$-test).

However, since $dP/dt_{\text{max}}$ is also dependent on heart rate, we constructed force-frequency curves to determine the “optimal” heart rate where $dP/dt_{\text{max}}$ peaks (Fig. 5). In the control group, the baseline optimal heart rate was 161 ± 6 beats/min and peak $dP/dt_{\text{max}}$ was 1,333 ± 109 mmHg/s. The optimal heart rate was reduced at 30 and 120 min of reperfusion to 159 ± 10 and 151 ± 4 beats/min, with peak $dP/dt_{\text{max}}$ of 995 ± 123 and 892 ± 118 mmHg/s, respectively. In the PerC group, the baseline optimal heart rate was 164 ± 6 beats/min and peak $dP/dt_{\text{max}}$ was 1,358 ± 76 mmHg/s. The optimal heart rate at 30 and 120 min of reperfusion was 164 ± 7 and 174 ± 4, with peak $dP/dt_{\text{max}}$ of 1,063 ± 67 and 995 ± 98 mmHg/s, respectively. There was no difference between groups in the decline in peak $dP/dt_{\text{max}}$, but the leftward shift of the FFR (drop in optimal heart rate) was significantly less with PerC ($P = 0.02$).

Assessment of arrhythmia. During the period of LAD occlusion, five animals in the control group had a ventricular arrhythmia requiring cardioversion, and five animals in the control group had a ventricular arrhythmia requiring cardioversion, and two of these animals had two episodes. In the PerC group, two animals had sustained arrhythmia requiring cardioversion, and these were single episodes ($P = 0.15, \chi^2$ test). During reperfusion, eight animals in the control group and three animals in the PerC group had one episode of rhythm disturbance requiring cardioversion ($P = 0.02, \chi^2$ test).

Experiment 2: Role of the $K_{\text{ATP}}$ Channel

Assessment of size of MI. There were no significant differences in LV mass (60.7 ± 1.9 and 60.0 ± 1.4 g for PerC and PerC + glibenclamide, respectively, $P = \text{NS}$), mass of area at risk (22.1 ± 2.5 and 22.2 ± 1.0 g for PerC and PerC + glibenclamide, respectively, $P = \text{NS}$), or the ratio between the groups. In the PerC + glibenclamide group, there was MI in 60 ± 6% of the area at risk compared with 32 ± 6% in the PerC-only group ($P = 0.02$).

Assessment of LV function. No animals required inotropic support during the study. In the PerC + glibenclamide group ($n = 6$), there was a 46 ± 5% reduction in PRSW at 120 min of reperfusion compared with baseline and an 18 ± 4% reduction in the PerC-only group ($P = 0.003, t$-test).

$dP/dt_{\text{max}}$ declined 40 ± 5% at 2 h of reperfusion in the PerC + glibenclamide group compared with 14 ± 7% in the PerC-only group ($P < 0.01, t$-test).

There were no differences between the groups in diastolic function (Table 2).

DISCUSSION

This study characterizes a novel strategy to achieve myocardial protection against I/R injury. Our data show that PerC, induced by intermittent limb ischemia administered after the onset of established myocardial ischemia and before reperfusion, preserves LV function, reduces malignant rhythm disturbance, and reduces the extent of MI resulting from prolonged coronary arterial occlusion. Furthermore, we show that this is mediated through a $K_{\text{ATP}}$ channel-dependent mechanism, since glibenclamide blocked the cardioprotection.

We previously showed that remote preconditioning induced by hindlimb ischemia reduced the magnitude of ventricular dysfunction during ischemia and subsequent infarct size after reperfusion in a similar porcine model (9). In that study, the preconditioning stimulus was administered before the onset of myocardial ischemia. Furthermore, we have shown that this stimulus is cardioprotective in experimental bypass and that it is clinically applicable and reduces myocardial injury in chil-
dren undergoing cardiac surgery. In the present study, we administered an identical stimulus during established ischemia. Similarly, there was a significant reduction in the extent of MI, but a particular feature of the effects of PerC was a reduction in functional disturbance during ischemia and reperfusion and fewer reperfusion-related ventricular arrhythmias.

**PerC and LV Function**

Although, in experimental studies, reduction of the extent of infarction is an important end point, we characterized in detail the changes in LV pressure, volume, and function over the time of ischemia and reperfusion in a closed-chest in vivo mammalian model of acute MI, so that the functional impact of this reduction in MI could also be demonstrated. Despite being administered after the onset of ischemia, PerC afforded protection against diastolic dysfunction but had no effect on systolic function during ischemia. Although the mechanism for this protection is unknown, diastolic dysfunction is a sensitive marker of even mild coronary hypoperfusion. To our knowledge, this has not been described previously. It is possible that this beneficial effect may be mediated by modification of the effects of ischemia on mechanical performance of ischemic myocardium, potentially through a neuronal mechanism. This observation requires further investigation. The major beneficial effects of PerC were observed during the reperfusion phase, however.

Reperfusion following a period of prolonged ischemia initiates a series of cellular processes that can contribute to cell death. In the present study, the onset of reperfusion was characterized by an acute reduction in volume and pressure in control and PerC animals. However, PerC significantly attenuated this process. It has been previously shown that postischemic hypercontracture amplifies reperfusion-induced necrosis, and temporary contractile blockade is sufficient to reduce the extent of infarction under these circumstances. Calcium overload appears to be a central mechanism of contracture in isolated cells, and, although speculative, the close relation between the extent of LV contracture and MI in the control group, and loss of this relation with PerC, raises the possibility that PerC modifies reperfusion-induced calcium overload. Regardless of the cellular mechanisms of this early protection and its relation to later functional recovery, PerC was also associated with improved ventricular function during the later reperfusion phase. PRSW, a robust load-independent index of global ventricular function, dP/dt max, and ventricular FFR were superior in the PerC group, suggesting reduced reperfusion injury and improved myocyte recovery.

**PerC and Arrhythmia**

The influence of preconditioning on arrhythmia is controversial and, possibly, species dependent. Although an experimental porcine study showed that preconditioning decreased the time to onset of ventricular arrhythmia, other studies showed that ischemic and pharmacological preconditioning reduces the propensity to ventricular arrhythmia with coronary ischemia in animal models, as well as patients undergoing coronary bypass surgery. In the present study, there was a tendency to reduced arrhythmia during LAD occlusion, and PerC led to a highly significant reduction in the number of episodes of malignant ventricular arrhythmia during reperfusion. Again, the ability to administer this stimulus during an established ischemic event has particular relevance to its potential clinical utility.

**Glibenclamide and PerC**

The protective effects of PerC are abolished by glibenclamide, suggesting that its effects are mediated, at least in part, via a KATP channel-dependent mechanism. Although only elucidating one step in the terminal part of the complex pathway presumed to mediate the cardioprotective effect of classical and remote preconditioning (1, 17), these findings support the hypothesis that PerC is utilizing a similar pathway. Importantly, previous studies have shown that glibenclamide itself does not exacerbate MI in swine; therefore, we do not believe that this reflects a nonspecific effect of glibenclamide.

**Mode and Mechanism of Protection**

In contrast to the observations of Gho et al. (5) in the original study of remote preconditioning, our data indicate that the remote ischemic stimulus does exert cardioprotective effects, even when applied after the onset of index ischemia. There may be several explanations for this finding. Most likely, there may be several explanations for this finding. Most likely, temporal aspects play an important role. In our study, the stimulus consisted of four 5-min periods of remote ischemia applied throughout the entire cardiac index ischemia vs. a single 15-min ischemic episode early during index ischemia in the original study. We believe that repetitive stimuli enhance the protective effect, but even a single episode of renal ischemia late during cardiac index ischemia appears to generate potent cardioprotection. Moreover, the type of organ used to induce remote protection (limb vs. intestines/kidneys) may influence the efficacy and quality of the protective “signal” sent to the target organ.

Our study does not in detail clarify the mechanism of cardioprotection achieved by PerC, but some assumptions can be made on the basis of our findings. Inasmuch as the pig has significant collateral blood flow during coronary artery occlusion, a circulating effector would have to exert its effect during reperfusion. No differences in end-diastolic or end-systolic volumes between groups were observed during index ischemia. However, in the earliest phase (<2 min) of reperfusion, reduced cardiac contracture is observed in the PerC group, presumably reflecting reduced reperfusion injury. This observation supports the hypothesis that protection is achieved during the first minutes of reperfusion. The impact on LV functional indexes during index ischemia may reflect responses to a circulating effector in the nonischemic perfused part of the myocardium or baroreceptor responses to the minor hemodynamic changes caused by occlusion of the limb perfusion. Most likely, these effects are not directly related to the reduction in reperfusion injury.

The present study does not elucidate whether glibenclamide blocks the effect of PerC by affecting the KATP channels in the limb and/or heart. Interestingly, Liem et al. (13) showed a role for adenosine in the initiation (within the mesenteric bed), as well as the effectuation (in the heart), of cardioprotection in a rat model of remote ischemic preconditioning.

However, in a rat model of I/R, we previously showed that hearts remotely preconditioned in vivo and subsequently explanted and mounted in a Langendorff system to undergo I/R...
with glibenclamide added to the perfusion solution developed larger infarcts (reversed the effect of remote preconditioning) than isolated hearts perfused with a normal Krebs-Henseleit solution (12). Hence, in that study, glibenclamide clearly prevented the remote preconditioning, at least partly, by blocking cardiac KATP channels.

Clearly, more studies are necessary to clarify the precise signaling mechanisms involved in this form of cardioprotection. Circulating humoral, neurogenic, and cellular mediators have been implicated in transfer of the remote preconditioning stimulus. Pell et al. (20) showed that renal ischemia also provided protection against infarction and observed a transient reduction in diastolic blood pressure at renal reperfusion that was thought to be mediated by adenosine release. In our studies, we observed a reduction in LV pressure and volume with each cycle of limb ischemia, but glibenclamide did not affect this, and the precise effect of these minor hemodynamic changes remains unclear. Although the infarct-related vessel is occluded, this does not itself exclude an effective humoral agent from recruiting cardioprotection through effects on adjacent coronary vasculature or myocardium. Furthermore, neuronal mechanisms are still potentially active, even with vessel occlusion.

Kerendi et al. (8) recently described remote postconditioning, where a single 5-min period of renal artery occlusion just before reperfusion reduced MI through an adenosine-mediated pathway in a rat model. We have chosen to use the term preconditioning for the phenomenon described in the present study, inasmuch as the stimulus was applied immediately after the onset of ischemia and well before reperfusion. The importance of our data in this context is that not only do we confirm the biological plausibility of achieving cardioprotection by peripheral tissue ischemia in a second experimental model and demonstrate the role of the KATP channel in this, but importantly, since limb ischemia can be delivered clinically and we have already shown its potential utility in surgery, it raises the exciting possibility that this technique could be used for direct clinical trials. Postconditioning and PerC widen the potential clinical utility of cardioprotective strategies of this type. The advantages of PerC lie in the fact that the protection can be recruited during the ischemic period, and, in contrast to local postconditioning, it does not require that the unstable ischemic heart be subjected to further shorter periods of ischemia. Furthermore, the exact relation between PerC and remote and local postconditioning is not clear. Indeed, late renal remote postconditioning, with renal reperfusion 60 s after myocardial reperfusion, is not effective in reducing MI. There may be important mechanistic differences between these various maneuvers, and defining the initiators and subcellular pathways involved may therefore allow the most comprehensive myocardial protection. Indeed, it would be important to address whether there are additive benefits in combining PerC with local postconditioning, for example.

Study Limitations
We did not study the detailed physiology of the signal in terms of duration and timing relative to the onset of ischemia and reperfusion, and although this may improve our understanding of the phenomenon, the major aim of our study was to validate the potential to use limb ischemia as a protective

stimulus and develop investigations in relevant human models and controlled clinical trials.

In conclusion, PerC induces protection against myocardial dysfunction, malignant arrhythmia, and MI, even though it is administered after established ischemia. These experimental data provide proof of the concept that this simple technique may provide a valuable adjunct to reperfusion therapy in patients presenting with established ischemia and evolving acute MI.

GRANTS
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