Rabbit heart can be “preconditioned” via transfer of coronary effluent

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Departments of 1Emergency Medicine and 2Physiology and 3Division of Cardiology, University of Massachusetts Medical School, Worcester, 01655; 4BioPAL, Inc., Wellesley Hills, Massachusetts 02481; 5Heart Institute, Good Samaritan Hospital, and 6Section of Cardiology, University of Southern California, Los Angeles, California 90017-2395

Dickson, Eric W., Mojca Lorbar, William A. Porcaro, Richard A. Fenton, Christopher P. Reinhardt, Anne Gysembergh, and Karin Przyklenk. Rabbit heart can be “preconditioned” via transfer of coronary effluent. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H2451–H2457, 1999.—Brief myocardial ischemia not only evokes a local cardioprotective or “preconditioning” effect but also can render remote myocardium resistant to sustained ischemia. We propose the following hypotheses: remote protection is initiated by a humoral trigger; brief ischemia-reperfusion will result in release of the humoral trigger (possibly adenosine and/or norepinephrine) into the coronary effluent; and transfer of this effluent to a virgin acceptor heart will elicit cardioprotection. To test these concepts, effluent was collected during normal perfusion from donor-control hearts and during preconditioning ischemia-reperfusion from donor-preconditioned (PC) hearts. After reoxygenation occurred and aliquots for measurement of adenosine and norepinephrine content were harvested, effluent was transfused to acceptor-control and acceptor-PC hearts. All hearts then underwent 40 min of global ischemia and 60 min of reperfusion, and infarct size was delineated by tetrazolium staining. Mean infarct size was smaller in both donor- and acceptor-PC groups (9% of left ventricle) than in donor- and acceptor-control groups (36% and 34%; P < 0.01). Protection in acceptor-PC hearts could not, however, be attributed to adenosine or norepinephrine. Thus preconditioning-induced cardioprotection can be transferred between rabbit hearts by transfusion of coronary effluent. Although adenosine and norepinephrine are apparently not responsible, these results suggest that remote protection is initiated by a humoral mechanism.

Adenosine A1,A3, and/or α1-adrenergic receptor (3,19,20,26).

A small number of studies have expanded on this initial concept and demonstrated that regional ischemia not only elicits a local preconditioning effect but also, interestingly, can protect remote, virgin myocardium from later, prolonged ischemia. This phenomenon of preconditioning the myocardium “at a distance” can be mediated by ischemia in a remote coronary vascular bed (25) or by intermittent ischemia in noncardiac tissues including kidney (9,11,12,24), mesentery (11), and skeletal muscle (5). The communicative mechanism(s) responsible for initiating this remote protection is, at present, unresolved, but both neuronal (11) and hormonal (8,10,29) triggers have been suggested.

We hypothesize (8,10,29) that remote preconditioning is mediated by an as yet unidentified humoral trigger signal. If so, we propose that brief myocardial ischemia-reperfusion will result in release of the humoral trigger into the coronary effluent and that transfer of this effluent to a virgin acceptor heart will protect the acceptor from sustained ischemia. Our primary objective was to test this theory in the isolated buffer-perfused rabbit heart model of global ischemia, employing reduction of infarct size as the established “gold standard” of conventional ischemic preconditioning as our index of cardioprotection. Finally, our secondary aim was to determine whether release of adenosine and/or norepinephrine into the transfused coronary effluent may represent the humoral trigger for evoking remote cardioprotection.

METHODS

Animals used in this study were maintained and used in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animals Resources, National Research Council (DHHS Publication No. (NIH) 85-23, Revised 1985) and guidelines of the Animal Care Advisory Committee of the University of Massachusetts Medical School.

Isolated buffer-perfused heart preparation. New Zealand White rabbits (1–1.5 kg, 6–8 wk of age, n = 40) were anesthetized with a single intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) and ventilated by mask with 100% O2, and their chests were opened via a midline thoracotomy. The hearts were quickly removed, immediately submersed in chilled physiological saline solution (PSS) consisting of (in mM) 118 NaCl, 4.7 KCl, 24 NaHCO3, 1.2 KH2PO4, 1.2 MgSO4·7H2O, 11 glucose, and 2.5 CaCl2 (anhydrous), and rapidly placed on a modified Langendorff apparatus. Perfusion was initiated within 1 min after exci-
sion to minimize the possibility of an ischemia-induced preconditioning effect during preparation (2).

PSS equilibrated with 95% O2-5% CO2 was warmed to 38°C and delivered by retrograde perfusion at a constant pressure of 80 mmHg. A latex balloon was inserted into the left ventricle (LV) through the left atrium and filled with saline to a baseline end-diastolic pressure (EDP) of 10 mmHg. The balloon volume was then held constant throughout the experiment. Heart rate was maintained at 210 beats/min by pacing via right ventricular electrodes (Grass Stimulator, Quincy, MA). During all episodes of global ischemia (described in detail in Experimental protocol), hearts were immersed in PSS warmed to 37°C. During periods of perfusion, heart temperature (monitored intermittently by a right ventricular temperature probe) was consistently maintained at 37.0–37.5°C. Coronary flow rate was measured every 5 min throughout the protocol by timed collection of coronary effluent. LV pressure (monitored via LV balloon) was recorded continuously throughout each experiment on a chart recorder (Grass Polygraph, Quincy MA).

Experimental protocol. Hearts were experimented on in a paired, donor/acceptor sequence. After a 20-min stabilization period, the initial (donor) heart was randomized to either the preconditioned (PC) or control group by coin toss. Donor-PC hearts underwent repeated brief PC ischemia (3 5-min episodes of global, no-flow ischemia, each interrupted by 10 min of reperfusion; n = 10), whereas the donor-control cohort (n = 10) received an equivalent period of uninterrupted perfusion (Fig. 1). During 30 min of the PC/control donor period (encompassing the beginning of the first brief reflow to the onset of the final reperfusion; Fig. 1), coronary effluent was collected from all donor hearts, maintained at 37°C, and reoxygenated for subsequent transfer (within 1 h) to the two acceptor cohorts. It must be emphasized that none of the acceptor hearts received antecedent ischemia; rather, the acceptor-PC group (n = 10) received perfusate from the donor preconditioned hearts, whereas acceptor controls (n = 10) received perfusate from the donor controls. Immediately after the donor/acceptor period was completed, all hearts were subjected to a 40-min index episode of sustained global, no-flow ischemia followed by 1 h of reperfusion (Fig. 1).

Infarct size determination. When the protocol was completed, hearts were briefly frozen and cut into four to five transverse slices. The heart slices were incubated for 20 min at 37°C in a 1% solution of triphenyltetrazolium chloride (Sigma, St. Louis, MO) to distinguish necrotic from viable myocardium (17, 28) and were stored for 48 h in 10% buffered Formalin. The hearts were then photographed, right ventricular tissue was trimmed from each slice, and the remaining LV tissue was weighed.

Photographic images of the heart slices were projected and traced at magnifications of approximately ×5–10. The area of necrosis (AN) in each LV slice was quantified by computerized planimetry, corrected for the weight of the slice, and summed for each heart. AN was then expressed as a percentage of the total LV weight. All tracing, planimetry, and infarct size determination were performed by two investigators (K. Przyklenk and A. Gysembergh) who remained blinded with regard to the group assignments until all analyses were completed.

Adenosine and norepinephrine concentrations. Immediately before transfer of perfusate to the acceptor groups was begun, a 10-ml aliquot of coronary effluent was collected from

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**Fig. 1.** Study design showing donor/acceptor sequence in control (top) and preconditioned hearts (bottom).
donor-PC and donor-control hearts and frozen at −80°C for later determination of adenosine and norepinephrine concentrations. Samples were intentionally obtained at this time to ensure that the measured concentrations of adenosine and norepinephrine accurately represented the levels of these agents in the transferred perfusate.

For analysis, samples were thawed to 5°C. Five milliliters of each sample were dehydrated overnight with a gentle stream of air at 35–40°C. The dehydrated samples were reconstituted with deionized water to 1 ml in volume and filtered (0.45 μm). The samples were analyzed for adenosine content by high-performance liquid chromatography (HPLC) using ultraviolet absorbence detection, a Hypersil C-18 column (Phenomenex, Torrance, CA), and a mobile phase consisting of 1.5% triethylamine, 2% methanol, and 0.4% phosphoric acid (pH 6.5, flow 1 ml/min). The adenosine contained in the coronary effluents is expressed in millimolar concentrations.

Norepinephrine in the coronary effluent was first concentrated using alumina. A 1-ml amount of each effluent sample was placed in a 2-ml conical tube containing 50 mg of acid-washed alumina, and then 0.5 ml of Tris buffer (1.5 M Tris base, 53.7 mM Na-EDTA; pH 8.6) was added to each effluent sample. All samples were then shaken in a reciprocal shaker for 10 min, allowing norepinephrine to bind to the alumina. After shaking, the alumina was washed twice (with shaking) with water, transferred into a microfilter tube (0.22 μm), and centrifuged at 1,000 g for 10 min. The norepinephrine was then eluted from the alumina using 100 μl of BAS eluting solution (BAS, West Lafayette, IN). A 20-μl aliquot of each sample was injected into an HPLC unit with electrochemical detection (Coulochem II; ESA, Chelmsford, MA). Separation was performed using a C-18 cartridge column (MD-150; ESA) with a flow of 0.5 ml/min and a mobile phase (MD-TM Mobile Phase; ESA). Quantitative analysis was performed by electrochemical detection (Coulochem II) at 170 mV. Values are expressed as nanomolar concentrations.

Buffer composition. We measured pH, PCO₂, PO₂, and Na⁺, K⁺, Cl⁻, K⁺, Ca²⁺ (Instrumentation Laboratories model 1640, Lexington, MA), and glucose concentrations (Precision G MediSense, Bedford, MA) in fresh perfusate and effluent collected from donor-control and donor-PC hearts.

End points and statistics. Coronary flow, LVEDP, and LV developed pressure (LVEDP; maximal systolic pressure minus diastolic pressure) were assessed repeatedly throughout the protocol and compared statistically among the four treatment groups at baseline, at the end of the donor/acceptor period (immediately before onset of sustained global ischemia), at 40 min into the test ischemia (EDP only), and at 10, 30, and 60 min postreperfusion by two-factor ANOVA with replication. If statistical significance was achieved, Dunnett’s post hoc test was applied to identify differences in donor-PC, acceptor-PC, and/or acceptor-control groups versus donor-control group. Infarct size (AN/LV wt) was compared among groups by ANOVA followed by Dunnett’s test. Composition (pH, PCO₂, PO₂, glucose and electrolyte concentrations) of fresh perfusate, effluent, from donor-control hearts, and effluent from donor-PC hearts was compared by ANOVA, whereas adenosine and norepinephrine concentrations in coronary effluent from donor-control and donor-PC hearts were compared by both t-test and the Mann-Whitney (nonparametric) test. For all comparisons, P values < 0.05 were considered statistically significant.

**RESULTS**

Exclusions. Of the 40 animals enrolled in the study, 2 hearts (1 donor-PC and 1 acceptor-PC heart) failed to complete the protocol because of persistent ventricular fibrillation on reperfusion. Infarct size was inadequately delineated in three hearts (1 donor-control, 1 donor-PC, and 1 acceptor-PC heart) because of technical errors in tetrazolium staining or photography. Thus a total of 35 hearts (9 donor-control, 10 acceptor-control, 8 donor-PC, and 8 acceptor-PC hearts) were included in the analysis.

**Hemodynamics.** Coronary flow was similar among groups at baseline and did not differ among the four cohorts at any time during the protocol (Table 1).

All groups, as expected, developed contracture during sustained ischemia-reflow, with LVEDP increasing from a baseline value of 10 mmHg to >30 mmHg (Table 1). LVEDP was similar throughout ischemia-refusion in both control cohorts. During sustained ischemia, contracture was exacerbated in the acceptor-PC group (55 ± 5 mmHg) versus the donor-control group (33 ± 2 mmHg; P < 0.05). This difference, however, was transient and did not persist after relief of ischemia. In contrast, in the donor-PC group, contracture was not altered during index ischemia but was significantly attenuated throughout reperfusion when compared with that in the donor-control group (Table 1).

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**Table 1. Hemodynamics**

<table>
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<th>Baseline</th>
<th>Preschemia</th>
<th>End Ischemia</th>
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<th>30-min Reflow</th>
<th>60-min Reflow</th>
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<td>Donor controls</td>
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<td>29 ± 2</td>
<td>24 ± 2</td>
<td>20 ± 1</td>
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<tr>
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<td>24 ± 2</td>
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<td>26 ± 2</td>
<td>23 ± 2</td>
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<tr>
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<td>19 ± 1</td>
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<td>40 ± 4</td>
<td>34 ± 4</td>
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<td>43 ± 5*</td>
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<td>53 ± 6</td>
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<td>34 ± 4</td>
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<tr>
<td>Donor PC</td>
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<td>41 ± 4</td>
<td>53 ± 6</td>
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<tr>
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<td>LVDP, % of baseline</td>
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<td>0</td>
<td>29 ± 5*</td>
<td>36 ± 4</td>
<td>49 ± 4</td>
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</table>

Values are means ± SE. Ischemia consisted of 40 min of global ischemia. PC, preconditioned; LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure. *P < 0.05 vs. donor controls, by 2-factor ANOVA and Dunnett’s test.
Baseline values of LVDP were comparable in all groups, ranging from 77 to 88 mmHg. LV function was maintained at >86% of baseline values throughout the intervention phase in the donor-control, acceptor-control, and acceptor-PC groups (Table 1). In contrast, donor-PC hearts, as expected, exhibited a prompt cessation of systolic contraction during each episode of brief global ischemia (data not shown) and a trend toward modest "stunning" after repeated brief ischemia [i.e., mean LVDP was 78 ± 8% of baseline; P = 0.3 (nonsignificant) vs. 92 ± 3% in donor-control hearts; Table 1].

In all groups, LVDP remained depressed after relief of sustained global ischemia. At 10 min after reflow, recovery of LV function was modestly improved in the donor-PC and acceptor-PC groups versus the donor-control group (25 ± 7 and 29 ± 5% vs. 6 ± 2% of baseline; P < 0.05; Table 1). This difference, however, was not maintained, because there were no differences in LVDP among groups at 30 or 60 min postreperfusion (Table 1).

Infarct size. Mean infarct size in donor-controls was 36 ± 5% of the total LV weight, consistent with that previously published in reports assessing infarct size in the setting of global ischemia (15, 17). Moreover, as expected, brief antecedent ischemia was cardioprotective, with infarct size in the donor-PC group averaging only 9 ± 2% (P < 0.01 vs. donor-control group; Fig. 2).

Transfer of control effluent had no effect on the extent of necrosis; mean AN/LV weight in acceptor-control hearts was 34 ± 4%. In contrast, in acceptor-PC hearts (i.e., hearts that received effluent from donor-PC group), infarct size was reduced to 9 ± 1%, significantly smaller (P < 0.01) than that observed in the donor-control cohort and comparable to the value of 9% in the donor-PC cohort (Fig. 2).

Buffer composition. Reduction of infarct size in the acceptor-PC group was not caused by differences in effluent composition. PO2, PCO2, and glucose and electrolyte concentrations were similar in fresh buffer, coronary effluent collected from donor-control hearts, and buffer from donor-PC hearts (Table 2).

Adenosine and norepinephrine concentration. Adenosine and norepinephrine levels in coronary effluent collected from donor-control hearts averaged 0.26 ± 0.02 µM and 0.78 ± 0.08 nM, respectively (Table 3), within the range of values reported in the literature for rabbit hearts (6, 7).

There was no increase in norepinephrine content in effluent collected from the donor-PC group; rather, norepinephrine levels in the transfused preconditioned effluent were significantly reduced versus that in the donor-control group, to a mean of 0.47 ± 0.11 nM (P < 0.05; Table 3).

Because concentrations of adenosine in effluent from donor-PC hearts were not normally distributed, data were compared between groups using both parametric and nonparametric tests. Neither the mean nor the median adenosine content (0.40 and 0.26 µM) differed significantly from the respective control values (0.26 µM).

Table 3. Adenosine and norepinephrine concentrations

<table>
<thead>
<tr>
<th></th>
<th>Transfused Control Effluent</th>
<th>Transfused PC Effluent</th>
<th>P Value, Control Effluent vs. PC Effluent</th>
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</thead>
<tbody>
<tr>
<td>[Adenosine], µM</td>
<td>0.26±0.02</td>
<td>0.40±0.09</td>
<td>0.2</td>
</tr>
<tr>
<td>(Mean ± SE)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (25th; 75th percentiles)</td>
<td>0.26 (0.21; 0.31)</td>
<td>0.26 (0.19; 0.51)</td>
<td>0.4</td>
</tr>
<tr>
<td>[Norepinephrine], nM</td>
<td>0.78±0.08</td>
<td>0.47±0.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(Mean ± SE)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (25th; 75th percentiles)</td>
<td>0.75 (0.62; 0.94)</td>
<td>0.40 (0.21; 0.61)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are concentrations of adenosine ([Adenosine]) or norepinephrine ([Norepinephrine]) in either effluent. Preconditioning did not significantly change [Adenosine] from control.

All data are presented as means ± SE. [Na+], [K+], and [Ca2+], electrolyte concentrations; [glucose], glucose concentration.
and 0.26 µM; Table 3). In addition, regression analysis revealed no relationship between adenosine concentration in the donor-transfused perfusate and infarct size for hearts in the acceptor-PC group, the acceptor-control group, or the combined acceptor populations ($r^2$ values of 0.07, 0.03, and 0.08, respectively; Fig. 3).

**DISCUSSION**

In this study, we report a significant reduction in infarct size in isolated buffer-perfused rabbit hearts transfused with coronary effluent obtained from donor hearts subjected to brief preconditioning ischemia. From these data, we deduce that a humoral trigger signal is released from preconditioned myocardium that, when delivered to acceptor hearts, evokes a cardioprotective effect. However, our results further suggest that adenosine and norepinephrine are not the triggers responsible for rendering the acceptor-PC group resistant to infarction.

Comparison with previous studies. The phenomenon of preconditioning at a distance was first described in the in vivo canine model of coronary artery occlusion, in which brief episodes of intermittent circumflex artery occlusion were reported to protect virgin myocardium perfused by the left anterior descending coronary artery from a subsequent sustained ischemic insult (25). More recently, other studies (5, 9, 11, 22, 24) have revealed that intermittent ischemia in remote, noncardiac tissues (including kidney, mesentery, and skeletal muscle) also render the heart resistant to infarction. These results raised the following question: Could the concept of "remote preconditioning" be further extended, such that protection could be transferred, via transfusion of effluent, from one discretely perfused organ to another or, potentially, from one animal to another? Results of two recent studies from our laboratory (8, 10) provided preliminary support for this theory. First, we found, in the in vivo rabbit model, that infarct size was reduced in animals that received whole blood from donor rabbits subjected to repeated brief circumflex and renal artery occlusion. However, it was not determined whether myocardial ischemia or renal ischemia, or both, were responsible (or necessary) for eliciting protection in the acceptor cohort (10). Second, in isolated rabbit hearts perfused using a constant-flow system, both donor- and acceptor-PC groups exhibited a significant, ~20% improvement in recovery of LVDP after relief of global ischemia compared with a donor-control group (8). In the current study, we expand on these observations and demonstrate by using reduction of infarct size, the hallmark of conventional, preconditioning-induced cardioprotection, as our end point, that the benefits of brief intermittent myocardial ischemia can indeed be transferred, via transfusion of effluent, from donor-PC to acceptor-PC hearts.

It is interesting to note that, in apparent contrast to our earlier findings, donor-PC and acceptor-PC groups exhibited only a transient (rather than sustained) improvement in recovery of LVDP after reflow. In addition, contracture after reflow was attenuated in the donor-PC group (but not the acceptor-PC group), an observation not made in our previous study. Although the reasons for these differences in outcome with regard to LV function are not clear, methodological differences between the two studies, specifically, measurement of function in hearts subjected to 30 min of global ischemia and 30 min of reperfusion in a constant-flow system (8) versus hearts perfused at constant pressure and subjected to a longer, 40-min episode of global ischemia and 1 h of reflow, may play a role. In any case, results of the current investigation underscore the concept that whereas preconditioning may, in some instances, be associated with an acute improvement in recovery of LV function (1–3), this is not a primary or independent consequence of preconditioning-induced cardioprotection (5); reduction of infarct can, as in the present study, be evoked without a concomitant, acute benefit on contractile performance (15, 17, 25, 26).

Identity of the protective, humoral trigger? Our current results are consistent with the concept that reduction of infarct size in the acceptor-PC hearts was initiated by a humoral trigger signal present in the transfused effluent collected from the donor-PC hearts. The obvious question is, what is the identity of the trigger signal?

One potential candidate, on the basis of studies of conventional ischemic preconditioning, is stimulation.
of α1-adrenergic receptors via release of norepinephrine (3). In the isolated buffer-perfused rat heart, a single 2-min period of brief preconditioning ischemia has been shown to result in a marked, approximately eightfold increase in norepinephrine content in the coronary effluent. Moreover, exogenous administration of α1-agonists (including norepinephrine) mimicked, whereas α1-antagonists abrogated, preconditioning-induced cardioprotection as assessed by recovery of LV function (3). Pharmacological stimulation and blockade of α1-receptors has yielded similar results in rabbit models employing infarct size as the primary end point (4, 27). However, in contrast to the rat, repeated brief (2 min) episodes of global ischemia reportedly fail to elicit release of norepinephrine from the isolated rabbit heart (7). These data have been interpreted to suggest that although, in rabbit, exogenous norepinephrine does not alter the collection period, it is in agreement with this conclusion.

It is perhaps not surprising, in light of these previous data (7), that norepinephrine release was not increased in response to repeated brief ischemia in our rabbit preparation. It is, however, interesting, that norepinephrine content was significantly lower in the effluent of donor-PC hearts versus donor-control hearts. If levels of norepinephrine in the perfusate reflect a continuous “leakage” of the agent into the effluent during normal perfusion, rather than substantive “release” of norepinephrine during episodes of reflow, our data may simply be explained by the fact that the 30-min collection period consisted of continuous perfusion in the donor-control group versus three 10-min interrupted periods of collection in the donor-PC group. Detailed temporal analysis of norepinephrine release throughout the collection period would, however, be required to resolve this issue.

A second obvious potential candidate for the humoral trigger in our model is adenosine. There is no question that adenosine is produced in ischemic myocardium via the catabolism of ATP and released in large quantities during the early minutes following reperfusion (12, 16). For example, previous studies employing isolated buffer-perfused rabbit hearts have reported an ~30-fold increase in adenosine concentration in the coronary effluent immediately on relief of a brief, 5-min ischemic insult (12). This liberation of adenosine, as well as subsequent stimulation of adenosine A1- and/or A2-receptors on myocyte membranes, has been identified as a trigger of infarct size reduction with both conventional ischemic preconditioning (13, 16, 17, 20, 26) and preconditioning at a distance as elicited by renal ischemia in the in vivo rabbit model (24).

Three pieces of evidence suggest that adenosine is not, however, responsible for initiating remote protection in the current study. First, there was no significant increase in adenosine concentration in the coronary effluent obtained from donor-PC hearts versus donor-control hearts. This observation is not surprising. Although adenosine is released in massive quantities immediately on reflow, this effect is transient (12, 16); indeed, it has been demonstrated that at 5 min after reperfusion, adenosine content in the coronary effluent does not differ from baseline values (12). In addition, it is well-established that adenosine release is markedly attenuated (i.e., by ~50%) after a second versus the first repeated brief ischemic insult (12). Thus our protocol for collection of coronary effluent (essentially “averaged” over a period of 30 min) would minimize any differences in adenosine concentrations between the two groups. Nonetheless, it could be argued that the modest trend toward increased adenosine content observed in some donor-PC samples might contribute to cardioprotection in the recipient, acceptor-PC hearts. However, even the highest adenosine concentration recorded in this study (0.98 µM) is well below the values observed immediately after relief of brief ischemia and, importantly, well below the 10–20 µM concentrations of exogenous adenosine used by previous investigators to mimic ischemic preconditioning (17, 26).

Finally, if adenosine content in the trans fused effluent were an important determinant of infarct size, one would anticipate that hearts in the acceptor-PC group (or acceptor-control group) receiving the highest concentrations of adenosine would develop the smallest infarcts. There was, however, no relationship between these two variables. It is therefore improbable that the low concentrations of adenosine transfused in the current protocol were responsible for the reduction of infarct size seen in the acceptor-PC hearts.

If adenosine and norepinephrine are not the humoral triggers for the transferred protection, what are the other possibilities? Stimulation of adenosine and/or α1-adrenergic receptors is not the only means by which preconditioning-induced cardioprotection may be initiated; rather, stimulation of a host of G protein-coupled receptors, including angiotensin II, bradykinin B2, muscarinic M2, endothelin-1, δ-opioid, and others, have all been implicated to play a contributory, additive, or redundant role in one or more models (26). Whether one or more of these other agonists is responsible for the protection achieved with transfer of perfusate in our model remains to be determined. Interestingly, pilot experiments from our group suggest that protection may be attenuated if transfused effluent from donor-PC hearts is first passed through a hydrophobic filter. However, confirmation of this observation, and identification of the filtered signal, awaits further study.

Clinical significance. Any extrapolation of these data beyond our current protocol must obviously be made with caution. Nonetheless, if remote preconditioning is indeed mediated by a hormone, its potential utility in clinical medicine may be significant. First, successful identification of the humoral trigger may be exploited in the design of pharmacologic therapy to protect the myocardium and mimic the benefits of ischemic preconditioning. Second, protection achieved with a preconditioning hormone may not be limited to the heart as with
conventional ischemic preconditioning (14, 18, 21); other organs and tissues may also be amenable to protection via the humoral trigger. Finally, release of a humoral signal in the setting of brief ischemia may serve as a diagnostic test in patients with coronary occlusive syndromes, thereby potentially improving patient outcome by facilitating earlier diagnosis and treatment.

In conclusion, cardioprotection by ischemic preconditioning, manifest as a reduction of infarct size, can be transferred via transfusion of coronary effluent between isolated buffer-perfused rabbit hearts. These data are consistent with the hypothesis that preconditioning at a distance is initiated by a humoral trigger signal. Adenosine or norepinephrine do not, however, appear to be the triggers responsible for evoking remote protection in this model.

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