Ischemic Preconditioning in Pigs: A Graded Phenomenon
Its Relation to Adenosine and Bradykinin

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Background—A threshold concept for ischemic preconditioning (IPC) has been proposed. It is unclear, however, whether IPC, above a certain threshold, is an all-or-nothing or a graded phenomenon.

Methods and Results—In 71 enflurane-anesthetized swine, severe left anterior descending coronary artery hypoperfusion for 90 minutes followed by 2 hours of reperfusion resulted in an infarct size (IS, by triphenyltetrazolium chloride) of 16.7±3.4% (SEM) of the area at risk. IPC by 2 minutes of low-flow ischemia and 15 minutes of reperfusion before the 90-minute target ischemia did not reduce IS (21.9±7.0%). IS was decreased to 9.0±2.6% (P<0.05) by 3 minutes of IPC and reduced further to 1.9±0.9% (P<0.05) by 10 minutes of IPC. The interstitial adenosine concentration (microdialysis, high-performance liquid chromatography) was unchanged with 2 and 3 minutes of IPC but increased with 10 minutes of IPC (by 573±144%). The interstitial bradykinin concentration (microdialysis, radioimmunoassay) remained unchanged with 2 minutes of IPC but increased to a similar extent with 3 minutes (by 198±32%) and 10 minutes (by 224±30%) of IPC. The IS reduction by 3 minutes of IPC was abolished by blockade of the bradykinin B2 receptor with intracoronary HOE 140 (16.6±4.3%) but not with intracoronary infusion of adenosine deaminase (8.4±2.5%, P<0.05).

Conclusions—IS reduction by IPC is a graded phenomenon. Whereas bradykinin is essential during preconditioning ischemia/reperfusion, blockade of the bradykinin B2 receptor abolished the IS reduction by 10 minutes of IPC. Combined infusion of HOE 140 and adenosine deaminase abolished the IS reduction by 10 minutes of IPC (15.4±6.7%).

Key Words: ischemia ■ adenosine ■ bradykinin

The delay of infarct size development by ischemic preconditioning is the most powerful endogenous cardioprotective phenomenon determined thus far.1 Endogenous activation of adenosine receptors in rabbits,2 dogs,3 and swine,4 α1-adrenergic receptor in dogs,5 bradykinin B2 receptors in rabbits,6 and opioid receptors in rats1 have been shown to be involved in the infarct size reduction achieved by ischemic preconditioning. In anesthetized rabbits, blockade of the bradykinin B2 receptor abolished the infarct size reduction by ischemic preconditioning with 1 cycle of 5 minutes of ischemia and 5 minutes of reperfusion preceding 30 minutes of sustained index ischemia.6 However, with 4 such cycles of preconditioning ischemia/reperfusion, blockade of the bradykinin B2 receptor no longer abolished the infarct size reduction by ischemic preconditioning.6 From these data, Downey and coworkers derived a threshold concept of ischemic preconditioning, in that a certain number of triggers during the preconditioning ischemia/reperfusion are necessary to achieve an infarct size reduction.

It remains unclear, however, whether ischemic preconditioning, above a certain threshold, is an all-or-nothing or a graded phenomenon.6,8–11 In anesthetized rabbits, infarct size was not significantly reduced further by 2 episodes10 or 4 episodes6 of 5 minutes of ischemia and 10 minutes of reperfusion over that by a single preconditioning episode of 5 minutes of ischemia and 10 minutes of reperfusion, whereas in another study, infarct size was significantly decreased further with 3 preconditioning cycles of 5 minutes of ischemia and 10 minutes of reperfusion over that by a single preconditioning cycle.11 Similarly, data in anesthetized pigs suggested, although it was not proven statistically, that infarct size reduction by ischemic preconditioning is a graded rather than an all-or-nothing phenomenon.9

We now tested in enflurane-anesthetized swine whether infarct size reduction by ischemic preconditioning depends on the strength of the preconditioning stimulus, and we therefore varied the duration of the preconditioning ischemia (3 minutes versus 10 minutes). In swine, the importance of adenosine and activation of ATP-dependent potassium channels for ischemic preconditioning has been documented4,12; the contribution of bradykinin to ischemic preconditioning in swine has not been determined thus far. We therefore measured the interstitial adenosine and bradykinin concentrations by microdialysis. In a second
step, we used adenosine deaminase and a bradykinin B₂-receptor antagonist to eliminate the contribution of either adenosine or bradykinin to preconditioning by 3 minutes versus 10 minutes of ischemia and 15 minutes of reperfusion preceding a sustained 90-minute index ischæmia.

Methods
The experimental protocols used in this study were approved by the Bioethical Committee of the district of Düsseldorf, and they adhere to the guiding principles of the American Physiological Society.

Experimental Model
The experimental model has been described extensively in a previous publication; in brief, in 71 enflurane-anesthetized Göttinger miniswine (20 to 40 kg), a micromanometer was placed in the left ventricle through the apex. Ultrasonic dimension gauges were implanted in the left ventricular myocardium to measure the thickness of the anterior and posterior (control) walls. The proximal left anterior descending coronary artery (LAD) was cannulated and perfused from an extracorporeal circuit. Left atrial pacing was performed at 10 bpm above sinus rhythm to avoid baroreflex-mediated changes in heart rate.

Regional Myocardial Blood Flow
Radiolaabeled microspheres (15 μm in diameter; ¹¹¹Ce, ¹³¹In, ⁹⁵Cr, ¹¹³Sn, ¹⁰³Ru, ⁹⁸Nb, or ⁴⁶Sc; NEN-DuPont Co) were injected into the coronary perfusion circuit to determine the regional myocardial blood flow and its distribution throughout the LAD perfusion bed (model 5912, Gammazint BF 5300 Packard).

Interstitial Adenosine and Bradykinin
Microdialysis probes (CMA/220, Bioanalytical Systems, Inc) were inserted into the LAD perfusion bed. Except for the first ischemia/reperfusion period, in which the effluent was collected over 2 or 3 or 15 minutes, respectively, all other dialysates were collected in 10-minute fractions. For the analysis of interstitial adenosine, a 15-μL aliquot of the microdialysis sample was subjected to high-performance liquid chromatography. The interstitial bradykinin was measured with a commercially available kit (Peninsula Laboratories Inc).

Adenosine Deaminase
The dose of adenosine deaminase (5 IU·mL⁻¹·min⁻¹) attenuated the cardioprotection achieved by ischemic preconditioning with 10 minutes of ischemia and 15 minutes of reperfusion in a previous study.

Blockade of the Bradykinin B₂ Receptor
In 4 separate dose-finding experiments, intracoronary infusion of bradykinin increased coronary inflow in a dose-dependent manner and, at a concentration of 70 nmol/L, by more than 2-fold. The concentration of HOE 140 that nearly completely blocked this increase in blood flow was 0.01 μg·kg⁻¹·min⁻¹ IC.

Morphology
At the end of each study, the heart was removed and sectioned from base to apex into 5 transverse slices in a plane parallel to the atroioventricular groove. The slices were immersed in a 0.09-mol/L sodium phosphate buffer (pH 7.4) containing 1.0% triphenyl tetrazolium chloride (Sigma) and 8% dextran (MW 77 800) for 20 minutes at 37°C to identify infarcted tissue. The amount of infarcted tissue is expressed as a percentage of the left ventricular area at risk as determined by the microsphere technique.

Experimental Protocols
A scheme of the protocols is presented in Figure 1.

In all swine, a 90-minute sustained index ischæmia was followed by 120 minutes of reperfusion to facilitate the identification of necrotic tissue. Duringangina, blood flow to the LAD was reduced to a level sufficient to reduce the regional work index by ~90%. During steady-state ischæmia, microspheres were injected into the LAD perfusion bed, and systemic hemodynamic and regional dimension data were recorded. A set of measurements was obtained within 1 minute. At 90 minutes of ischæmia, measurements were repeated, and the myocardium was reperfused for 120 minutes.

Protocol A: Infarct Size Reduction by Preconditioning Ischæmia of Different Durations

Control Group (n=7)
After control measurements, coronary inflow was reduced to achieve a 90% reduction in regional myocardial function.

Ipc2 Group (n=5)
After control measurements, the myocardium was subjected to 2 minutes of preconditioning ischæmia with a 90% reduction in regional myocardial function and then reperfused for 15 minutes. After reperfusion, coronary inflow was once again reduced to the same level as during the preconditioning ischæmia. Thereafter, this protocol was identical to that of the control group.

Ipc3 Group (n=7)
This protocol was identical to that of the IPC2 group, except that the preconditioning ischæmia was of 3-minute duration.

Ipc10 Group (n=7)
This protocol was identical to that of the IPC2 group, except that the preconditioning ischæmia was of 10-minute duration.

Protocol B: Contribution of Adenosine and Bradykinin to Infarct Size Reduction

Dea-Ipc3 Group (n=7)
After control measurements, the intracoronary infusion of adenosine deaminase was started 10 minutes before ischæmia and maintained until the end of the 90 minutes of sustained ischæmia. Except for the adenosine deaminase infusion, this protocol was identical to that of the IPC3 group. In a previous study using the same model, adenosine deaminase per se did not alter infarct size resulting from 90 minutes of ischæmia, but it attenuated the preconditioning achieved by 10 minutes of ischæmia and 15 minutes of reperfusion.
TABLE 1. Systemic Hemodynamics and Regional Myocardial Function and Blood Flow

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<th>Group</th>
<th>Control</th>
<th>Preconditioning Ischemia</th>
<th>15 min Reperfusion</th>
<th>5 min Ischemia</th>
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<td>0.12±0.02</td>
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</table>

LVpP indicates left ventricular peak pressure; CAP, mean coronary arterial pressure; CBF, mean coronary blood flow; WI, work index; and mTMF, mean transmural blood flow.

*P<0.05 vs control.
†P<0.05 vs preceding value.

HOE-Control Group (n=8)
After control measurements, bradykinin B₁ receptors were blocked by infusion of HOE 140. The infusion was started 30 minutes before ischemia and maintained until the end of the 90 minutes of sustained ischemia. Otherwise, this protocol was identical to that of the control group.

HOE-IPc3 Group (n=9)
After control measurements, the infusion of HOE 140 was started and maintained until the end of the 90 minutes of sustained ischemia. Otherwise, this protocol was identical to that of the IPC3 group.

HOE-IPc10 Group (n=8)
This protocol was identical to that of the HOE-IPc3 group, except that the preconditioning ischemia was of 10-minute duration.

HOE-Dea-Control Group (n=7)
After control measurements, the intracoronary infusion of HOE 140 was started. After 20 minutes of HOE 140 infusion, the additional adenosine deaminase infusion was started. Both infusions were maintained until the end of the 90 minutes of sustained ischemia. Otherwise, this protocol was identical to that of the control group.

HOE-Dea-IPc10 Group (n=6)
After control measurements, the intracoronary infusion of HOE 140 was started. After 20 minutes of HOE 140 infusion, the additional adenosine deaminase infusion was started. Both infusions were maintained until the end of the 90 minutes of sustained ischemia. Otherwise, this protocol was identical to that of the IPC10 group.

Data Analysis and Statistics
Hemodynamic and functional parameters were digitized and recorded over a 20-second period during each microsphere injection by use of CORDAT II software. Parameters reported are left ventricular peak pressure, mean coronary arterial pressure and blood flow, a regional work index, and mean transmural blood flow. Calculation of all parameters was done on a beat-to-beat basis, and data were then averaged. Biochemical parameters include the interstitial adenosine and bradykinin concentrations at baseline, peak values during the preconditioning cycle of ischemia/reperfusion, and peak values during the sustained ischemia.

Statistical analysis was performed with SYSTAT software. Hemodynamic and biochemical data were compared by 2-way ANOVA for repeated measures. Area at risk and infarct size were compared by 1-way ANOVA. When significant differences were detected, individual mean values were compared by post hoc tests. All data are reported as mean±SEM, and a value of P<0.05 was accepted as indicating a significant difference in mean values. Linear regression analyses between subendocardial blood flow at 5 minutes of ischemia and infarct size were performed in all groups. Regression lines were compared by ANCOVA.

Results
Data on systemic hemodynamics, regional myocardial function, and blood flow are summarized in Tables 1 and 2. Heart rate was held constant by left atrial pacing at 104±4 bpm. Regional myocardial function of the posterior control wall remained stable throughout the experimental protocol in each group.

Protocol A: Infarct Size Reduction by Preconditioning Ischemia of Different Durations
Systemic hemodynamics, regional myocardial blood flow, and function were not different among groups under resting and ischemic conditions (Table 1). At the end of the 15-minute reperfusion period, regional myocardial function...
tended to be decreased in the IPc3 group but was significantly depressed in the IPc2 and IPc10 groups.

**Interstitial Adenosine and Bradykinin**

The interstitial adenosine concentration was significantly increased only with 10 minutes of preconditioning ischemia (Figure 2). In contrast, the interstitial bradykinin concentration increased to a similar extent during 3 minutes and 10 minutes of preconditioning ischemia (Figure 2).

**Myocardial Infarction**

The area at risk was comparable among the control, IPc2, IPc3, and IPc10 groups, averaging 48.8±3.2%, 40.3±1.6%, 46.4±2.6%, and 42.6±1.3%, respectively. After 90 minutes of sustained ischemia and 120 minutes of reperfusion, 16.7±3.4% of the area at risk was infarcted. Infarct size remained unchanged (21.9±7.0%) in the IPc2 group. Infarct size was reduced to 9.0±2.6% in the IPc3 group (P<0.05 versus control and IPc2 groups) and further reduced to 1.9±0.9% in the IPc10 group (P<0.05 versus control, IPc2, and IPc3 groups). The slopes of the relationships between infarct size and subendocardial blood flow in the control and IPc2 groups were not significantly different (Figure 3). The slope was significantly reduced, however, in the IPc3 group (P<0.05) and was decreased even further in the IPc10 group (P<0.05 versus control, IPc2, and IPc3 groups).

**Protocol B: Contribution of Adenosine and Bradykinin to Infarct Size Reduction**

Systemic hemodynamics, regional myocardial blood flow, and function were not different among groups under control and ischemic conditions (Table 2).

**Interstitial Adenosine and Bradykinin**

Infusion of adenosine deaminase reduced the interstitial adenosine concentration to values <0.5 μmol/L. Thereafter, the interstitial adenosine concentration remained at this reduced level throughout the remaining experimental protocol (Figure 4). HOE 140 per se did not alter the interstitial adenosine concentration. Whereas with 3 minutes of preconditioning ischemia, adenosine concentration remained unchanged, it was significantly increased with 10 minutes of preconditioning ischemia. During the sustained 90-minute index ischemia, the interstitial adenosine concentration increased to a similar extent in the control and IPc3 groups, whereas in the IPc10 group, the increase in the interstitial adenosine concentration during the sustained ischemia was attenuated. HOE 140 and adenosine deaminase infusion did not alter the interstitial bradykinin concentration under control conditions. During the preconditioning ischemia/reperfusion, the interstitial bradykinin concentration was increased; the increase in the interstitial bradykinin concentration was not related to the duration of the preconditioning ischemia (Figure 5). During the prolonged index ischemia, the interstitial bradykinin concentration increased to a similar extent in all groups of swine (Figure 5).

**Myocardial Infarction**

The area at risk was comparable among the DEA-IPc3, HOE-control, HOE-IPc3, HOE-IPc10, HOE-Dea-control, and HOE-Dea-IPc10 groups, averaging 46.6±2.7%, 47.4±2.5%, 42.9±2.9%, 45.2±3.3%, 48.7±4.6%, and 46.0±1.2%, respectively. Infusion of adenosine deaminase did not attenuate the infarct size reduction achieved by 3 minutes of ischemia and 15 minutes of reperfusion (8.4±2.5%). In the presence of HOE 140, after the 90 minutes of sustained ischemia and 120 minutes of reperfusion, 14.0±1.8% of the area at risk was infarcted. With preconditioning by 3 minutes of ischemia and 15 minutes of reperfusion in the presence of HOE 140, infarct size was no longer reduced (16.6±4.3%). In contrast, the reduction in infarct size achieved by 10 minutes of ischemia and 15 minutes of reperfusion was not affected by HOE 140.
TABLE 2. Systemic Hemodynamics and Regional Myocardial Function and Blood Flow

<table>
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<th>Group</th>
<th>Control</th>
<th>Drug Treatment</th>
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<td>178±21†</td>
<td>18±10*†</td>
<td>15±8*</td>
</tr>
<tr>
<td>HOE-Dea-Control</td>
<td>262±32</td>
<td>217±32</td>
<td>NA</td>
<td>NA</td>
<td>2±5†</td>
<td>3±3*</td>
</tr>
<tr>
<td>HOE-Dea-IPc10</td>
<td>249±19</td>
<td>232±20</td>
<td>19±10*†</td>
<td>96±19†</td>
<td>11±7†</td>
<td>11±10*</td>
</tr>
<tr>
<td>mTMF, mL · min⁻¹ · g⁻¹</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dea-IPc3</td>
<td>NA</td>
<td>1.00±0.12</td>
<td>0.12±0.02†</td>
<td>0.75±0.10†</td>
<td>0.12±0.02†</td>
<td>0.16±0.03†</td>
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<tr>
<td>HOE-Control</td>
<td>0.63±0.05</td>
<td>0.74±0.05</td>
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<td>NA</td>
<td>0.11±0.01†</td>
<td>0.12±0.01*</td>
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<td>HOE-IPc3</td>
<td>NA</td>
<td>0.72±0.07</td>
<td>0.12±0.01†</td>
<td>0.81±0.10†</td>
<td>0.11±0.14†</td>
<td>0.13±0.02‡</td>
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<td>HOE-IPc10</td>
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<td>0.71±0.08</td>
<td>0.14±0.02‡</td>
<td>0.88±0.16‡</td>
<td>0.15±0.03‡</td>
<td>0.16±0.03‡</td>
</tr>
<tr>
<td>HOE-Dea-Control</td>
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<td>0.70±0.05</td>
<td>NA</td>
<td>NA</td>
<td>0.11±0.02†</td>
<td>0.12±0.02*</td>
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<tr>
<td>HOE-Dea-IPc10</td>
<td>NA</td>
<td>0.75±0.05</td>
<td>0.11±0.03‡</td>
<td>0.71±0.17†</td>
<td>0.10±0.03‡</td>
<td>0.12±0.03‡</td>
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Abbreviations as in Table 1.
*P<0.05 vs control.
†P<0.05 vs preceding value.
‡P<0.05<0.05 vs drug treatment.

(3.5±1.1%, still P<0.05 versus control and HOE-control groups). Combined infusion of HOE 140 and adenosine deaminase slightly reduced infarct size (11.0±3.1%, P=NS versus control group) and completely abolished the infarct size reduction achieved by 10 minutes of ischemia and 15 minutes of reperfusion (15.4±2.6%). The reduction in the slope of the relationship between infarct size and subendocardial blood flow (Figure 6) by preconditioning with 3 minutes of ischemia and 15 minutes of reperfusion was abolished by HOE 140 but not by adenosine deaminase infusion (still P<0.05 versus control group). HOE 140, however, did not alter the relationship between infarct size and subendocardial blood flow with preconditioning by 10 minutes of ischemia and 15 minutes of reperfusion (still P<0.05 versus control group). With combined infusion of HOE 140 and adenosine deaminase, the relationships between infarct size and subendocardial blood flow in the control group and the 10-minute ischemic preconditioning group were not significantly different (Figure 7).

Discussion

Critique of Methods

The present experiments were performed in swine because infarct development in this species, as a result of the sparsity of the innate collateral circulation, most closely resembles that observed in humans.16

Hypoperfusion at low flow made it possible that adenosine deaminase and HOE 140 could be administered throughout the ischemic period, ensuring a homogeneous delivery at a high concentration but without effects on systemic hemodynamics. Also, infarct development could be related to ischemic subendocardial blood flow, and thus, a more sensitive end point than infarct size per se could be used, because even
triggers in ischemic preconditioning. But rather qualitatively reflect the involvement of these 

Therefore, the adenosine and bradykinin concentrations in the 

exchange rate of the microdialysis membrane in vivo might 

be different from the exchange rate determined in vitro.

Within microdialysis, absolute concentrations of substances 

within the interstitial space cannot be determined, because complete equilibration between the buffer within the dialysis membrane and the interstitial fluid does not occur at perfusion rates of 0.5 to 5.0 μL/min. Furthermore, the exchange rate of the microdialysis membrane in vivo might be different from the exchange rate determined in vitro. Therefore, the adenosine and bradykinin concentrations in the present study cannot be taken in exactly quantitative terms but rather qualitatively reflect the involvement of these triggers in ischemic preconditioning.

Ischemic Preconditioning: A Graded Phenomenon

Clearly, a threshold exists below which ischemic preconditioning does not reduce infarct size, and in our preparation, this threshold is somewhere between 2 and 3 minutes in duration of the preconditioning ischemia. Also, in anesthetized rabbits, 2 cycles of 2 minutes of coronary occlusion with 10 minutes of reperfusion each did not reduce infarct size after 30 minutes of coronary occlusion, but a single cycle of

5 minutes of ischemia and 10 minutes of repertusion reduced infarct size significantly.

Controversy remains as to whether or not, above such threshold, ischemic preconditioning is a graded or an all-or-nothing phenomenon. In anesthetized dogs and rabbits, the infarct size reduction by 1 single episode of 5 minutes of ischemia with 10 minutes of reperfusion was as effective as preconditioning with 24 or 12 such episodes of preconditioning ischemia/reperfusion. In contrast, in anesthetized swine, 1 single episode of 10 minutes of ischemia and 30 minutes of reperfusion reduced infarct size from 71.3 ± 4.4% to 54.3 ± 10.2% ($P=NS$), but infarct size was further reduced when 2 such preconditioning episodes were used (25.6 ± 3.9%, $P<0.05$). The level of significance for protection with 1 single preconditioning episode was possibly missed only because of the small number of animals studied.

Figure 4. Infusion of adenosine deaminase (Dea) reduced interstitial adenosine concentrations to values <0.5 μmol/L. Thereafter, interstitial adenosine concentrations remained at this reduced level throughout remaining experimental protocol. HOE 140 per se did not alter interstitial adenosine concentration. Interstitial adenosine concentration was not altered with 3 minutes of preconditioning ischemia but was significantly increased with 10 minutes of preconditioning ischemia. During index ischemia, interstitial adenosine concentrations increased to a similar extent in control and IPC3 groups; in IPC10 group, increase in interstitial adenosine concentration was attenuated. *$P<0.05$ vs resting conditions (REST), §$P<0.05$ vs all other groups.

in collateral-deficient species, some flow variations during total coronary artery occlusion occur. With microdialysis, absolute concentrations of substances within the interstitial space cannot be determined, because complete equilibration between the buffer within the dialysate membrane and the interstitial fluid does not occur at perfusion rates of 0.5 to 5.0 μL/min. Furthermore, the exchange rate of the microdialysis membrane in vivo might be different from the exchange rate determined in vitro. Therefore, the adenosine and bradykinin concentrations in the present study cannot be taken in exactly quantitative terms but rather qualitatively reflect the involvement of these triggers in ischemic preconditioning.

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Figure 5. HOE 140 and adenosine deaminase (Dea) infusion did not significantly alter interstitial bradykinin concentrations. During preconditioning ischemia/reperfusion period, interstitial bradykinin concentrations increased; these increases in interstitial bradykinin concentrations were not related to duration of preconditioning ischemia. During prolonged index ischemia, interstitial bradykinin concentration increased to a similar extent in all groups of swine. *$P<0.05$ vs REST.

Figure 6. Relationships of subendocardial blood flow and infarct size in swine undergoing 90 minutes of index ischemia without and with 3 minutes and 10 minutes of preconditioning ischemia in presence of HOE 140. Adenosine deaminase did not affect reduction in slope of relationships between infarct size and subendocardial blood flow with preconditioning by 3 minutes of ischemia and subendocardial blood flow with preconditioning by 3 minutes of ischemia was abolished by HOE 140 ($y=−279.2x+31.5, n=7, r=0.74, P<0.05$ vs control group). Reduction in slope of relationships between infarct size and subendocardial blood flow with preconditioning by 3 minutes of ischemia was abolished by HOE 140 ($y=−279.2x+31.5, n=9, r=0.74$). HOE 140, however, did not alter relationship between infarct size and subendocardial blood flow achieved with preconditioning by 10 minutes of ischemia ($y=−51.3x+6.4, n=8, r=0.46, P<0.05$ vs HOE-control and HOE-IPC3 groups).

Figure 7. Relationships of subendocardial blood flow and infarct size in swine undergoing 90-minute index ischemia without and with preconditioning by 10 minutes of ischemia in presence of HOE 140 and adenosine deaminase (Dea). With combined infusion of HOE 140 and Dea, relationships between infarct size and subendocardial blood flow in control and preconditioned hearts were not significantly different ($y=−201x+25.7, n=7, r=0.87$ vs $y=−308x+34.4, n=6, r=0.88$).
Similarly, in a recent study in anesthetized rabbits, 1 single episode of 5 minutes of ischemia with 10 minutes of reperfusion reduced infarct size compared with placebo (36% versus 60%) but to a much lesser extent than 3 such episodes (0.6%).

The previous studies varied the number of preconditioning cycles of constant duration to vary the intensity of the preconditioning stimulus, whereas in the present study, the duration of a single preconditioning ischemic episode was varied; therefore, results are not necessarily transferable.

**Sources of Adenosine and Bradykinin**

The most obvious pool of adenosine is the breakdown of cytosolic AMP in cardiomyocytes (for review, see Reference 19). In previous studies, the interstitial adenosine concentration increased no earlier than after 5 minutes of ischemia. In accordance with previously published data in anesthetized dogs,[21] rabbits,[22] and pigs,[23] the interstitial adenosine concentration during the sustained ischemia was attenuated in the 3-minute and 10-minute ischemic preconditioning groups. Such attenuation of the increase in the interstitial adenosine concentration is also observed when ischemic preconditioning is abolished by adenosine receptor blockade, and therefore, it is not a reflection of the cardioprotective effect.[24]

The cellular origin of bradykinin during ischemia is unclear. The reduction in oxygen supply is unlikely to cause the release or production of bradykinin, especially because the endothelium is more resistant to ischemia/reperfusion injury than the cardiomyocyte itself.[25] With the reduction in flow, however, the mechanical forces on the endothelium are altered. Experimental data for the dependence of bradykinin release/production on mechanical forces, however, are lacking. Altered proton release during flow restriction could also increase the bradykinin concentration, because a reduction in plasma pH can activate plasma kallikrein and reduce kinin breakdown.[26] Indeed, coronary venous pH, as a gross measure of proton production, was lower during the initial preconditioning ischemic period in the IPc3 group (from 7.392±0.015 under control conditions to 7.347±0.017 with 3 minutes of ischemia, P<0.05) and the IPc10 group (from 7.352±0.017 to 7.204±0.025 with 10 minutes of ischemia, P<0.05) than in the IPc2 group (from 7.391±0.025 to 7.371±0.028 with 2 minutes of ischemia, P=NS), and only in the IPc3 and IPc10 groups was an increased interstitial bradykinin concentration measured during the preconditioning cycle of ischemia/reperfusion. Although the source/trigger of bradykinin release/production remains unclear, in a previous study in anesthetized dogs, increased coronary venous bradykinin concentration was measured as early as after 3 to 5 minutes of ischemia.[27]

**Contribution of Adenosine and Bradykinin to Ischemic Preconditioning**

The results of the present study support the threshold concept proposed by Downey and coworkers.[6] In accordance with their study, bradykinin was of major importance only during a less intense preconditioning stimulus, ie, a shorter duration of preconditioning ischemia, whereas during a more intense preconditioning stimulus, ie, a more prolonged period of preconditioning ischemia, adenosine was of greater importance. Also, the additive interaction of triggers was confirmed in the present study, in that only combined blockade of the bradykinin B2 receptor by HOE 140 and increased breakdown of endogenous adenosine by adenosine deaminase completely abolished the infarct size reduction achieved by preconditioning with 10 minutes of ischemia and 15 minutes of reperfusion.

In conclusion, infarct size reduction by a single episode of preconditioning ischemia/reperfusion is a graded rather than an all-or-nothing phenomenon in the anesthetized swine in situ. Although bradykinin is essential during preconditioning ischemia of shorter duration, adenosine is more important during preconditioning ischemia of longer duration.

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