Angiotensin-Converting Enzyme Inhibitors Potentiate Preconditioning Through Bradykinin B₂ Receptor Activation in Human Heart

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Objectives. This study was designed to determine whether angiotensin-converting enzyme (ACE) inhibitors play a role in cardioprotection in a human model of preconditioning.

Background. Recent studies have suggested that bradykinin may contribute to the protective effects of preconditioning in animal models. ACE inhibitors are known to inhibit the degradation of bradykinin and hence may be able to potentiate the effect of preconditioning.

Methods. We examined the effects of the ACE inhibitors captopril and lisinopril in combination with a subthreshold preconditioning stimulus (i.e., insufficient to have any protective effects alone). Human atrial trabeculae were superfused with Krebs buffer and paced at 1 Hz. They were subjected to a full or subthreshold preconditioning stimulus consisting of either 3 or 1.5 min of simulated ischemia and 7 min of reoxygenation. In each instance, this stimulus was followed by 90 min of simulated ischemia and 2 h of reoxygenation. In addition, the subthreshold preconditioned group had 20 min of previous ACE inhibitor treatment.

Results. Recovery of contractile function (percent of baseline) was 22 ± 1% (mean ± SEM) in the control group versus 61 ± 1% in the preconditioned group. The subthreshold preconditioned group and the ACE inhibitor-alone groups did not exhibit any protection; however, in combination, the protection was significant (71 ± 4% in the captopril group, 58 ± 8% in the lisinopril group, p < 0.005) compared with the control group. There was no significant difference between these values and recovery after the full preconditioning stimulus. Furthermore, Hoe 140, a specific bradykinin B₂ receptor antagonist, abolished the protection.

Conclusions. To our knowledge, these are the first results in human muscle to suggest that ACE inhibitors may augment ischemic preconditioning, possibly through B₂ receptor activation.

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The introduction of angiotensin-converting enzyme (ACE) inhibitor therapy has had an enormous impact on clinical practice, far beyond initial expectations when first introduced as antihypertensive agents. As well as their use in the management of heart failure, they are now being implicated as myocardial and vascular “protective” agents that not only prevent left ventricular remodeling, but also reduce myocardial ischemic events. These anti-ischemic and antiremodeling effects were somewhat unexpected, and the exact mechanisms involved are uncertain. Large trials, such as the Studies of Left Ventricular Dysfunction (SOLVD) (1) and Survival and Ventricular Enlargement (SAVE) (2) trials, have shown a significant effect of ACE inhibitors on the prevention of myocardial ischemic events. More recent evidence comes from trials such as Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico III (GISSI-3) (3), in which the lisinopril-treated group had a significant reduction in early mortality (6 weeks), and Fourth International Study of Infarct Survival (ISIS-4) (4), which showed a 7% decrease in 5-week mortality in the captopril group.

There is accumulating evidence for a direct protective role for ACE inhibitors in the ischemic/reperfused myocardium (5–9), although the published reports are conflicting. Experiments to date have largely demonstrated this protective effect if the ACE inhibitor is given as pretreatment before infarction. It was proposed that ACE inhibitors protect in this manner by inhibiting the breakdown of bradykinin (9) because Hoe 140 has been shown to reverse the protective effect (6,7). A recent study by Miki et al. (10), showed that captopril potentiates the infarct size-limiting effect of ischemic preconditioning through bradykinin B₂ receptors in a rabbit model.

Ischemic preconditioning is another phenomenon that offers the myocardium an extremely powerful means of self-protection. During ischemia, locally produced mediators, such as adenosine, catecholamines and bradykinin, may act as triggers of preconditioning, in which resistance to ischemic injury is enhanced by previous exposure to a brief episode of ischemia followed by reperfusion. The importance of these mediators requires further elucidation, although the involvement of the adenosine receptor in the human heart was previously confirmed by our group (11). The question remains, Is there a role for some of the other putative mediators of preconditioning in the human heart?
There is now increasing awareness that ACE inhibitor activity is mediated in part through increased local bradykinin levels. Intracardiac production of bradykinin has been shown to be increased in myocardial ischemia (8,12,13), and, furthermore, recent reports suggest that infarct size limitation in the rabbit is blocked by the B2 receptor antagonist Hoe 140 and mimicked by kinin administration (7).

It has also been reported that direct intracoronary infusion of bradykinin can reduce infarct size in dogs (14) and also mimic preconditioning by reducing the severity of ischemia-induced arrhythmias (5). These effects have been proposed to be mediated through prostacyclin or nitric oxide, or both, and increasing cyclic guanosine monophosphate.

We recently developed a model of preconditioning using isolated superfused isometrically contracting human atrial trabeculae. This model has the advantage of avoiding complications of collateral flow, and the mix of necrotic and viable tissue within each specimen is comparable to the situation in evolving acute myocardial infarction. Previous studies have established that in this model, the protective effects of preconditioning can be induced by activation of adenosine A1 receptors (11) and that protein kinase C (PKC) and the activation of the ATP-dependent potassium channel (KATP) are also involved (15), confirming results obtained in animal studies.

The aim of the present study was to determine whether ACE inhibitors, (with and without sulfhydryl groups) could contribute to the protective effects of preconditioning. Furthermore, using a specific B2 receptor antagonist, Hoe 140, our aim was to determine whether any protective effect observed is related to enhanced bradykinin B2 receptor activation after reduced kinin degradation.

**Methods**

**Experimental model.** Experiments were performed in trabeculae derived from human atrium. Specimens of right atrial appendage were obtained from the right atrial cannulation site in patients undergoing coronary artery bypass grafting (46 patients, 37 men, 9 women; 39 to 79 years old, mean age 64). All patients had chronic stable angina, and those with right ventricular failure or atrial arrhythmias or taking antiarrhythmic medication were excluded. Patients taking oral hypoglycemic agents and those receiving ACE inhibitors were also excluded. Ethical approval for this procedure was obtained from the hospital ethics committee. The specimens were transported to the laboratory in oxygenated modified Tyrode’s solution.

Atrial trabeculae (diameter 0.9 mm, length ≥ 3 mm) were dissected under magnification in a dish superfused with Tyrode’s solution, tied at one end with a 7/0 silk suture and removed together with a small portion of atrial wall at the free end. The silk suture was then used to attach the muscle to a fixed post in an organ bath while the free end was attached to a force transducer (Gould Statham UCT 2) using a snare around the wedge of atrial wall. The muscles were suspended horizontally in the organ bath through which there was a continuous flow of superfusate oxygenated with a 95% O2/5% CO2 gas mixture. The superfusate was a modified Tyrode's solution of the following composition (mmol/liter): NaCl 118.5, KCl 4.8, NaHCO3 24.8, KH2PO4 1.2, MgSO4·7H2O 1.44, CaCl2·2H2O 1.8, glucose 10.0 and pyruvic acid 10.0. In the substrate-free Tyrode’s solution, 7 mmol/liter choline chloride was substituted for glucose and pyruvic acid to maintain constant osmolarity. All reagents were AnaR grade from BDH Chemicals, Poole, England, except for pyruvic acid from Sigma Chemicals. The organ bath was covered to prevent gas exchange with the atmosphere. Gas tensions in the organ bath were analyzed intermittently using an automated blood gas analyzer (AVL 993, AVL Medical Instruments, Switzerland).

The pH was maintained between 7.35 and 7.45, the partial pressure of O2 between 50 and 60 kPa and partial pressure of CO2 between 4.0 and 6.0 kPa. During simulated ischemia, the superfusate was free of substrate and was bubbled with 95% N2/5% CO2 to lower the partial pressure O2 in the organ bath to 6 to 8 kPa. (pH 7.24 to 7.34). The organ bath and preorgan bath heat exchanger were both water jacketed and circulated (Technic circulator C 85-A, Cambridge, UK) to maintain a constant temperature of 37.0 ± 0.1°C, which was monitored through a thermocouple in the bath. The length and width of the trabecular muscles were recorded at the end of the experiment using an eyepiece graticule in an overhead microscope (Prior, UK), and all specimens were then weighed. The cross-sectional area was calculated by dividing muscle mass by length times density, assuming a cylindric shape and a density of 1.0 mg/mm3. To ensure that initial comparisons of developed tensions and rest force were not affected by variable muscle size, cross-sectional area of the muscles was used to calculate tensions. In addition, all muscles with a cross-sectional area >1.2 mm2 were excluded from the study (16).

**Muscle stimulation and recording of data.** Once horizontally suspended, trabeculae were paced by field stimulation at 1 Hz by parallel flattened platinum electrodes with an isolated stimulator (Digitimer DS2, Hertfordshire, UK) triggered by a programmable computerized clock. The pulse width was fixed at 5 ms and the pulse amplitude set at twice threshold (6 to 8 mV). Contractile force was amplified and recorded on paper (Universal Amplifier and RS3400 ink pen recorder, Gould).

**Chemicals.** Captopril and lisinopril were obtained from Sigma. Captopril was dissolved in normal saline and added to Tyrode’s solution to give a final concentration of 10 mmol/liter, and lisinopril was similarly dissolved to give a final concentra-
tion of 20 μmol/liter—concentrations comparable to plasma levels in patients taking these particular ACE inhibitors (17). Hoe 140 was diluted to a final concentration of 20 nmol/liter, a concentration known to be sufficient to effectively antagonize B₂ receptors.

**Experimental protocols.** Trabeculae were initially stimulated at 1 Hz unstretched for 30 min to allow time for recovery. They were subsequently stretched in a stepwise manner over 15 min to a length developing 90% of maximal force and allowed to equilibrate for a further 30 min. All groups eventually underwent a period of simulated ischemia that consisted of 90 min of hypoxic substrate-free superfusion and rapid pacing at 3 Hz, followed by reperfusion for 120 min with oxygenated Tyrode’s solution and pacing at 1 Hz. The preconditioning protocol consisted of 3 min of hypoxic substrate-free superfusion with trabeculae paced at 3 Hz, followed by 7 min of reperfusion with oxygenated Tyrode’s solution with substrate and pacing at 1 Hz. A subthreshold preconditioning protocol was established that failed to protect alone. This protocol consisted of 90 s of pacing at 3 Hz with hypoxic substrate-free perfusate and was also followed by 7 min of reperfusion with oxygenated Tyrode’s solution. Figure 1 shows the experimental protocols for the 10 groups, all of which were finally subjected to 90 min of simulated ischemia:

1) Control protocol = stabilization period followed by 90 min of ischemia; 2) ACE inhibitor protocol = superfusion with captopril or lisinopril for 20 min before 90 min of ischemia; 3) preconditioning protocol = preconditioning followed by 90 min of ischemia; 4) subthreshold preconditioning protocol = subthreshold preconditioning followed by ischemia; 5) ACE inhibitor plus subthreshold preconditioning protocol = 20 min of superfusion with captopril or lisinopril, followed by subthreshold preconditioning; and 6) Hoe 140 plus subthreshold preconditioning protocol = superfusion with Hoe 140 for 20 min before subthreshold preconditioning; and 7) Hoe 140 plus ACE inhibitor plus subthreshold preconditioning protocol = superfusion with Hoe 140 for 10 min before addition of captopril or lisinopril for 20 min, followed by subthreshold preconditioning. For all these groups n = 6, including six experiments for each ACE inhibitor, with a maximum of two trabeculae from each heart.

**Statistical analysis.** Results are presented as mean value ± SEM. Statistical differences between groups were evaluated by two-way analysis of variance followed by a Fisher protected least significant difference post hoc test with respect to treatment and time; p ≤ 0.05 was considered significant.
compared with 61

6 time points. Recovery was 22

3-min ischemic preconditioning protocol, although captopril

ACE inhibitor in combination with a subthreshold

preconditioning-alone (23 ± 4%); this group had a level of

recovery similar to that of the control group (no pretreatment).

ACE inhibitor pretreatment alone had no protective effects

in contrast to the marked protection observed when combined

with subthreshold preconditioning, as seen in Figure 3. In

addition, Hoe 140 pretreatment alone did not adversely affect

recovery compared with that in the control group, with a

maximal recovery in this group of 20 ± 5%.

Figures 4 and 5 illustrate the effects of pretreatment with the B<sub>2</sub> receptor antagonist Hoe 140 on the captopril-

Table 1. Physical Characteristics and Baseline Functional Data of Trabeculae Study Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Length (mm)</th>
<th>Mass (g)</th>
<th>Diameter (mm)</th>
<th>Rest Force (g)</th>
<th>Developed Force (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.4 ± 0.3</td>
<td>0.006 ± 0.001</td>
<td>0.71 ± 0.04</td>
<td>0.65 ± 0.11</td>
<td>1.20 ± 0.16</td>
</tr>
<tr>
<td>Cap</td>
<td>4.5 ± 0.3</td>
<td>0.006 ± 0.001</td>
<td>0.73 ± 0.04</td>
<td>0.77 ± 0.10</td>
<td>1.51 ± 0.18</td>
</tr>
<tr>
<td>Lis</td>
<td>4.9 ± 0.5</td>
<td>0.005 ± 0.001</td>
<td>0.77 ± 0.06</td>
<td>0.70 ± 0.09</td>
<td>1.45 ± 0.13</td>
</tr>
<tr>
<td>PC</td>
<td>4.2 ± 0.3</td>
<td>0.005 ± 0.001</td>
<td>0.71 ± 0.05</td>
<td>0.71 ± 0.10</td>
<td>1.38 ± 0.19</td>
</tr>
<tr>
<td>sPC</td>
<td>4.5 ± 0.4</td>
<td>0.005 ± 0.001</td>
<td>0.67 ± 0.04</td>
<td>0.70 ± 0.10</td>
<td>1.35 ± 0.20</td>
</tr>
<tr>
<td>Cap+sPC</td>
<td>5.0 ± 0.4</td>
<td>0.006 ± 0.001</td>
<td>0.71 ± 0.05</td>
<td>0.77 ± 0.11</td>
<td>1.26 ± 0.21</td>
</tr>
<tr>
<td>Lis+sPC</td>
<td>4.7 ± 0.3</td>
<td>0.006 ± 0.001</td>
<td>0.70 ± 0.02</td>
<td>0.66 ± 0.11</td>
<td>1.19 ± 0.21</td>
</tr>
<tr>
<td>Hoe+sPC</td>
<td>4.6 ± 0.4</td>
<td>0.005 ± 0.001</td>
<td>0.69 ± 0.05</td>
<td>0.68 ± 0.12</td>
<td>1.28 ± 0.17</td>
</tr>
<tr>
<td>Hoe+Cap+sPC</td>
<td>4.5 ± 0.3</td>
<td>0.006 ± 0.001</td>
<td>0.67 ± 0.06</td>
<td>0.75 ± 0.12</td>
<td>1.47 ± 0.22</td>
</tr>
<tr>
<td>Hoe+Lis+sPC</td>
<td>4.7 ± 0.4</td>
<td>0.006 ± 0.001</td>
<td>0.71 ± 0.05</td>
<td>0.67 ± 0.13</td>
<td>1.48 ± 0.19</td>
</tr>
</tbody>
</table>

Data presented are mean value ± SEM. Cap = captopril; Hoe = Hoe 140; Lis = lisinopril; PC = preconditioning; sPC = subthreshold preconditioning.

**Results**

*Exclusions.* Three muscles were excluded because of a calculated cross-sectional area >1.2 mm<sup>2</sup>. There were no other exclusions, and all samples completed the protocol to which they were randomly assigned.

**Analysis of data.** The physical characteristics of the trabeculae and baseline functional data were similar in all 10 groups (Table 1), including rest and developed force. Therefore, experimental data on developed force are presented graphically as percent of baseline developed force.

The addition of an ACE inhibitor at the beginning of the experiment led to a minor, but nonsignificant, reduction in developed force. The 3-min or 90-s preconditioning protocols resulted in a significant reduction in developed force that recovered to baseline on reperfusion before the long ischemic insult.

Figures 2 to 5 show the results for developed force as a percent of baseline. Time 0 indicates the beginning of simulated ischemia; therefore, reperfusion with oxygenated Tyrode's solution extends from 90 to 210 min. As indicated in the diagram of the protocols (Fig. 1), simulated ischemia is preceded in all groups by a stabilization period and various pretreatments (indicated on the graphs by an arrow). As noted, there was no significant difference in developed force between the 10 groups at the end of the stabilization period, and this baseline function is denoted as 100% developed force.

Figure 2 illustrates the synergistic effect of pretreatment with an ACE inhibitor in combination with a subthreshold preconditioning stimulus. The level of protection observed is of a similar magnitude to that obtained with the standard 3-min ischemic preconditioning protocol, although captopril was significantly more protective than lisinopril at the last two time points. Recovery was 22 ± 1% in the control group compared with 61 ± 1% in the preconditioned group, 71 ± 4% in the captopril plus subthreshold preconditioned group and 58 ± 8% in the lisinopril plus subthreshold preconditioned group (at the end of reperfusion, i.e., 210 min). There was no protection in the group receiving subthreshold ischemic pre-
lisinopril-treated groups, respectively. In Figure 4 it can be seen that Hoe 140 clearly abrogates the protective effects of captopril in combination with subthreshold preconditioning, with a functional recovery of 35 ± 5%. However, there is some residual protective effect observed, although this was not significant. Figure 5 also shows that Hoe 140 abrogates the protective effects of lisinopril in combination with subthreshold ischemic preconditioning (31 ± 5%).

Discussion

Study findings. This study shows that ACE inhibition in combination with a subthreshold preconditioning stimulus resulted in a significant degree of protection, in terms of recovery of function, when the trabeculae were subjected to a subsequent 90-min period of simulated ischemia. The degree of protection provided by combined subthreshold preconditioning and ACE inhibition is of a comparable degree to that after a full ischemic preconditioning protocol. Furthermore, the cardioprotective effect was abolished by pretreatment with the B<sub>2</sub> receptor antagonist Hoe 140. These results support the hypothesis that generation of bradykinin during preconditioning contributes, through B<sub>2</sub> receptor activation, to the protection observed in terms of improved posts ischemic functional recovery. However, pretreatment with ACE inhibitor alone did not result in protection, perhaps because a degree of ischemia is required to release bradykinin initially, or other mediators, such as adenosine, are required in addition to trigger preconditioning.

Figure 3. Developed force expressed as percent of baseline force over time (min), comparing the combination of ACE inhibitor plus subthreshold preconditioning (sPC) with ACE inhibitor alone. The percent of recovery in the ACE inhibitor plus subthreshold preconditioning groups is significantly greater than that in the ACE inhibitor-alone groups (*p < 0.005). Open circles = lisinopril; solid circles = lisinopril plus subthreshold preconditioning; solid squares = captopril; open squares = captopril plus subthreshold preconditioning.

Figure 4. Developed force expressed as percent of baseline force over time (min), illustrating that pretreatment with Hoe 140 abrogates the protective effect of captopril in combination with subthreshold preconditioning (sPC) (solid circles) (*p < 0.005). Recovery of the Hoe 140-pretreated group was not statistically significant from that for the control group. Solid squares = Hoe 140 plus captopril plus subthreshold preconditioning; open squares = subthreshold preconditioning; PC = preconditioning.

There are several clinical studies suggesting that preconditioning occurs in humans (18–20), with evidence from in vitro studies using isolated human ventricular cardiomyocytes that were protected against a 90-min period of simulated ischemia by a preconditioning protocol (21). The other studies are from our own group (11,15) using the same model as that in the present study. These studies have demonstrated that preconditioning of human atrial trabeculae results in a significantly better posts ischemic recovery of contractile function than non-preconditioned trabeculae and, in common with animal models of preconditioning, have confirmed a role for adenosine receptors and PKC (11,15).

Human atrial model. The present study used isolated human atrial trabeculae to examine the mechanism of preconditioning in human myocardium. Atrial specimens make stable preparations, and sampling was part of the routine procedure for coronary artery bypass graft surgery. However, there are differences between atrial and ventricular tissue, and the results from one may not be applicable to the other.

The present study used a period of hypoxic superfusion in combination with rapid pacing to simulate ischemia rather than the “true” ischemia of ischemic preconditioning. However, there is a great deal of evidence (22–25) in animal models of both regional and global hypoxia and in cell cultures that hypoxia is as effective as ischemia in inducing preconditioning. We know that preconditioning attenuates contractile dysfunction as well as reducing infarct size. For example, Jenkins et al. (26), who measured both infarct volume and functional recovery after ischemia, showed that the improved recovery of
subthreshold preconditioning; PC

plus lisinopril plus subthreshold preconditioning; open squares

levels in our samples, Hoe 140 is the most specific and potent

system. They demonstrated the presence of kallikrein (a potent

enzymatic activity) in primary cultures of neonatal rat atrial and ventricular cardiocytes and in their incubation medium. Heart slices were shown to release kallikrein without depletion of total tissue kallikrein, suggesting pool replenishment. In fact, kallikrein was found in epicardial slices and in the Krebs solution bathing the slices (which were washed and reincubated in Krebs solution)—and this release was inhibited by pretreatment with puromycin—suggesting primary synthesis. Presumably, ischemia results in pH and other changes that cause activation of kallikrein within the myocardium and the subsequent local availability of bradykinin (33). Hence, this work supports the hypothesis that local production of kinin contributed to the protection observed.

Figure 5. Developed force expressed as percent of baseline force over time (min), illustrating that pretreatment with Hoe 140 abrogates the protective effect of lisinopril in combination with subthreshold preconditioning (sPC) (solid circles) (*p < 0.005). Solid squares = Hoe 140 plus lisinopril plus subthreshold preconditioning; open squares = subthreshold preconditioning; PC = preconditioning.

global left ventricular function produced by preconditioning is proportional to a reduction in infarction, and Cohen et al. (27) and Przyklenk et al. (28) were able to correlate improved recovery of systolic shortening with reduced infarct size in vivo models of regional ischemia. Clearly, these studies demonstrate that the enhanced recovery of contractile function due to preconditioning that follows a prolonged period of ischemia results from a reduction in infarct size and is not due to a reduction in stunning. Our model used a 90-min period of simulated ischemia as the ischemic insult, a period more likely to result in cell death than stunning, which is induced by shorter periods of 5 to 15 min of ischemia. Hence, there is evidence that hypoxia can be as effective as ischemia in preconditioning, that recovery of contractile function is a good correlate for the degree of myocardial necrosis and that this experimental model is representative of the preconditioning phenomenon.

Role of bradykinin. Although we did not measure kinin levels in our samples, Hoe 140 is the most specific and potent B2 receptor antagonist currently known. It has virtually the same affinity for B1 receptors as bradykinin itself and does not react with receptors for other peptides. Furthermore, kinin release from ischemic myocardium has been well documented in the isolated rat heart in vitro (13,29) and the canine myocardium in vivo (30,31). Baumgarten et al. (13) showed that kinin release after 15 min of coronary occlusion was enhanced by pretreatment with an ACE inhibitor, ramiprilat, in isolated rat hearts. Furthermore, Nolly et al. (32) demonstrated that the heart contains an independent kallikrein–kinin system. They demonstrated the presence of kallikrein (a potent kinin-generating enzyme) mRNA in rat atria and ventricles and kallikrein activity in primary cultures of neonatal rat atrial and ventricular cardiocytes and in their incubation medium. Heart slices were shown to release kallikrein without depletion of total tissue kallikrein, suggesting pool replenishment. In fact, kallikrein was found in epicardial slices and in the Krebs solution bathing the slices (which were washed and reincubated in Krebs solution)—and this release was inhibited by pretreatment with puromycin—suggesting primary synthesis. Presumably, ischemia results in pH and other changes that cause activation of kallikrein within the myocardium and the subsequent local availability of bradykinin (33). Hence, this work supports the hypothesis that local production of kinin contributed to the protection observed.

Relevance of the sulfhydryl moiety. Protection was observed for both a sulphydryl-containing ACE inhibitor, captopril, and a non–sulphydryl-containing one, lisinopril. However, for the last two time points, recovery in the captopril group was significantly greater than that in the lisinopril group, although both ACE inhibitors in combination with subthreshold preconditioning provided significant protection from 150 min of reperfusion onward. The greater beneficial effect of captopril over lisinopril could theoretically be due to the free radical scavenging ability conferred by the presence of the —SH moiety. Another mechanism by which ACE inhibitors may provide protection is through activation of the KATP channel. Sargent et al. (34) found that the sulphydryl-containing ACE inhibitor zofenopril induced cardioprotection through modulation of the KATP channel. Indeed, the cardioprotection observed in ischemic rat hearts with zofenopril was abolished by the KATP blockers glyburide and 5-hydroxydecanoate.

Our results show that ACE inhibition alone is insufficient to confer any protection after 90 min of ischemia. This result is consistent with previously published studies (35–38). However, the situation is not clear-cut, and there are some reports of infarct size limitation by the same agents (6,39). Our results are compatible with a current concept of preconditioning as a phenomenon that may have several different endogenous mediators, such as adenosine and bradykinin, that stimulate PKC to a certain threshold sufficient to trigger the intracellular pathways necessary for providing protection against a further ischemic insult (33). Recently, Goto et al. (33) found that Hoe 140 blocked the protection from one 5-min period of preconditioning but could not block protection when a more profound preconditioning stimulus was used in a rabbit model. Hence, it is possible that the adenosine and bradykinin released during the subthreshold ischemia is insufficient to trigger protection unless the level of bradykinin is augmented by ACE inhibition, as illustrated in Figure 6.

It is possible that the interaction between adenosine and bradykinin may not simply be additive, but a study (40) in smooth muscle cells found that stimulation of adenosine A1 and bradykinin receptors results in a synergistic increase in inositol 1,4,5-triphosphate and free calcium levels.

Intracellular signaling pathways. The present study does not address the issue of the cardioprotective mechanism
downstream to activation of the B₂ receptor. This receptor is coupled to phospholipases A₂ and C through G proteins (41,42), and stimulation of this receptor leads to the production of two important vasoactive substances—prostacyclin and nitric oxide. However, neither cyclooxygenase inhibitors (43) nor the nitric oxide synthase inhibitor nitro-L-arginine methyl ester (12,44) attenuated infarct size limitation by preconditioning in a rabbit model of infarction. In contrast Goto et al. (12) demonstrated that polymyxin B, a blocker of PKC, abolished infarct size limitation by treatment with a kinin. Furthermore, it was recently observed (45) that B₂ receptor stimulation caused significant inositol triphosphate production in isolated cardiac myocytes, suggesting that simultaneously generated diacylglycerol could activate PKC. Hence, these observations suggest that PKC activation plays a role in bradykinin-mediated protection.

**Role of angiotensin II.** The present study does not take into account the simultaneous suppression of angiotensin II production, which has been reported in some recent studies (46) to contribute to ischemic preconditioning; and angiotensin II receptor activation can mimic preconditioning (47). Hence, the effect of ACE inhibition in the reduction of angiotensin II levels would tend to lead to an underestimation of the protective effect observed with respect to bradykinin because some of the angiotensin II–mediated component could theoretically be reduced. In terms of implications for future therapy, development of a specific kininase inhibitor without the ACE inhibitory component could be valuable in this setting. Furthermore, agents such as angiotensin II receptor antagonists would not have this bradykinin-potentiating effect.

**Conclusions.** We demonstrated in a human model that ACE inhibitors can potentiate the effects of a subthreshold preconditioning stimulus and that B₂ bradykinin receptors may play a role in this protection. To our knowledge, this is the first report to allude to a potential role for bradykinin as a mediator in human preconditioning, giving a possible clue to the mechanisms involved in the reduction of fatal ischemic events in patients treated with ACE inhibitors observed in the multicenter trials.

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**References**


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**Figure 6.** Summary depicting putative mediators involved in triggering PKC in ischemic preconditioning (PC), ultimately leading to myocardial protection. ACEi = ACE inhibitor; sPC = subthreshold preconditioning. Adapted, with permission, from Goto et al. (33).


