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# Captopril Potentiates the Myocardial Infarct Size-Limiting Effect of Ischemic Preconditioning Through Bradykinin $B_2$ Receptor Activation

TAKAYUKI MIKI, MD, TETSUJI MIURA, MD, PHD, NOBUYUKI URA, MD, PHD, TAKASHI OGAWA, MD, KATSUO SUZUKI, MD, KAZUAKI SHIMAMOTO, MD, PHD, OSAMU IIMURA, MD, PHD

*Sapporo, Japan*

***Objectives.*** **To investigate the role of kinin in preconditioning against infarction, the present study assessed the effect of captopril, a kininase II inhibitor, on preconditioning and arterial plasma kinin levels.**

***Background.*** **Recent studies suggest a possible contribution of kinin to preconditioning against infarction. However, its role and the site of kinin production remain uncharacterized.**

***Methods.*** **Six groups of rabbits (n = 6 to 13) underwent 30-min coronary occlusion and 3-h reperfusion. The infarct size and area at risk were determined by tetrazolium staining and fluorescent particles, respectively. Arterial blood was sampled under baseline conditions, before the 30-min ischemia and after reperfusion for radioimmunoassay of the kinin level.**

***Results.*** **Infarct size expressed as a percentage of area at risk (%IS/AR) was 42.9 ± 2.9% (mean ± SEM) in the control group, 34.5 ± 3.3% in the group preconditioned with 2 min of ischemia/5 min of reperfusion and 41.7 ± 5.1% in the group given captopril (1 mg/kg body weight) alone before the 30-min ischemia. These %IS/AR values were not significantly different between the three groups. However, a combination of captopril and subsequent preconditioning with 2 min of ischemia markedly limited %IS/AR to 21.2 ± 2.4%. This potentiation of 2 min of preconditioning by captopril was not observed when 2 μg/kg body weight of Hoe 140, a specific bradykinin $B_2$ receptor antagonist, was administered before preconditioning (%IS/AR = 41.2 ± 5.7%), whereas Hoe 140 alone did not modify infarct size (%IS/AR = 38.5 ± 5.1%). Arterial plasma kinin levels were comparable between the control rabbits, the group given captopril alone and the group that received captopril plus 2 min of preconditioning at baseline (3.8 ± 1.0, 6.3 ± 1.9 and 5.2 ± 1.7 pg/ml, respectively), and there was no significant change in kinin levels after the captopril injection or the combination of captopril plus 2 min of preconditioning.**

***Conclusions.*** **The present results indicate that captopril is capable of potentiating preconditioning without increasing the arterial kinin level and that the beneficial effect of captopril can be inhibited by Hoe 140. These findings support the hypothesis that kinin produced locally in the heart during preconditioning may contribute to the cardioprotective mechanism through bradykinin receptor activation.**

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Myocardial tolerance to ischemic injury is markedly enhanced by exposing the myocardium to a brief episode of ischemia followed by a period of reperfusion. This phenomenon, called *preconditioning*, has been widely confirmed in various animal models (1,2), and its presence is implicated in the human heart by circumstantial evidence, including attenuation of ST segment elevation after repetitive coronary balloon inflation during percutaneous transluminal coronary angioplasty (3), and deceleration of ischemia-induced adenosine triphosphate depletion in the myocardium after preconditioning (4). Although the mediator in the preconditioning-like phenomenon in human hearts is still poorly understood, the key role of an adenosine (probably $A_1/A_3$) receptor has been demonstrated in the mechanism of preconditioning against infarction in most of the animal species examined to date (1,2,5–7). However, there are a number of other substances that are thought to be released during preconditioning ischemia/reperfusion that may contribute to the cardioprotection against infarction. One such substance is kinin, which is known to be produced in a severely ischemic myocardium (8,9), but its physiologic significance is still not clear. Recently, Wall et al. (10) reported that infarct size limitation by preconditioning in the rabbit was blocked by a bradykinin $B_2$ receptor antagonist, Hoe 140 (11), and was mimicked by kinin administration. These results suggest that kinin may also be an important mediator, as well as adenosine, in the mechanism of preconditioning.

However, it has not been demonstrated whether kinin is indeed produced in the myocardium during a brief period of preconditioning ischemia and contributes to cardioprotection. To gain insight into this issue, the present study assessed the effect of captopril, a kininase II (i.e., angiotensin-converting enzyme [ACE]) inhibitor, on the infarct size limiting effect of

From the Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan. This study was supported in part by Grant-in-Aid for Scientific Research 04670547 (Dr. Miura) from the Ministry of Education, Science and Culture, Tokyo, Japan. Hoe 140 was provided by Bernwald A. Scholkens, MD, Hoechst AG, Frankfurt, Germany.

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Address for correspondence: Dr. Tetsuji Miura, Second Department of Internal Medicine, Sapporo Medical University, S-1, W-16, Chuo-ku, Sapporo, 060 Japan.



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**Abbreviations and Acronyms**

ACE = angiotensin-converting enzyme
ANOVA = analysis of variance
%IS/AR = infarct size expressed as percentage of area at risk

preconditioning in a rabbit model of myocardial infarction. If production of kinin during preconditioning is indeed a crucial mechanism, inhibition of kinin degradation by captopril (12) can be expected to enhance the cardioprotective effect of preconditioning, and that effect should be abolished by a kinin antagonist. To easily detect the possible potentiation of the cardioprotection by captopril, the duration of preconditioning ischemia was selected as 2 min, which has been shown (7) to very modestly protect the heart against infarction. We also assessed the effect of captopril on arterial plasma kinin levels to test whether captopril modifies preconditioning through increasing circulating plasma kinin levels.

## Methods

**Surgical preparation.** The study conformed to the Guidelines of Sapporo Medical University on Research Animal Use. The surgical preparation in the present study was essentially the same as that in our previous studies (1,6,7). Male albino rabbits (Japanese White) weighing 1.8 to 2.9 kg were intravenously anesthetized by pentobarbital (30 mg/kg body weight), and additional anesthesia was given during the experiment as needed. A tracheostomy was performed, and mechanical ventilation was provided by a Harvard respirator (model 683) using room air and oxygen supplement. Tidal volume, respiratory rate and oxygen supplement were adjusted to maintain arterial blood gas within the normal physiologic range. A fluid-filled catheter was inserted into the carotid artery and connected to a Nihon-Kohden SCK-580 transducer to monitor blood pressure. Another catheter was placed in the jugular vein for infusing drugs. Electrocardiographic electrodes were placed on the chest wall, corresponding to $CC_5$ leads. The heart was exposed by left lateral thoracotomy, and 4-0 silk thread was passed around a marginal branch of the left circumflex coronary artery with a tapered needle. The ends of the silk thread were passed through a small vinyl tube to make a snare. The rabbits were subjected to pretreatment (see Experimental protocol), and the coronary artery was occluded by the coronary snare. Myocardial ischemia was confirmed by regional cyanosis and ST segment change on the electrocardiogram. After 30 min of coronary occlusion, the coronary artery was reperfused by releasing the snare. Reperfusion was confirmed by the restoration of color to the ischemic region. Three hours after reperfusion, the rabbit underwent heparinization with 2,000 U of heparin and was killed by a pentobarbital overdose. The heart was quickly removed and processed for postmortem analysis.

**Postmortem analysis.** The heart was mounted on a Langendorff apparatus and perfused with saline to wash out the remaining blood. The coronary branch was reoccluded and fluorescent particles (3 to 30 $\mu$m in diameter, Duke Scientific Co.) were infused into the perfusate to negatively mark the area at risk (the territory of the occluded coronary artery). The heart was then frozen and sectioned into ~2-mm transverse slices. The heart slices were incubated with 1% triphenyl tetrazolium in 100 mmol/liter sodium phosphate buffer (pH 7.4) for 20 min to visualize infarcts (13). The slices were mounted on a glass press and compressed to exactly 2 mm. A clear acetate sheet was overlaid on the glass press, and then the infarcts (i.e., regions unstained by tetrazolium) and areas at risk (i.e., regions deficient of fluorescent particles) were traced on the acetate sheet under room light and under ultraviolet light, respectively. The traces were enlarged by 200% using a Xerox copy machine. The areas of the infarct and region at risk were measured by PIAS II, a computer-assisted image analysis system (PIAS Co., Osaka, Japan), and each volume was calculated by multiplying the area by the thickness of the heart slice.

**Experimental protocol.** After hemodynamic variables had been stabilized for 10 min, baseline hemodynamic variables were measured, and the rabbits then underwent pretreatment and 30 min of coronary occlusion/3 h of reperfusion. In the first series of experiments, 40 rabbits were randomly divided into four groups (n = 10 for each group): Control group, 2'PC group, Cap group and Cap/2'PC group. The Control group was subjected to the 30 min of ischemia without any pretreatment. In the 2'PC group, the heart was preconditioned with 2 min of ischemia and 5 min of reperfusion before the 30-min coronary occlusion. The Cap group received an intravenous injection of 1 mg/kg of captopril 22 min before the coronary occlusion. The Cap/2'PC group received the same dose of captopril and was preconditioned with 2 min of ischemia/5 min of reperfusion. Because the mean of the size of area at risk in the Cap/2'PC group was slightly smaller than those in the other groups (although the differences were not statistically significant), an additional five rabbits were added to this group. In the Control, Cap and Cap/2'PC groups, arterial blood for kinin assay was sampled immediately after the baseline hemodynamic measurements; the second sample was taken 2 min before the 30-min coronary artery occlusion (20 min after captopril injection in the Cap and Cap/2'PC groups); the third sample was taken 5 min after reperfusion. The blood sample was drawn from the arterial line directly into a syringe containing a mixture of aprotinine, polybrene, soybean trypsin inhibitor, phenanthroline and EDTA to inhibit both kinin production and degradation (14).

In the second series of experiments, 14 rabbits were randomly assigned to the Hoe (n = 7) and Cap/Hoe/2'PC groups (n = 7). In addition, for every two rabbits assigned to these groups, we prepared one contemporary control; thus, three rabbits were subjected to no pretreatment, and four were preconditioned with 2 min of ischemia. We did not completely randomize the 21 rabbits into the four groups in this series because the Control and 2'PC groups in the first series of the experiment had already provided good infarct size data for

**Table 1.** Summary of Hemodynamic Variables

| Group | n | Baseline | After Tx | Ischemia | Reperfusion 5′ | Reperfusion 180′ |
|---|---|---|---|---|---|---|
| HR (beats/min) | | | | | | |
| Control | 13 | 256 ± 11 | 260 ± 11 | 262 ± 11 | 268 ± 10 | 255 ± 7 |
| 2′PC | 13 | 272 ± 11 | 273 ± 11 | 275 ± 11 | 268 ± 9 | 257 ± 12 |
| Cap | 9 | 283 ± 7 | 287 ± 8 | 284 ± 8 | 280 ± 9 | 282 ± 13 |
| Cap/2′PC | 13 | 262 ± 5 | 266 ± 7 | 263 ± 7 | 259 ± 7 | 263 ± 11 |
| Hoe | 6 | 253 ± 15 | 250 ± 14 | 256 ± 14 | 254 ± 9 | 271 ± 19 |
| Cap/Hoe/2′PC | 7 | 286 ± 13 | 288 ± 8 | 289 ± 8 | 287 ± 10 | 293 ± 13 |
| MAP (mm Hg) | | | | | | |
| Control | 13 | 87 ± 4 | 89 ± 4 | 84 ± 5 | 69 ± 5* | 59 ± 4* |
| 2′PC | 13 | 88 ± 3 | 86 ± 3 | 85 ± 3 | 77 ± 3* | 67 ± 4* |
| Cap | 9 | 83 ± 2 | 74 ± 2 | 74 ± 3 | 58 ± 3* | 53 ± 4* |
| Cap/2′PC | 13 | 90 ± 3 | 80 ± 4 | 76 ± 3* | 66 ± 4* | 63 ± 4* |
| Hoe | 6 | 80 ± 6 | 82 ± 5 | 86 ± 5 | 74 ± 8 | 57 ± 8* |
| Cap/Hoe/2′PC | 7 | 86 ± 5 | 78 ± 4 | 79 ± 4* | 69 ± 3* | 60 ± 4* |
| RPP/100 | | | | | | |
| Control | 13 | 259 ± 20 | 254 ± 14 | 252 ± 22 | 220 ± 24 | 185 ± 14* |
| 2′PC | 13 | 272 ± 15 | 266 ± 15 | 264 ± 14 | 237 ± 13 | 203 ± 11* |
| Cap | 9 | 272 ± 10 | 258 ± 9 | 249 ± 9 | 206 ± 13* | 198 ± 16* |
| Cap/2′PC | 13 | 270 ± 11 | 253 ± 14 | 240 ± 14 | 210 ± 13* | 212 ± 15* |
| Hoe | 6 | 235 ± 27 | 236 ± 23 | 250 ± 25 | 219 ± 28 | 191 ± 27 |
| Cap/Hoe/2′PC | 7 | 280 ± 23 | 262 ± 14 | 263 ± 17 | 237 ± 15 | 222 ± 15 |

* $p < 0.05$ versus baseline values in each study group (see Experimental protocol for explanation of study groups). Data presented are mean value ± SD. After Tx = 1 min before coronary occlusion; HR = heart rate; Ischemia = 2 min after coronary occlusion; MAP = mean arterial pressure; Reperfusion 5′ and 180′ = 5 and 180 min after reperfusion, respectively; RPP = rate-pressure product.

nonpreconditioned and preconditioned hearts. In the Hoe group, 2 μg/kg of Hoe 140 was injected intravenously 12 min before coronary occlusion. The Cap/Hoe/2′PC group received captopril and Hoe 140 before preconditioning with 2 min of ischemia/5 min of reperfusion. In this group, the timing of the injection and the dose of captopril and Hoe 140 were the same as that in the Cap and the Hoe group, respectively. The 2-μg/kg dose of Hoe 140 was selected because half of this dose has been shown (10) to abolish preconditioning with 5 min of ischemia. We also confirmed in pilot experiments that 2 μg/kg of Hoe 140 almost completely blocked for at least 60 min the hypotensive response to an injection of 100 ng/kg of bradykinin into the left atrium, which was a decrease in blood pressure of ~30 mm Hg in untreated rabbits.

**Exclusion criteria.** We excluded rabbits if they developed ventricular fibrillation that did not terminate spontaneously within 1 min or if diastolic blood pressure after recovery from ventricular fibrillation was <40 mm Hg. Our previous study (6) showed that hypotension below this level may cause an extension of infarction during the reperfusion period in the present model. No pharmacologic agents were used for defibrillation or to maintain blood pressure.

**Plasma kinin assay.** After plasma was separated by centrifugation of the blood sample at 4°C, kinin was extracted with ethanol and assayed by a highly sensitive radioimmunoassay method, as previously reported (14).

**Chemicals.** Captopril was purchased from Sigma Co., and Hoe 140 was provided by Hoechst AG, Germany.

**Statistical analysis.** Hemodynamic variables and alterations in plasma kinin levels were compared between groups by two-way repeated measures analysis of variance with a multivariate linear model (SigmaStat, Jandel Scientific) to correct for the differing group sizes (15). Differences in infarct size data between groups were tested by one-way ANOVA. When the overall difference was significant, a multiple comparison was performed by the Student-Newman-Keuls post hoc test. Results are expressed as mean value ± SEM.

## Results

Because baseline hemodynamic variables, heart weight and the size of area at risk were comparable between the study groups in the first series of experiments and those in the second series, the results of both series were combined and presented together as follows.

**Exclusion of rabbits.** According to the exclusion criteria, we excluded five rabbits from the following analysis; two rabbits in the Cap/2′PC groups, one in the 2′PC group, one in the Cap group and one in the Hoe group. The two rabbits in the Cap/2′PC were not defibrillated, and the other three failed to maintain diastolic blood pressure >40 mm Hg after recovery from ventricular fibrillation.

**Hemodynamic variables.** Hemodynamic variables in the six study groups are summarized in Table 1. Heart rate, mean blood pressure and rate-pressure product were comparable in all groups at baseline. Two-way repeated measures ANOVA indicated no significant changes in heart rate but a significant time-related reduction in mean blood pressure and rate-pressure product during the experiments. No group-related effects were detected for either mean blood pressure or

**Table 2.** Summary of Infarct Size Data

| Group* | n | Heart Weight (g) | Area at Risk ($cm^3$) | Infarct Size ($cm^3$) | % Infarct Area at Risk |
|---|---|---|---|---|---|
| Control | 13 | 6.3 ± 0.3 | 0.78 ± 0.06 | 0.34 ± 0.04 | 42.9 ± 2.9 |
| 2'PC | 13 | 6.8 ± 0.3 | 0.79 ± 0.04 | 0.27 ± 0.03 | 34.5 ± 3.3 |
| Cap | 9 | 7.0 ± 0.3 | 0.78 ± 0.08 | 0.35 ± 0.06 | 41.7 ± 5.1 |
| Cap/2'PC | 13 | 6.4 ± 0.2 | 0.65 ± 0.06 | 0.14 ± 0.02† | 21.2 ± 2.4† |
| Hoe | 6 | 6.2 ± 0.4 | 0.75 ± 0.11 | 0.33 ± 0.08 | 41.2 ± 5.7 |
| Cap/Hoe/2'PC | 7 | 6.6 ± 0.3 | 0.73 ± 0.11 | 0.31 ± 0.07 | 38.5 ± 5.1 |

*See Experimental protocol for explanation of study groups. †$p < 0.05$ versus Control group. Data presented are mean value ± SD.

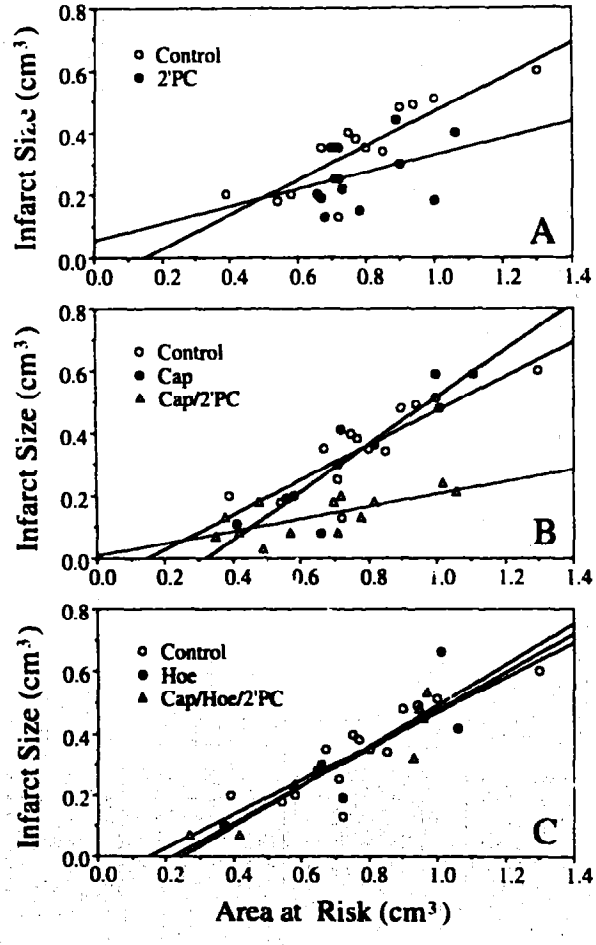
rate-pressure product (p = NS). These results suggest that the effects of preconditioning, captopril and Hoe 140, and a combination of these, did not significantly alter the time course of these hemodynamic variables in the present rabbit model of infarction.

**Infarct size data.** Infarct size data are summarized in Table 2. Heart weight and size of the area at risk were similar in all groups. Infarct size as a percentage of the area at risk (%IS/AR) was 42.9 ± 2.9% in the Control group and 41.7 ± 5.1% in the Cap group (p = NS). The %IS/AR was smaller in the 2'PC group (34.5 ± 3.3%) than the Control group, although the difference did not reach statistical significance. In contrast, %IS/AR in the Cap/2'PC group was 21.2 ± 2.4%, which was significantly smaller than that in the Control and Cap groups. Before three rabbits were added (see Experimental protocol) to this group to make the size of area at risk more comparable (thus, the area at risk was 0.60 ± 0.07 $cm^3$, n = 10), %IS/AR was 20.2 ± 3.0% ($p < 0.05$ vs. Control group). These findings indicate that pretreatment with captopril potentiated the infarct size-limiting effect of 2 min of preconditioning. In the Hoe group and Cap/Hoe/2'PC group, %IS/AR was 41.2 ± 5.7% and 38.5 ± 5.1%, respectively, both of which were similar to the control infarct size, and these values were significantly larger than the %IS/AR in the Cap/2'PC group.

Because %IS/AR is an index that can be influenced by the risk area size in the rabbit model of infarction (16), the effect of captopril on preconditioning was also assessed in light of the infarct size–risk area size relation. As shown in Figure 1, the regression line between the infarct size and risk area size shifted slightly toward smaller infarcts in the 2'PC group, but this shift was not statistically significant (panel A). Although captopril alone did not shift the regression line between infarct size and risk area size, the regression line in the Cap/2'PC group was significantly less steep than that of the Control group (Fig. 1, panel B). Hoe 140 alone did not shift the regression line, but this agent blocked the downward shift of the regression line by captopril plus 2 min of preconditioning (Fig. 1, panel C). This analysis also shows the significant infarct size limitation by the combination of captopril and 2 min of preconditioning, which was inhibitable by Hoe 140.

**Plasma kinin levels.** Plasma kinin levels at baseline were 3.8 ± 1.0, 6.3 ± 1.9 and 5.2 ± 1.7 in the Control, Cap and

**Figure 1.** Scatterplots of the relation between infarct size and size of area at risk. **Panel A,** Control group versus 2'PC group. The **regression line** of the 2'PC group (y = 0.27x + 0.06, r = 0.36) was not different from that of the Control group (y = 0.55x −0.09, r = 0.87). **Panel B,** Control group versus Cap and Cap/2'PC groups. The **regression line** of the Cap/2'PC group (y = 0.20x + 0.01, r = 0.70) was significantly less steep than that of the Control group, whereas there was no significant difference between the regression line of the Cap group (y = 0.76x − 0.24, r = 0.91) and that of the Control group. **Panel C,** Control group versus Hoe group and Cap/Hoe/2'PC group. The **regression lines** of the Hoe (y = 0.65x − 0.16, r = 0.85) and Cap/Hoe/2'PC groups (y = 0.61x − 0.13, r = 0.94) did not differ from that of the Control group. See Experimental protocol for explanation of study groups.



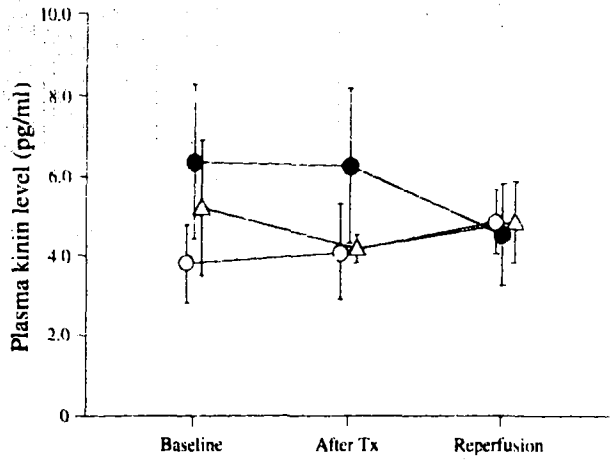



**Figure 2.** Time course of changes in plasma kinin levels in arterial blood. Baseline plasma kinin levels were comparable in the Control **(open circles)**, Cap **(solid circles)** and Cap/2'PC groups **(triangles)**, and there was no significant alteration in the plasma kinin levels before and after the coronary occlusion in these three groups. After Tx = immediately before coronary occlusion; Reperfusion = 5 min after coronary reperfusion. See Experimental protocol for explanation of study groups.

Cap/2'PC groups, respectively (p = NS). In all three groups, there was no significant alteration in arterial plasma kinin levels during the time course of preischemic and reperfusion periods, as shown by Figure 2.

## Discussion

In the present study, captopril potentiated the infarct size-limiting effect of preconditioning with 2 min of ischemia, and this cardioprotective effect was abolished by Hoe 140, a specific bradykinin $B_2$ receptor antagonist. However, plasma kinin levels in arterial blood were not changed by the administration of captopril or its combination with preconditioning. These results support the hypothesis that generation of kinin in the heart during preconditioning contributes, through $B_2$ receptor activation, to infarct size limitation by preconditioning.

**Kinin production in heart.** A limitation of the present study is the absence of direct data of kinin production in the ischemic myocardium. It is not technically possible to collect venous blood from the territory of the left marginal artery in the rabbit heart, and blood sampling from the coronary sinus does not allow accurate assessment of kinin metabolism in the ischemic region of the heart. Accordingly, we took an approach that excluded involvement of systemic circulating kinin in preconditioning. Hoe 140, a specific $B_2$ receptor antagonist (11), completely abolished the cardioprotection of captopril and preconditioning, suggesting a contribution of the $B_2$ receptor. In contrast, the dose of captopril used in the present study did not change arterial kinin levels with or without the combination of preconditioning (Fig. 2), although different doses of ACE inhibitors have been shown (17,18) to increase circulating plasma kinin levels in humans. However, the finding that arterial kinin levels were not elevated by captopril does not necessarily contradict the successful inhibition of kininase II by captopril in the heart because the effect of kininase II inhibition on kinin levels depends on the rate of kinin production, which, presumably, was high in the ischemic myocardium. Several earlier studies demonstrated that kinin production in the myocardium is accelerated by ischemia both in vivo (8,9) and in vitro (19,20). Baumgarten et al. (19) showed that the kinin release after 15 min of coronary occlusion was enhanced by pretreatment with an ACE inhibitor, ramiprilat, in isolated rat hearts. In contrast, the slight blood pressure decrease after captopril (Table 1) can be explained by the suppression of angiotensin II production by inhibiting ACE. Taken together, the present findings suggest that the kinin that contributed to the cardioprotection of preconditioning was produced locally in the rabbit heart.

**Effect of captopril in nonpreconditioned hearts.** In contrast to the marked cardioprotective effect of a combination of captopril and preconditioning, captopril alone failed to limit infarct size. This result is consistent with the observations in our previous study (21) as well in others (22–24). However, there are also reports of infarct size limitation by the same agents (25–28), and the reason for the discrepancy remains unclear.

**Potentiation of preconditioning: captopril versus nucleoside transport.** Like captopril in the present study, nucleoside transport inhibitors were shown to potentiate preconditioning with 2 min of ischemia against infarction. In our previous studies (7,29), pretreatment of rabbits with dipyridamole, dilazep and R75231 before preconditioning with 2 min of ischemia limited infarct to ~30% of the infarct size in the untreated control rabbits, whereas any of the nucleoside transport inhibitors alone failed to modify infarct size. Furthermore, the effect of nucleoside transport inhibitors was inhibited by 8-phenyl-theophylline (7), suggesting an adenosine-mediated mechanism. In contrast, the captopril-induced cardioprotection of 2 min of preconditioning was abolished by Hoe 140, which is a specific antagonist of the $B_2$ receptor and has no known antagonistic action on an adenosine receptor. The extent of infarct size limitation by the combination of dipyridamole or captopril with 2 min of preconditioning was comparable to that by preconditioning with 5 min of ischemia. A possible explanation of these findings may be that accumulation of either kinin or adenosine in the cardiac interstitium up to a certain threshold could trigger the cardioprotective mechanism, although the levels of both substances are below the threshold during 2 min of preconditioning without captopril or nucleoside transport inhibitors.

**Mechanism of preconditioning downstream to $B_2$ receptor activation.** The present study cannot specify the cardioprotective mechanism downstream to activation of the $B_2$ receptor. The $B_2$ receptor is coupled with phospholipases $A_2$ and C through G proteins (12,30), and stimulation of this receptor leads to the production of two important vasoactive substances—prostacyclin and nitric oxide. However, neither cyclooxygenase inhibitors (i.e., aspirin and meclofenamate) (31) nor the nitric oxide synthase

inhibitor, nitro-L-arginine methyl ester (32,33) attenuated infarct size limitation by preconditioning in the rabbit model of infarction. In contrast, polymyxin B, a blocker of protein kinase C, reportedly abolished infarct size limitation by pretreatment with kinin in a study by Goto et al. (32). Although Goto et al. (32) did not try other protein kinase C inhibitors on kinin-induced cardioprotection in that particular study, they demonstrated that polymyxin B, staurosporine (34) and chelerythrine (35) are capable of blocking preconditioning against infarction in the in vitro and in vivo rabbit model of infarction. Furthermore, a recent study using isolated cardiac myocytes showed that $B_2$ receptor stimulation caused significant inositol triphosphate production (36), suggesting that simultaneously generated diacylglycerol could activate protein kinase C. Taken together, this circumstantial evidence suggests that protein kinase C activation may play a key role in cardioprotection after $B_2$ receptor activation.

The important role of kinin in preconditioning against ischemia-induced arrhythmia has also been pointed out. Vegh et al. (37,38) found that the antiarrhythmic effect of preconditioning was blocked by Hoe 140 and mimicked by an intracoronary infusion of kinin in canine hearts. However, in contrast with the antiinfarct effect of preconditioning (32,33), the antiarrhythmic effect of preconditioning was blocked by $N^G$-methyl-L-arginine, suggesting an involvement of nitric oxide (39). Furthermore, a recent study (40) showed a proarrhythmic effect of the protein kinase C activator during myocardial hypoxia/reoxygenation. Thus, $B_2$ receptor activation before ischemic insult might enhance myocardial tolerance against both infarction and ischemic arrhythmia, but the underlying subcellular mechanism may be different (i.e., protein kinase C activation and nitric oxide production), depending on the end points.

**Role of angiotensin II?** The present study used captopril to decelerate degradation of kinin by kininase II in the myocardium. A methodologic disadvantage of this approach is the simultaneous suppression of angiotensin II production. However, it is more likely that the effect of captopril on angiotensin II levels would cause an underestimation of the role of endogenous kinin in preconditioning. A preliminary study (41) suggested that angiotensin II production may be responsible for a part of cardioprotection by preconditioning. Furthermore, a recent study (42) showed that angiotensin II receptor activation can mimic preconditioning. Thus, a part of cardioprotection by the combination of captopril and preconditioning might have been canceled by removing the angiotensin II-mediated component. If a specific kininase inhibitor without ACE inhibitory action is available, it might be able to potentiate preconditioning more than captopril and other ACE inhibitors.

**Conclusions.** The present study provided evidence that kinin endogenously produced in the heart during preconditioning contributes to the cardioprotective mechanism of preconditioning through $B_2$ receptor activation. The relative importance of plasma and the cardiac kallikrein–kinin system and the subcellular mechanism subsequent to $B_2$ receptor activation warrant further investigation.

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