Infarct Size Limitation by Nicorandil: Roles of Mitochondrial $K_{\text{ATP}}$ Channels, Sarcolemmal $K_{\text{ATP}}$ Channels, and Protein Kinase C

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OBJECTIVES This study aimed to examine: 1) whether nicorandil protects the ischemic myocardium by activating sarcolemmal adenosine triphosphate (ATP)-sensitive K$^+$ (sarcK$\text{ATP}$) channels or the mitochondrial K$\text{ATP}$ (mitoK$\text{ATP}$) channels, and 2) whether protein kinase C (PKC) activity is necessary for cardioprotection afforded by nicorandil.

BACKGROUND Nicorandil is a hybrid of nitrate and a K$\text{ATP}$ channel opener that activates the sarcK$\text{ATP}$ and mitoK$\text{ATP}$ channels. Both of these K$\text{ATP}$ channels are regulated by PKC, and this kinase may be activated by nitric oxide and also by oxygen free radicals (OFR) generated after mitoK$\text{ATP}$ channel opening.

METHODS In isolated rabbit hearts, infarction was induced by 30-min global ischemia/2-h reperfusion with monitoring of the activation recovery interval (ARI), an index of action potential duration. Protein kinase C translocation was assessed by Western blotting.

RESULTS Nicorandil did not change ARI before ischemia, but it accelerated ARI shortening after the onset of ischemia and reduced infarct size by 90%. A sarcK$\text{ATP}$ channel selective blocker, HMR1098, abolished acceleration of ischemia-induced ARI shortening by nicorandil and eliminated 40% of nicorandil-induced infarct size limitation. A mitoK$\text{ATP}$ channel selective blocker, 5-hydroxydecanoate, abolished the protection afforded by nicorandil without affecting ARI. Cardioprotection by nicorandil was inhibited neither by an OFR scavenger, N-2-mercaptopropionyglycine nor by a PKC inhibitor, calphostin C, at a dose that was capable of inhibiting PKC-$\varepsilon$ translocation after preconditioning.

CONCLUSIONS Both the sarcK$\text{ATP}$ and mitoK$\text{ATP}$ channels are involved in anti-infarct tolerance afforded by nicorandil, but PKC activation induced by nitric oxide or OFR generation, if any, does not play a crucial role. (J Am Coll Cardiol 2002;40:1523–30) © 2002 by the American College of Cardiology Foundation

Nicorandil is currently the only adenosine triphosphate (ATP)-sensitive K$^+$ (K$\text{ATP}$) channel opener that is used for patients with coronary artery disease. Cardioprotective effects of K$\text{ATP}$ channel openers, including nicorandil, have been demonstrated in various animal models of myocardial ischemia/reperfusion (1–4). Furthermore, clinical studies (5–8) have shown that nicorandil infusion attenuates electrocardiographic changes during coronary occlusion and that this agent improves functional recovery of the reperfused myocardium in patients with acute myocardial infarction. Most recently, the Impact Of Nicorandil in Angina (IONA) study, which was a double-blind placebo-controlled trial in which more than 5,000 patients were recruited, demonstrated that nicorandil improves the prognosis of stable angina pectoris (9). However, the molecular mechanisms of these beneficial effects of nicorandil remain unclear.

Because nicorandil is a hybrid agent with K$\text{ATP}$ channel opener and nitrate properties (10,11), it can activate the sarcolemmal K$\text{ATP}$ (sarcK$\text{ATP}$) and mitochondrial K$\text{ATP}$ (mitoK$\text{ATP}$) channels and also release nitric oxide (NO). Furthermore, there may be some interaction between these mechanisms to protect ischemic cardiomyocytes. First, it is possible that NO released from nicorandil activates the mitoK$\text{ATP}$ channels (12). Second, protein kinase C (PKC) may mediate cardioprotection triggered by both NO and the mitoK$\text{ATP}$ channels. A recent study by Nakano et al. (13) showed that anti-infarct tolerance of the myocardium afforded by a NO donor, S-nitroso-N-acetyl-DL-penicillamine (SNAP), was abolished by a PKC inhibitor, chelerythrine. It has also been suggested that opening of the mitoK$\text{ATP}$ channel induces a burst of free radicals from the mitochondria and, thus, activates PKC (14,15). On the other hand, it has been known for some time that PKC regulates the activity of sarcK$\text{ATP}$ (16) and mitoK$\text{ATP}$ channels (17). Taken together, the findings suggest that cardioprotection by nicorandil may not be simply due to opening of sarcK$\text{ATP}$ or mitoK$\text{ATP}$ channels but may involve a complex interaction among these K$\text{ATP}$ channel subtypes, NO and PKC.

In the present study, we used a novel sarcK$\text{ATP}$ channel selective blocker, HMR1098 (18,19), and a mitoK$\text{ATP}$ channel blocker, 5-hydroxydecanoate (5-HD), to differen-
tiate the contribution of each KATP channel subtype to cardioprotection by nicorandil. The role of NO derived from nicorandil could not be directly examined because no selective agent is available for inhibiting the mechanism of NO release from nicorandil (20,21). However, contributions of PKC and free radicals to the nicorandil-induced cardioprotection were assessed by using calphostin C, a PKC inhibitor, and N-2-mercaptopropionylglycine (MPG), a free radical scavenger. Effects of pharmacologic agents on the sarcKATP channel were monitored by measuring PKC and free radicals to the nicorandil-induced cardioprotection. PROTOCOL 2.

METHODS

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institute of Health (NIH publication No. 85-23, revised 1996) and was approved by the Animal Use Committee of Sapporo Medical University.

Experiment 1: effects of KATP channel blockers, calphostin C and MPG, on infarct size-limiting effects of nicorandil. PREPARATION. Isolated rabbit hearts were prepared as previously described (24,25). In brief, hearts were excised from male albino rabbits (Japanese White) that had been anesthetized and mechanically ventilated. Each excised heart was quickly mounted on a Langendorff apparatus with a water jacket and perfused at a pressure of 75 mm Hg with a noncirculating modified Krebs-Henseleit buffer at 38°C (NaCl, 118.5 mM; KCl, 4.7 mM; MgSO4, 1.2 mM; KH2PO4, 1.2 mM; NaHCO3, 24.8 mM; CaCl2, 2.5 mM; and glucose, 10 mM), which was continuously oxygenated by 95% O2 and 5% CO2. A fluid-filled latex balloon connected to a transducer (Nihon-Kohden, Tokyo, Japan) with PE-160 tubing was inserted into the left ventricle via the left atrium to monitor ventricular pressure, and the balloon volume was adjusted to maintain baseline end-diastolic pressure within 0 to approximately 5 mm Hg. A unipolar electrode was attached to the surface of the left ventricle by using an elastic net, and an epicardial electrocardiogram and its electronically obtained first derivatives were continuously recorded. The ARI was determined as the interval between the times of minimum derivative of the QRS and maximum derivative of the T-wave (26,27). Right atrial pacing was performed at 200 beats/min when the spontaneous rate was lower. Coronary flow was measured by collection of perfusate in a graduated cylinder. The heart was excluded from the study if the left ventricular systolic pressure was below 70 mm Hg after a 20-min stabilization period.

Experimental protocols. PROTOCOL 1. After a 20-min stabilization period, each heart was subjected to 30-min global ischemia and 2-h reperfusion. Before global ischemia, hearts were untreated or received nicorandil (100 μM) with or without 100 μM 5-HD, or 5 μM HMR1098. Nicorandil was infused for 10 min before the onset of ischemia. Infusion of the KATP channel blockers was started 5 min before the onset of nicorandil administration and continued for 15 min.

PROTOCOL 2. Hearts were untreated or received nicorandil with or without 200 nM calphostin C or 300 μM MPG before 30-min global ischemia/2-h reperfusion. Nicorandil was administered for 10 min as in protocol 1, and calphostin C and MPG were infused for 15 min before ischemia. In this protocol, ARI was not determined.

Determination of infarct size. After 2 h of reperfusion, the heart was removed from the Langendorff apparatus, weighed, frozen, and cut into 2-mm-thick sections from apex to base. The uppermost slice containing the valves was not used for infarct size determination. The heart slices were incubated in 1% solution of triphenyltetrazolium chloride in 100 mM sodium phosphate buffer (pH 7.4) for 15 min at 37°C. Each slice was traced on a clear acetate sheet, and the traces were read by a Macintosh G3 computer using a Hewlett Packard ScanJetIIC scanner (Hewlett Packard, Palo Alto, California). The area of infarct and the left ventricle (i.e., area at risk) in each slice were measured using NIH Image, an image analysis program, and multiplied by the thickness of the heart slice to obtain their volumes.

Experiment 2: effects of calphostin C on PKC translocation by ischemic preconditioning. PREPARATION AND EXPERIMENTAL PROTOCOL. This series of experiments was performed to confirm that 200 nM calphostin C, which is well above the reported IC50 of this PKC inhibitor (i.e., 50 nM), is sufficient to inhibit PKC in rabbit hearts in experiment 1. Because PKC-α and PKC-δ are cardioprotective isoforms in the rabbit and rat, respectively (28–30), we assessed the effects of calphostin C on these PKC isoforms. Rabbit hearts were perfused as in experiment 1.
and subjected to one of three treatments: PC with two cycles of 5-min ischemia/5-min reperfusion, calphostin C plus PC, and calphostin C infusion for 15 min. Calphostin C infusion was started 5 min before PC in the group that received combination of calphostin C and PC. In each study group, left ventricular biopsy samples (0.5 to approximately 1.0 g) were taken from the hearts before and after treatment. The tissues were frozen in liquid nitrogen and stored at −70°C until Western blotting according to the method reported previously (31). In brief, frozen samples were homogenized in cold buffer containing 50 mM Tris–HCl (pH 7.4), 5 mM EDTA, 10 mM EGTA, 50 mM NaF, 50 μg/ml phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 0.3% β-mercaptoethanol. The supernatant at centrifugation at 1,000 g for 10 min was recentrifuged at 100,000 g for 60 min. The 100,000 g supernatant was used as a cytosolic fraction. Particulate fraction samples were obtained by treating the 100,000 g pellet with 0.3% Triton X-100 and recentrifugation at 10,000 g for 10 min. Protein concentration was determined using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, California).

**Western blotting of PKC.** Samples of cytosolic and particulate fractions were electrophoresed on a 12.5% polyacrylamide gel and blotted onto polyvinylidene difluoride membranes (Millipore Co., Bedford, Massachusetts). The blots were blocked with 5% nonfat dry milk in buffer containing 100 mM NaCl, 10 mM Tris–HCl (pH 7.4), and 0.1% Tween 20 for 1 h. The blots were then incubated with 1,000-fold-diluted antibody against PKC-ε or PKC-δ (Transduction Laboratories, Lexington, Kentucky). Protein kinase C was then visualized using an ECL detection kit (Amersham, Little Chalfont, Minnesota) and quantified by using Sigma-Gel, gel analysis software (SPSS Inc., Chicago, Illinois).

**Chemicals.** Nicorandil and HMR1098 were kindly provided by Chugai Pharmaceutical Co. (Tokyo, Japan) and Aventis Pharmaceuticals (Frankfurt, Germany), respectively. Calphostin C and 5-HD were purchased from Sigma (St. Louis, Missouri).

**Statistics.** All data are presented as means ± SE. Hemodynamic variables in study groups were compared by two-way repeated measures analysis of variance. One-way analysis of variance with the Student Newman–Keuls post-hoc test was used to test for differences in infarct size between groups. The difference was considered significant if the p value was <0.05.

**RESULTS**

**Experiment 1. PROTOCOL 1. Hemodynamic parameters and ARI.** Of 44 hearts used in this series of experiments, two hearts in the control group and one heart each in the 5-HD–treated groups were excluded according to the exclusion criteria. Heart rate, left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), coronary flow (Table 1), and ARI (Fig. 1) were comparable in study groups under baseline conditions. In the untreated control group, ARI was significantly shortened during ischemia, indicating activation of the sarcK<sub>ATP</sub> channel, and LVDP after reperfusion was depressed by approximately 70%. Before ischemia, nicorandil did not modify heart rate, LVDP, or LVEDP but significantly increased coronary flow by approximately 40%. Nicorandil had no effect on ARI before the onset of ischemia but significantly accelerated ischemia-induced shortening of ARI (Fig. 1). Infusion of 5-HD did not modify the time course of ARI after ischemia (Fig. 1A). In contrast, HMR1098 blunted ischemia-induced ARI shortening and abolished the effects of nicorandil on ARI change during ischemia (Fig. 1B). Posts ischemic recovery of LVDP and LVEDP were improved in the nicorandil–treated group (Table 1). These beneficial effects of nicorandil were abolished by 5-HD and HMR1098, though these K<sub>ATP</sub> channel blockers alone did not aggravate the posts ischemic contractile dysfunction.

**INfarCT SIZE DATA.** The volumes of myocardial infarct size are summarized in Table 2. Nicorandil reduced infarct size as a percentage of the left ventricle (%IS/LV) from 53.2 ± 4.1% in controls to 5.3 ± 1.6% (p < 0.05). Although neither 5-HD nor HMR1098 alone changed infarct size, 5-HD completely abolished and HMR1098 significantly attenuated the infarct size–limiting effect of nicorandil (%IS/LV = 53.0 ± 6.3 and 21.3 ± 5.0, respectively).

**PROTOCOL 2. Hemodynamic parameters.** In this protocol, 31 hearts were used, and one heart was excluded according to the exclusion criteria. As shown in Table 3, no significant effects of calphostin C and MPG pretreatment on heart rate, LVDP, LVEDP, and coronary flow were detected. Neither of these agents interfered with coronary dilation by nicorandil. Nicorandil suppressed elevation of LVEDP after ischemia/reperfusion regardless of pretreatment with calphostin C and MPG.

**INfarCT SIZE DATA.** As shown in Table 4, an infarct size–limiting effect of nicorandil was observed as in protocol 1, and neither calphostin C nor MPG abolished this cardioprotection. Calphostin C and MPG alone did not modify infarct size.

**Experiment 2. Ischemic preconditioning (PC) induced translocation of PKC-ε from the cytosol to the particulate fraction (Fig. 2). However, significant translocation of PKC-ε was not observed in hearts pretreated with calphostin C. These results confirm that 200 nM calphostin C is sufficient to inhibit PKC-ε in isolated rabbit hearts. In contrast with PKC-ε, PKC-δ did not translocate to the particulate fraction after PC (data not shown), as previously reported for rabbit hearts by Ping et al. (28).

**DISCUSSION**

Contribution of sarcK<sub>ATP</sub> and mitoK<sub>ATP</sub> channels to protection afforded by nicorandil. In the present study, the infarct size–limiting effect of nicorandil was completely
abolished by 5-HD but only partially inhibited by HMR1098. The dose of HMR1098 was more than fourfold higher than its reported IC_{50} for the sarcK_{ATP} channel (18,19), and it was sufficient to inhibit both ischemia-

induced ARI shortening and its acceleration by nicorandil (Fig. 1B). Therefore, it is highly unlikely that the dose of HMR1098 was insufficient to block the sarcK_{ATP} channel in the present preparation. In contrast with HMR1098,
Table 2. Summary of Infarct Size Data in Protocol 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>LV (cm³)</th>
<th>Infarct (cm³)</th>
<th>%IS/LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>2.56 ± 0.10</td>
<td>1.38 ± 0.13</td>
<td>53.2 ± 4.1</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>7</td>
<td>2.49 ± 0.15</td>
<td>0.13 ± 0.04*</td>
<td>5.3 ± 1.6*</td>
</tr>
<tr>
<td>5-HD</td>
<td>6</td>
<td>2.53 ± 0.13</td>
<td>1.42 ± 0.15*</td>
<td>56.1 ± 6.2</td>
</tr>
<tr>
<td>5-HD + nicorandil</td>
<td>6</td>
<td>2.40 ± 0.13</td>
<td>1.30 ± 0.20</td>
<td>53.0 ± 6.3</td>
</tr>
<tr>
<td>HMR</td>
<td>6</td>
<td>2.36 ± 0.11</td>
<td>1.16 ± 0.08</td>
<td>49.7 ± 4.1</td>
</tr>
<tr>
<td>HMR + nicorandil</td>
<td>5</td>
<td>2.35 ± 0.14</td>
<td>0.52 ± 0.14*</td>
<td>21.3 ± 5.0*</td>
</tr>
</tbody>
</table>

Mean ± SE. *p < 0.05 vs. control; †p < 0.05 vs. 5-HD + nicorandil and HMR + nicorandil.
HMR = HMR1098; LV = left ventricle; 5-HD = 5-hydroxydecanoic acid; %IS/LV = infarct size as a percentage of left ventricle.

5-HD at a dose of 100 μM had no effect on shortening of ARI during ischemia (Fig. 1), suggesting a lack of its effect on the sarcK_ATP channel. Taken together, the present results suggest that the mitoK_ATP channel plays a primary role in infarct size limitation by nicorandil and that the sarcK_ATP channel has a limited but significant contribution to the cardioprotection.

Whether these two K_ATP channel subtypes share a common effector or play distinctive roles in nicorandil-induced protection is not clear. However, two speculations can be made regarding the present results. First, activation of the sarcK_ATP channel may facilitate opening of the mitoK_ATP channel. Second, activation of the mitoK_ATP channel may produce signals to the sarcK_ATP channel and also to a sarcK_ATP channel-independent cardioprotective mechanism. There is some evidence to argue for and against either of these two possibilities. Hyperpolarization, which sarcK_ATP channel opening would induce, has been shown to activate phospholipase D (32), which could trigger a signaling cascade to the mitoK_ATP channel (22,23). On the other hand, mitoK_ATP channel opening triggers release of free radicals (14,15,33) and possibly other signaling molecules from the mitochondria (34,35), which could indirectly modulate the sarcK_ATP channel and other cardioprotective mechanisms. Nevertheless, it is notable that nicorandil is not the only agent of which protective effects depend on both the sarcK_ATP and mitoK_ATP channels. Actually, both of these K_ATP channels have been shown to contribute to cardioprotection afforded by PKC activation (19) and to

Table 3. Summary of Hemodynamic Parameters in Protocol 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Baseline</th>
<th>After Tx</th>
<th>Reperfusion 30 Min</th>
<th>Reperfusion 120 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>221 ± 9</td>
<td>225 ± 8</td>
<td>218 ± 10</td>
<td>209 ± 1</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>5</td>
<td>231 ± 13</td>
<td>227 ± 9</td>
<td>212 ± 11</td>
<td>206 ± 2</td>
</tr>
<tr>
<td>Cal C</td>
<td>5</td>
<td>215 ± 2</td>
<td>201 ± 5</td>
<td>200 ± 0</td>
<td>200 ± 0</td>
</tr>
<tr>
<td>Cal C + nicorandil</td>
<td>5</td>
<td>207 ± 9</td>
<td>206 ± 11</td>
<td>200 ± 0</td>
<td>200 ± 0</td>
</tr>
<tr>
<td>MPG</td>
<td>5</td>
<td>216 ± 5</td>
<td>213 ± 3</td>
<td>208 ± 0</td>
<td>208 ± 0</td>
</tr>
<tr>
<td>MPG + nicorandil</td>
<td>5</td>
<td>239 ± 4</td>
<td>239 ± 4</td>
<td>226 ± 8</td>
<td>211 ± 6</td>
</tr>
<tr>
<td>LVDP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>115 ± 6</td>
<td>120 ± 6</td>
<td>38 ± 3</td>
<td>39 ± 6</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>5</td>
<td>122 ± 8</td>
<td>116 ± 9</td>
<td>89 ± 6*</td>
<td>72 ± 6*</td>
</tr>
<tr>
<td>Cal C</td>
<td>5</td>
<td>106 ± 10</td>
<td>102 ± 10</td>
<td>33 ± 3</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>Cal C + nicorandil</td>
<td>5</td>
<td>119 ± 8</td>
<td>108 ± 6</td>
<td>83 ± 14*</td>
<td>62 ± 12*</td>
</tr>
<tr>
<td>MPG</td>
<td>5</td>
<td>102 ± 4</td>
<td>96 ± 4</td>
<td>38 ± 6</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>MPG + nicorandil</td>
<td>5</td>
<td>100 ± 7</td>
<td>100 ± 7</td>
<td>47 ± 8</td>
<td>50 ± 6</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>31 ± 5</td>
<td>21 ± 5</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>5</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>2 ± 1*</td>
<td>0 ± 0*</td>
</tr>
<tr>
<td>Cal C</td>
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<td>1 ± 1</td>
<td>1 ± 1</td>
<td>35 ± 10</td>
<td>39 ± 9</td>
</tr>
<tr>
<td>Cal C + nicorandil</td>
<td>5</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1*</td>
<td>0 ± 0*</td>
</tr>
<tr>
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<td>25 ± 10</td>
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<tr>
<td>MPG + nicorandil</td>
<td>5</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>8 ± 3*</td>
<td>2 ± 3*</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>62 ± 6</td>
<td>63 ± 6</td>
<td>39 ± 6</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>5</td>
<td>73 ± 4</td>
<td>94 ± 5*</td>
<td>68 ± 2*</td>
<td>49 ± 5*</td>
</tr>
<tr>
<td>Cal C</td>
<td>5</td>
<td>67 ± 5</td>
<td>66 ± 4</td>
<td>38 ± 5</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Cal C + nicorandil</td>
<td>5</td>
<td>66 ± 6</td>
<td>100 ± 4*</td>
<td>54 ± 2*</td>
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<td>36 ± 3</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>MPG + nicorandil</td>
<td>5</td>
<td>65 ± 3</td>
<td>103 ± 3*</td>
<td>50 ± 4</td>
<td>36 ± 4</td>
</tr>
</tbody>
</table>

Mean ± SE. *p < 0.05 vs. control.
After Tx = after treatment; Cal C = calphostin C; LVEDP = left ventricular end-diastolic pressure; LVDP = left ventricular developed pressure; MPG = N-2-mercaptopropionylglycine.
protection by preconditioning in some experimental preparations (36,37).

PKC and cardioprotective effects of nicorandil. Nicorandil is an NO donor, though its NO-releasing mechanism is independent of glutathione-S-transferase unlike that in organic nitrates (20,21). There is recent evidence supporting the notion that NO protects the ischemic myocardium by PKC-mediated mechanisms. Ping et al. (28) demonstrated that PKC-ε and PKC-η were activated by two structurally different NO donors (i.e., SNAP and dimethylendiamine/NO) in rabbit hearts. A study by Nakano et al. (13) has shown that infarct size-limiting effect of SNAP was abolished by a PKC inhibitor, chelerythrine, and by MPG, suggesting that both PKC and free radicals are involved in NO-induced anti-infarct tolerance. Furthermore, production of free radicals by nicorandil administration in the heart was suggested in a recent study using formation of 2,3-dihydroxybenzoic acid as an index of hydroxyl free radical production (33). Because free radicals are activators of PKC, these findings are consistent with the recent hypothesis that nitrate-derived oxiradicals trigger PKC-mediated cardioprotection against infarction (28,38).

However, such a PKC-mediated mechanism is unlikely to be important in infarct size limitation by nicorandil. In contrast with the reported cardioprotection by SNAP, the infarct size-limiting effect of nicorandil was not eliminated by calphostin C or by MPG (Table 4). The dose of calphostin C was fourfold higher than its IC50 to inhibit PKC activity (i.e., 50 μM). Furthermore, this dose was sufficient to prevent PKC translocation by preconditioning (Fig. 2) and to abolish cardioprotective signaling from activated adenosine receptors (25). The MPG dose used in the present study has been confirmed by our previous study (39) to be sufficient for abolishing the free radical-induced trigger mechanism of preconditioning. Taken together, the results suggest that, even if NO-mediated PKC activation contributes to opening of mitoKATP channel by nicorandil, such a PKC activation is unlikely to be crucial, and direct activation of the mitoKATP channel by this agent is of primary importance in the anti-infarct tolerance.

The role of PKC in cardioprotection by KATP channel openers has been examined for diazoxide, a mitoKATP channel-selective opener, which lacks nitrate property. Our previous study (25) and a study by Pain et al. (14) have shown that PKC inhibitors (calphostin C and chelerythrine) did not abolish infarct size-limiting effect of diazoxide in rabbit hearts. In contrast, the same PKC inhibitors reportedly eliminated the protective effects of diazoxide against ischemia/reperfusion injury in buffer-perfused rat hearts (40). This discrepancy cannot be clearly explained but may reflect species differences in regulatory mechanisms of PKC and the mitoKATP channel.

Activation of the mitoKATP and sarcKATP channels by nicorandil. Although a number of investigators have been interested in the mitoKATP channel in cardiomyocytes, the molecular structure of this channel has not yet been characterized. In isolated cardiomyocytes neither a dominant-negative construct of Kir6.1 nor that of Kir6.2 affected mitoKATP channel activity (41). Regarding the sulfonylurea receptor (SUR) subtype in the mitoKATP channel, Liu et al. (42) recently showed that responses of this channel to KATP channel openers (diazoxide, pinacidil) and blockers (glibenclamide, 5-HD, HMR1098) resembled those of a SUR1/Kir6.1 complex expressed in HEK293 cells. It is notable that nicorandil did not activate the pancreatic β-cell KATP channels consisting of SUR1/Kir6.1 complex (43) but afforded cardioprotection against infarction. Furthermore, this protective effect was inhibitable by 5-HD (Table 2) as was diazoxide-induced protection (25). These findings suggest that nicorandil binds to a specific site of SUR in the mitoKATP channel, which is not commonly present in the SUR1.
Recently, Sato et al. (44) showed that up to 1 mM nicorandil failed to open the sarcK<sub>ATP</sub> channel, though the mitoK<sub>ATP</sub> channel was activated by 100 μM of nicorandil in isolated rabbit cardiomyocytes. These features of nicorandil are very similar to those of diazoxide, a "mitoK<sub>ATP</sub> channel-selective" opener. The observation by Sato et al. (44) is actually consistent with our finding that nicorandil did not modify ARI before ischemia. Activation of the sarcK<sub>ATP</sub> channel by nicorandil, which is indicated by accelerated ARI shortening, after the onset of ischemia is presumably due to enhanced sensitivity of the sarcK<sub>ATP</sub> channel to a K<sub>ATP</sub> channel opener by accumulation of cytosolic adenosine diphosphate (45).

Conclusions. This study indicates that both the sarcK<sub>ATP</sub> and mitoK<sub>ATP</sub> channels contribute to anti-infarct tolerance afforded by nicorandil and that PKC activation induced by NO or oxygen-free radical generation, if any, does not play a crucial role. Whether these two K<sub>ATP</sub> channel subtypes link to a common effector or play distinctive roles in nicorandil-induced protection warrants further investigation.

**References**


