Exogenous NO triggers preconditioning via a cGMP- and mitoK<sub>ATP</sub>-dependent mechanism

Qining Qin, Xi-Ming Yang, Lin Cui, Stuart D. Critz, Michael V. Cohen, Natasha C. Browner, Thomas M. Lincoln, and James M. Downey

Departments of Physiology, Cell Biology and Neuroscience, and Medicine, College of Medicine, University of South Alabama, Mobile, Alabama 36688

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Qin, Qining, Xi-Ming Yang, Lin Cui, Stuart D. Critz, Michael V. Cohen, Natasha C. Browner, Thomas M. Lincoln, and James M. Downey. Exogenous NO triggers preconditioning via a cGMP- and mitoK<sub>ATP</sub>-dependent mechanism. Am J Physiol Heart Circ Physiol 287: H712–H718, 2004. First published March 25, 2004; 10.1152/ajpheart.00954.2003.—Exogenous nitric oxide (NO) triggers a preconditioning-like effect in heart via a pathway that is dependent on reactive oxygen species. This study examined the signaling pathway by which the NO donor S-nitroso-N-acetylpenicillamine (SNAP, 2 μM) triggers its anti-infarct effect. Isolated rabbit hearts experienced 30 min of regional ischemia and 120 min of subsequent reperfusion. Infarct size was determined by triphenyltetrazolium chloride staining. Infarct size was reduced from 30.5 ± 3.0% of the risk zone in control hearts to 10.2 ± 2.0% in SNAP-treated hearts. Bracketing the SNAP infusion with either the guanylyl cyclase blocker 1H-[1,2,4]oxadiazolo[4,3-α]quinazolin-1-one (2 μM) or the mitochondrial ATP-sensitive K<sup>+</sup> (mitoK<sub>ATP</sub>) channel blocker 5-hydroxydecanoate (200 μM) completely blocked the infarct-sparing effect of SNAP (34.3 ± 3.8 and 32.2 ± 1.6% infarction, respectively). Pretreatment of hearts with 8-(4-chlorophenylthio)-guanosine 3′,5′-cyclic monophosphate (10 μM), which is a cell-permeable cGMP analog that activates protein kinase G, mimicked the preconditioning effect of SNAP by reducing infarct size to 7.5 ± 1.1% of the risk zone. This salutary effect was abolished by either the free radical scavenger N-(2-mercaptopropionyl)glycine (1 mM) or 5-hydroxydecanoate (100 μM; 28.9 ± 2.7 and 33.6 ± 5.0% infarction of the risk zone, respectively). To confirm these functional data and the effect of SNAP on the guanylyl cyclase-protein kinase G signaling pathway, cGMP levels were measured. SNAP increased the level from 0.18 ± 0.04 to 0.61 ± 0.14 pmol/mg of protein (P < 0.05). These data suggest that exogenous NO triggers the preconditioning effect by initiating a cascade of events including stimulation of guanylyl cyclase to make cGMP, activation of protein kinase G, opening of mitoK<sub>ATP</sub> channels, and, finally, production of reactive oxygen species.

mitochondrial ATP-sensitive potassium channel; protein kinase G; S-nitroso-N-acetylpenicillamine; guanylyl cyclase

ISCHEMIC PRECONDITIONING CAUSES the heart to become resistant to infarction from ischemia. Protection from ischemic preconditioning consists of two phases: the early or classical phase, which lasts from 1 to 4 h (3, 32), and a late or delayed phase, which appears 12–24 h later and has a duration of up to 3 days (6, 7). Nitric oxide (NO) has been convincingly shown to trigger and mediate delayed preconditioning (10, 11, 44). However, the role of NO in classic ischemic preconditioning is controversial (15, 30, 43, 46, 49, 50). Cardiac myocytes express both the inducible and constitutive isoforms of NOS (5, 12), NO is produced when NO synthase (NOS) converts L-arginine to citrulline.

Vegh et al. (46) were the first to propose that endogenous NO might be involved in preconditioning. In open-chest dogs, Vegh and colleagues noted that N<sup>ω</sup>-nitro-l-arginine methyl ester (L-NAME, 10 mg/kg) administered before and after brief cycles of ischemia-reperfusion attenuated the antiarrhythmic effect of ischemic preconditioning. However, Weselcouch et al. (49) reported that inhibition of NO synthesis by L-NAME (30 μM), which is a potent NOS inhibitor, did not alter the effect of ischemic preconditioning on functional recovery in isolated, perfused rat hearts. Indeed, in studies from our lab, neither 100 μM (27) nor 200 μM (29) L-NAME would block the infarct-limiting effect of ischemic preconditioning in isolated rabbit hearts when applied 5 min before ischemic preconditioning and continually throughout the period of index ischemia. In a recent study from Yellon’s group (9), two cycles of ischemic preconditioning could reduce infarct size in wild-type mice, whereas four cycles of ischemic preconditioning were required to limit infarct size in endothelial NOS (eNOS) knockout mice. Because endogenous NO only lowered the threshold for the protection of ischemic preconditioning, they concluded that it acts in parallel with other pathways to trigger protection.

Although the role of endogenous NO in ischemic preconditioning remains to be resolved, there is ample evidence that exogenous NO that results from administration of a NO donor is cardioprotective (24, 28, 29, 33, 41, 52). We recently proposed that opening of mitochondrial ATP-sensitive K<sup>+</sup> (mitoK<sub>ATP</sub>) channels triggers preconditioning by causing mitochondria to produce reactive oxygen species (ROS) that act as second messengers to activate downstream kinases such as PKC and cause cells to enter the preconditioned state (37). We also found that the NO donor S-nitroso-N-acetylpenicillamine (SNAP) triggered a preconditioning effect in an ROS-dependent manner (34). In embryonic chicken ventricular myocytes, exogenous NO conferred protection that was blocked by either the ROS scavenger N-(2-mercaptopropionyl)glycine (MPG) or the selective mitoK<sub>ATP</sub> blocker 5-hydroxydecanoate (5-HD). NO itself is a radical species and reacts with superoxide to produce highly reactive peroxynitrite. Therefore, it is possible that NO (or peroxynitrite) directly activates the downstream kinases to trigger protection.

Recent evidence suggests that the mechanism may be more complicated. Bradykinin (BK) is one of the physiological triggers of ischemic preconditioning (48), and in isolated rabbit
cardiomyocytes, BK administration results in ROS production (29). We found that the pathway responsible for BK-induced ROS in cardiac myocytes includes NOS, guanylyl cyclase, and protein kinase G (PKG) and that all of the latter reside upstream from mitoKATP channels (36). Furthermore, both L-NAME (200 μM) and the guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazole[4,3-a]quinoxalin-1-one (ODQ) blocked the infarct-limiting effect of BK in isolated rabbit heart (29).

In the present study, we wanted to critically test the hypothesis that NO protects the heart through the guanylyl cyclase-cGMP cascade and that these events reside upstream of the ROS-producing event that triggers ischemic preconditioning in the intact isolated heart.

METHODS

This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals (34a).

Surgical preparation. New Zealand White rabbits of either sex were used. As previously described (34), hearts from animals anesthetized with pentobarbital sodium (30 mg/kg) were exposed through a left thoracotomy, and a suture was passed around a prominent coronary artery branch visible on the epicardial surface. The heart was then removed, mounted on a Langendorff apparatus, and perfused with modified Krebs-Henseleit bicarbonate buffer that contained (in mM) 118.5 NaCl, 24.8 NaHCO3, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, and 10 glucose. The buffer was gassed with 95% O2-5% CO2, which resulted in a pH of 7.4–7.5. The temperature of the perfusate was maintained at 38°C. A fluid-filled latex balloon connected to a transducer with polyethylene-240 tubing was inserted into the left ventricle. Balloon volume was adjusted to set the left ventricular end-diastolic pressure equal to 5 mmHg at the beginning of the experiment. Atrial pacing was started to keep the heart rate at 200 beats/min if the spontaneous rate was too low. Total coronary flow was measured by timed collection of perfusate that dripped from the right heart into a graduated cylinder. The heart was allowed to stabilize for 20 min before the experiment was started.

Experimental protocols. Figure 1 shows details of the experimental protocols. Ten groups of hearts were studied. All hearts were subjected to an index ischemia of 30 min of coronary branch occlusion.
and subsequent 2 h of reperfusion. Control hearts were subjected to only the index ischemia plus reperfusion. The second group was preconditioned with 5 min of global ischemia plus 10 min of reperfusion before the index ischemia. In the third and fourth groups, either L-NAME (200 μM) or ODQ (2 μM), respectively, was infused for 15 min starting 5 min before the preconditioning ischemia. In the fifth group, hearts were treated with SNAP (2 μM) for 5 min and received 10 min of washout before the index ischemia. In the sixth and seventh groups, a 5-min infusion of SNAP (2 μM) was bracketed by either ODQ (2 μM) or 5-HD (200 μM), respectively. The eighth group was treated for 20 min before the index ischemia with 8-(4-chlorophenylthio)-guanosine-3′,5′-cyclic monophosphate (8-pCPT-cGMP, 10 μM), which is a cell-permeable cGMP agonist. A washout period of 5 min was interposed between the infusion and the index ischemia. The ninth group was also treated with 8-pCPT-cGMP for 20 min, but MPG (1 mM) was coinfused beginning 5 min before 8-pCPT-cGMP treatment and ending with onset of the index ischemia. The tenth group was treated with a combination of 8-pCPT-cGMP and 5-HD (100 μM). In a previous study on isolated rabbit hearts, we found that neither L-NAME (200 μM) nor ODQ (2 μM) affected infarct size when infused alone (36). Similarly, neither MPG nor 5-HD by itself influenced infarct size (37). Therefore, we did not rework these groups in the present study.

Measurement of infarct size. At the end of the experiment the coronary artery was reoccluded, and 2 to 9-μm fluorescent microspheres (Duke Scientific; Palo Alto, CA) were infused into the perfusate to demarcate the ischemic zone (region at risk) as the area of tissue without fluorescence. The heart was weighed, frozen, and cut into 2-mm-thick slices. The slices were incubated in 1% triphenyltetrazolium chloride (TTC) in sodium phosphate buffer (pH 7.4) at 37°C for 20 min. TTC stains noninfarcted myocardium brick red. The slices were then immersed in 10% formalin to preserve the stained (viable) and unstained (necrotic) tissues. The risk zone was identified by illuminating the slices with UV light. The areas of infarct and risk zone were determined by planimetry of each slice, and volume was calculated by multiplying each area by the slice thickness and then summing these values for each heart. Infarct size is expressed as a percentage of the risk zone.

Measurement of cGMP levels in SNAP-treated isolated hearts. Left ventricular tissue from three additional hearts was biopsied before and 5 min after treatment with SNAP (2 μM). Heart tissue was frozen in liquid nitrogen until it was pulverized. The heart-tissue powder was treated with cell lysis buffer (0.1 N HCl-50% methanol), and the suspension was centrifuged at 14,000 g for 3 min at 4°C. The supernatant was collected for cGMP measurements, and the pellet was saved for protein assay. cGMP levels were determined by radioimmunoassay according to the method of Harper and Brooker (23).

Chemicals. MPG, 5-HD, and SNAP were purchased from Sigma (St. Louis, MO); L-NAME and ODQ were from Alexis (Qbiogene; Carlsbad, CA), and 8-pCPT-cGMP was from BIOLOG (Life Science; Bremen, Germany). Either distilled water or DMSO was used to dissolve the drugs and prepare stock solutions. The final DMSO concentration was kept at <1%.

Data analysis. Baseline hemodynamic variables in experimental groups were compared with those in the control group by one-way ANOVA, and changes with time in any given group were compared with baseline by ANOVA with repeated measures. Infarct size data were compared by one-way ANOVA, and cGMP levels were compared by an unpaired t-test. Differences were considered to be significant if the P value was <0.05.

RESULTS

Hemodynamics. Hemodynamics are presented in Table 1. At baseline, there were no differences between groups except for faster heart rates in the preconditioned (PC) and 8-pCPT-cGMP+MPG groups and a higher coronary flow in the SNAP+ODQ group. L-NAME and ODQ significantly depressed left ventricular developed pressure and coronary flow. As was expected, values for developed pressure and coronary flow in all groups were significantly lower than baseline values during coronary occlusion with partial recovery during reperfusion. Infarct-size data. Table 2 contains the infarct size data. There were no significant differences in risk zone volume among the groups. Infarction in control hearts averaged 30.5 ± 3.0% of the risk zone (Figs. 2 and 3). Although 200 μM L-NAME could block the protection of BK (36), this dose of L-NAME still could not block ischemic preconditioning (see Fig. 2). Similarly, ODQ did not block the anti-infarct effect of ischemic preconditioning, which indicates that endogenous NO is not involved in ischemic preconditioning in the isolated rabbit heart. Comparable to ischemic preconditioning, SNAP (2 μM) protected the heart and reduced infarction to 10.2 ± 3.0%.

Table 1. Hemodynamic parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Treatment</th>
<th>30-Min Ischemia</th>
<th>30-Min Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart Rate, beats/min</td>
<td>LVDP, mmHg</td>
<td>Coronary Flow, ml min⁻¹ g⁻¹</td>
<td>Heart Rate, beats/min</td>
</tr>
<tr>
<td>Control</td>
<td>197 ± 6</td>
<td>106 ± 4</td>
<td>9.6 ± 4.1</td>
<td>195 ± 6</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>242 ± 5</td>
<td>125 ± 13</td>
<td>10.8 ± 0.6</td>
<td>228 ± 8</td>
</tr>
<tr>
<td>PC + L-NAME</td>
<td>208 ± 10</td>
<td>120 ± 2</td>
<td>9.6 ± 0.2</td>
<td>184 ± 6</td>
</tr>
<tr>
<td>PC + ODQ</td>
<td>204 ± 8</td>
<td>116 ± 9</td>
<td>9.2 ± 0.5</td>
<td>200 ± 2</td>
</tr>
<tr>
<td>SNAP</td>
<td>206 ± 6</td>
<td>122 ± 5</td>
<td>10.2 ± 0.4</td>
<td>207 ± 8</td>
</tr>
<tr>
<td>SNAP + ODQ</td>
<td>204 ± 8</td>
<td>118 ± 7</td>
<td>12.1 ± 0.8</td>
<td>202 ± 3</td>
</tr>
<tr>
<td>SNAP + 5-HD</td>
<td>201 ± 7</td>
<td>109 ± 7</td>
<td>9.8 ± 0.6</td>
<td>205 ± 7</td>
</tr>
<tr>
<td>8-pCPT-cGMP</td>
<td>202 ± 5</td>
<td>117 ± 8</td>
<td>9.8 ± 0.8</td>
<td>193 ± 3</td>
</tr>
<tr>
<td>8-pCPT-cGMP +</td>
<td>227 ± 10</td>
<td>122 ± 12</td>
<td>9.0 ± 0.7</td>
<td>208 ± 6</td>
</tr>
<tr>
<td>MPG</td>
<td>192 ± 4</td>
<td>125 ± 7</td>
<td>9.7 ± 0.5</td>
<td>187 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVDP, left ventricular developed pressure; PC, preconditioning; L-NAME, Nω-nitro-L-arginine methyl ester; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a]quinolizin-1-one; SNAP, N5-nitro-N-acetylpenicillamine; 5-HD, 5-hydroxydecanoate; 8-pCPT-cGMP, 8-(4-chlorophenylthio)-guanosine-3′,5′-cyclic monophosphate; MPG, Nω-(2-mercaptopropionyl)glycine. *P < 0.001; †P < 0.05, significance of difference between time point and baseline; ‡P < 0.05, significance of difference between experimental group and control.

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2.0%, and this protection was blocked by ODQ. ODQ is a potent inhibitor of soluble guanylyl cyclase, which when activated by NO produces the second messenger cGMP. The potent inhibitor of soluble guanylyl cyclase, which when activated by NO produces the second messenger cGMP. The present study demonstrated that exogenous NO mimics preconditioning by a cGMP- and mitoK<sub>ATP</sub> channel-dependent signaling pathway. This protection could be duplicated by activating PKG with the cGMP analog 8-pCPT-cGMP. The data indicate that NO exerts its protective effect through the PKG pathway rather than acting directly as a free radical second messenger. Furthermore, PKG was found to be upstream of mitoK<sub>ATP</sub> channels, where presumably it acts to open the channels and cause mitochondria to produce ROS that ultimately trigger the preconditioned state. Interestingly, neither l-NAME nor ODQ blocked protection from ischemic preconditioning.

It is known that ROS can directly trigger the preconditioned state and that ROS are involved in the mechanism of ischemic preconditioning (4, 45). Several studies suggest that ROS are formed by mitochondria in response to mitoK<sub>ATP</sub> channel opening and that ROS are second messengers that trigger the preconditioned state (19, 37, 51). Nakano et al. (34) showed that the protection provided by SNAP was blocked by MPG, which indicates that NO generated by SNAP was acting via free radicals to trigger preconditioning. Because NO can react with superoxide to form the radical species peroxynitrite.

**DISCUSSION**

The present study demonstrated that exogenous NO mimics preconditioning by a cGMP- and mitoK<sub>ATP</sub> channel-dependent signaling pathway. This protection could be duplicated by activating PKG with the cGMP analog 8-pCPT-cGMP. The data indicate that NO exerts its protective effect through the PKG pathway rather than acting directly as a free radical second messenger. Furthermore, PKG was found to be upstream of mitoK<sub>ATP</sub> channels, where presumably it acts to open the channels and cause mitochondria to produce ROS that ultimately trigger the preconditioned state. Interestingly, neither l-NAME nor ODQ blocked protection from ischemic preconditioning.

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**Table 2. Infarct size data**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, kg</th>
<th>Heart Weight, g</th>
<th>Risk Zone, cm³</th>
<th>Infarct Size cm³</th>
<th>% of risk zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.2±0.1</td>
<td>5.6±0.1</td>
<td>1.20±0.08</td>
<td>0.37±0.05</td>
<td>30.5±1.0</td>
</tr>
<tr>
<td>PC</td>
<td>2.2±0.1</td>
<td>7.3±0.5</td>
<td>1.57±0.17</td>
<td>0.18±0.06</td>
<td>10.4±2.5</td>
</tr>
<tr>
<td>PC + l-NAME</td>
<td>2.3±0.1</td>
<td>7.7±0.5</td>
<td>1.27±0.04</td>
<td>0.15±0.02</td>
<td>12.2±1.7</td>
</tr>
<tr>
<td>PC + ODQ</td>
<td>2.2±0.1</td>
<td>5.8±0.5</td>
<td>1.19±0.14</td>
<td>0.12±0.04</td>
<td>8.9±2.6</td>
</tr>
<tr>
<td>SNAP</td>
<td>2.1±0.1</td>
<td>5.5±0.5</td>
<td>1.14±0.13</td>
<td>0.12±0.04</td>
<td>10.2±2.0</td>
</tr>
<tr>
<td>SNAP + ODQ</td>
<td>2.2±0.1</td>
<td>5.6±0.4</td>
<td>1.50±0.20</td>
<td>0.55±0.12</td>
<td>34.3±3.8</td>
</tr>
<tr>
<td>SNAP + 5-HD</td>
<td>2.2±0.1</td>
<td>5.4±0.2</td>
<td>1.11±0.07</td>
<td>0.36±0.04</td>
<td>32.2±1.6</td>
</tr>
<tr>
<td>8-pCPT-cGMP</td>
<td>2.2±0.1</td>
<td>5.4±0.4</td>
<td>1.08±0.12</td>
<td>0.09±0.02</td>
<td>7.5±1.1</td>
</tr>
<tr>
<td>8-pCPT-cGMP + MPG</td>
<td>2.2±0.0</td>
<td>6.3±0.3</td>
<td>1.10±0.14</td>
<td>0.31±0.04</td>
<td>28.9±2.7</td>
</tr>
<tr>
<td>8-pCPT-cGMP + 5-HD</td>
<td>2.2±0.1</td>
<td>5.5±0.3</td>
<td>1.35±0.05</td>
<td>0.46±0.08</td>
<td>33.6±5.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 hearts/group. *P < 0.05; †P < 0.001, significance of difference between experimental group and control.
mitoKATP channels. PKG could be acting directly on the which phosphorylates a substrate that results in opening of cGMP. The rise in the cGMP level then would activate PKG, complex, and that NO activates guanylyl cyclase to make protection could be eliminated by the free radical scavenger trite could mimic preconditioning in isolated rat hearts and this hypothesis, Altug et al. (2) reported that exogenous peroxynitrite could mimic preconditioning in isolated rat hearts and this protection could be eliminated by the free radical scavenger MPG. The present data suggest that the mechanism is more complex, and that NO activates guanylyl cyclase to make cGMP. The rise in the cGMP level then would activate PKG, which phosphorolyses a substrate that results in opening of mitoKATP channels. PKG could be acting directly on the channels or on a protein upstream of the channels.

Others have looked at the role of NO donors and cGMP in preconditioning. Lochner et al. (29) reported that cGMP was increased in isolated perfused rat hearts treated with either of the NO donors SNAP or sodium nitroprusside. In an isolated, perfused mouse heart model, Bell and colleagues (8) found that up to a concentration of 2 μM, SNAP attenuated infarct size; 2 μM is the concentration used in the present study. In cultured rat ventricular myocytes, Rakhit et al. (38) noted that SNAP mimicked ischemic preconditioning, and this protection was completely abolished by ODQ but was not affected by either the PKC inhibitor chelerythrine or the mitoKATP channel blockers glibenclamide or 5-HD. Lebuffe et al. (27) also found that exogenous NO was able to precondition embryonic chick ventricular myocytes, but this protection could be blocked by 5-HD, which suggests that NO can activate mitoKATP channels. Ockaili et al. (35) reported that sildenafil (Viagra), which elevates cGMP levels in cells, could reduce infarct size in rabbit hearts, and this protection was also blocked by 5-HD. Our data support and extend these prior observations.

The functional effect of the NO donor SNAP may be influenced by dose and its effect on cGMP. For example, its dose-related effect on myocardial contractility appears to be dependent on differential actions on cyclic AMP and GMP activities. SNAP exerts a negative inotropic effect on rat cardiomyocytes at a high concentration (100 μM) by stimulating cGMP activation of PKG (47). However, it increases the contractile response at a low concentration (1 μM) by a cGMP-independent activation of adenylyl cyclase. Subsequently, it was shown that exogenous NO attenuated the contractile response by a PKG-troponin I mechanism (26).

We have shown that SNAP at a dose of 2 μM significantly increased cGMP levels in heart tissue. Furthermore, the protection provided by SNAP was abolished by the soluble guanylyl cyclase inhibitor ODQ. Although we could not find a selective PKG inhibitor that was feasible for use in intact hearts, the potent PKG activator 8-pCPT-cGMP mimicked the protection of SNAP. In intact cells, 8-pCPT-cGMP is known to activate PKG with negligible effects on PKA- or cGMP-dependent phosphodiesterases (1, 13, 18, 20). Data from cells complement these observations in intact hearts. In isolated cardiomyocytes, two highly specific PKG antagonists were noted to abort the trigger mechanism of preconditioning by blocking mitochondrial ROS production that was initiated by a preconditioning mimetic drug. Thus the 8-bromoguanosine-3′,5′-cyclic monophosphorothioate R was blocked the effect of BK (36). And the highly selective DT-3, which is a cell-permeable peptide designed specifically to inhibit PKG (17), blocked the ability of both BK and acetylcholine to induce ROS production (T. Krieg, L. Cui, W. R. G. Dostmann, J. M. Downey, and M. V. Cohen, unpublished observation). These data suggest the involvement of PKG in the protection that SNAP provides.

In the present study, cardiac protection from both 8-pCPT-cGMP and SNAP could be blocked by the mitoKATP channel blocker 5-HD, which confirms a dependence on the ability of SNAP to open these channels. It should be noted that the role of 5-HD as a selective opener of mitoKATP channels and even the existence of these channels have recently been questioned (16). Prior investigations have demonstrated that the infarct-sparing effect of SNAP involved ROS production (27, 34). It is unknown how PKG might open mitoKATP channels. In vascular smooth muscle, NO was reported to activate surface KATP channels or on a protein upstream of the channels.
blocked the infarct-limiting effect of BK (36), this dose still could not block the protection provided by ischemic preconditioning in the present study. During ischemia, several metabolites including adenosine, BK, and opioids are released into the myocardium. It is believed that all of these converge on PKC or protein tyrosine kinases and that a finite threshold of protein kinase stimulation is required before a preconditioning state can be realized. Although BK seems to utilize NO of protein kinase stimulation is required before a preconditioning state can be realized. Although BK seems to utilize NO as a second messenger in its signaling pathway, adenosine, another trigger, probably does not. We have found that adenosine triggers protection by a mechanism that uses neither ROS nor mitoK\textsubscript{ATP} channels (14). In the isolated heart there are no kininogens in the perfusate, and as a result we have been unable to show a BK component of ischemic preconditioning, whereas it was easily demonstrated in the blood-perfused in situ model (21). Similarly, we have been able to demonstrate an opioid component in only the in situ rabbit heart (31). Thus it seems likely that the isolated rabbit heart differs markedly from the in situ heart in that adenosine is the predominant preconditioning trigger in the non-blood-perfused model, and thus failure to find an endogenous NO component is not surprising. We, however, would expect there to be a strong NO component of ischemic preconditioning in the in situ rabbit heart.

GRANTS

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