**cAMP-Independent Dilation of Coronary Arterioles to Adenosine**

**Role of Nitric Oxide, G Proteins, and K<sub>ATP</sub> Channels**

Travis W. Hein, Lih Kuo

**Abstract**—Adenosine is known to play an important role in the regulation of coronary blood flow during metabolic stress. However, there is sparse information on the mechanism of adenosine-induced dilation at the microcirculatory levels. In the present study, we examined the role of endothelial nitric oxide (NO), G proteins, cyclic nucleotides, and potassium channels in coronary arteriolar dilation to adenosine. Pig subepicardial coronary arterioles (50 to 100 \( \mu \)m in diameter) were isolated, cannulated, and pressurized to 60 cm H<sub>2</sub>O without flow for in vitro study. The arterioles developed basal tone and dilated dose dependently to adenosine. Disruption of endothelium, blocking of endothelial ATP-sensitive potassium (K<sub>ATP</sub>) channels by glibenclamide, and inhibition of NO synthase by \( N^G \)-nitro-L-arginine methyl ester and of soluble guanylyl cyclase by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one produced identical attenuation of vasodilation to adenosine. Combined administration of these inhibitors did not further attenuate the vasodilatory response. Production of NO from coronary arterioles was significantly increased by adenosine. Pertussis toxin, but not cholera toxin, significantly inhibited vasodilation to adenosine, and this inhibitory effect was only evident in vessels with an intact endothelium. Tetraethylammonium, glibenclamide, and a high concentration of extraluminal KCl abolished vasodilation of denuded vessels to adenosine; however, inhibition of calcium-activated potassium channels by iberiotoxin had no effect on this dilation. Rp-8-Br-cAMPS, a cAMP antagonist, inhibited vasodilation to cAMP analog 8-Br-cAMP but failed to block adenosine-induced dilation. Furthermore, vasodilations to 8-Br-cAMP and sodium nitroprusside were not inhibited by glibenclamide, indicating that cAMP- and cGMP-induced dilations are not mediated by the activation of K<sub>ATP</sub> channels. These results suggest that adenosine activates both endothelial and smooth muscle pathways to exert its vasodilatory function. On one hand, adenosine opens endothelial K<sub>ATP</sub> channels through activation of pertussis toxin–sensitive G proteins. This signaling leads to the production and release of NO, which subsequently activates smooth muscle soluble guanylyl cyclase for vasodilation. On the other hand, adenosine activates smooth muscle K<sub>ATP</sub> channels and leads to vasodilation through hyperpolarization. It appears that the latter vasodilatory process is independent of G proteins and of cAMP/cGMP pathways. (Circ Res. 1999;85:634-642.)

**Key Words:** adenosine ■ microcirculation ■ nitric oxide ■ K<sup>+</sup> channel

The nucleoside adenosine is a potent vasodilator that has been suggested to play a major role in the control of coronary blood flow during metabolic stress, such as hypoxia, ischemia, and increase in myocardial oxygen consumption. Recently, adenosine receptors have been characterized in coronary vascular smooth muscle and endothelial cells; however, the cellular signal pathway responsible for the vasodilation has not been systematically examined. The mechanisms implicated in adenosine-induced dilation of coronary arteries are controversial. For example, evidence suggests that endothelial production of nitric oxide (NO), stimulation of receptor-coupled G proteins, and elevation of intracellular cAMP levels are involved in the dilation of isolated coronary arteries to adenosine. In an intact heart preparation, the increased coronary flow by adenosine was partly inhibited by the ATP-sensitive potassium (K<sub>ATP</sub>) channel blocker glibenclamide and by inhibition of adenyl cyclase activity, suggesting the putative role of K<sub>ATP</sub> channels and cAMP in this process. Conversely, evidence against the involvement of endothelium, NO, and K<sub>ATP</sub> channels, and cAMP in vasodilation to adenosine was also reported. Despite these discrepancies, it is important to note that the findings derived from both isolated conduit vessel and intact heart studies are generally confounded by various undefined factors. For example, because of the lack of vascular tone in vitro, the study of vasodilation in conduit vessels is performed with various pharmacological constrictors that potentially influence the adenosine response by
activating and superimposing different contractile mechanisms. Most importantly, large-vessel studies are not representative of the resistance microvessels, where the blood flow is actually controlled, and thus it is difficult to extrapolate the results from conduit vessel to microvessel because of the functional differences. In addition, the limitation of in vivo studies is also apparently caused by the inevitable influences from neurohumoral and local control mechanisms. Therefore, the cellular mechanism and signal transduction pathway for the vasomotor response to adenosine in coronary resistance vessels are still unclear.

Recently, Kuo and Chancellor reported that dilation of isolated coronary arterioles to adenosine is attenuated by endothelial removal, NO synthase inhibitor, and glibenclamide, suggesting the involvement of endothelial NO release and K_{ATP} channel activation. However, the release of NO from these microvessels has not been directly assessed, and the cellular pathways for NO and K_{ATP} channel activation leading to vasodilation have not been elucidated. Whether cyclic nucleotides (cAMP/cGMP) are involved in this signal transduction process is also unclear. It has been shown that inhibition of G protein signal transduction by pertussis toxin (PTX) prevents the opening of K_{ATP} channels in pancreatic cells and cardiac myocytes, suggesting the involvement of PTX-sensitive G proteins in K_{ATP} channel activation. However, it is not known whether this transduction process also occurs in the vascular tissue, especially in response to adenosine. Therefore, in the present study, we focused on the cellular mechanism and signal transduction pathway for NO and K_{ATP} channel activation leading to adenosine-induced dilation. The role of endothelial NO, G proteins, cyclic nucleotides, and potassium channels in vasodilation to adenosine was examined in the isolated and pressurized coronary resistance vessels.

Materials and Methods

General Preparation
Pigs (8 to 12 weeks old of either sex) were sedated with an intramuscular injection of tiletamine and zolazepam (1:1, 4.4 mg/kg) and xylazine (2.2 mg/kg) and then anesthetized and heparinized with an intravenous administration of pentobarbital sodium (20 mg/kg) and heparin (1000 U/kg), respectively, via the marginal ear vein. Pigs were intubated and ventilated with room air. After a left thoracotomy was performed, the heart was electrically fibrillated, and the following studies were performed to elucidate the possible mechanisms involved in the coronary arterial dilation to adenosine.

Isolation and Cannulation of Microvessels
The techniques for identification and isolation of porcine coronary microvessels were described previously. In brief, a mixture of India ink and gelatin in physiological salt solution (PSS) containing (in mmol/L) NaCl 145.0, KCl 4.7, CaCl_2 2.0, MgSO_4 1.17, NaH_2PO_4 1.2, glucose 5.0, pyruvate 2.0, EDTA 0.02, and MOPS 3.0 was perfused into the left anterior descending artery (0.3 mL) and the right coronary artery (0.2 mL) to remove endothelial cells. To allow development of basal tone, preparations with leaks were excluded from further study.

Experimental Protocols for Mechanistic Study of Adenosine-Induced Dilation
Cannulated arterioles were bathed in PSS-albumin at 36°C to 37°C to allow development of basal tone. After vessels developed a stable basal tone (~40 to 60 minutes), the dose-diameter relationship for adenosine (0.1 mmol/L to 10 μmol/L) was established. The role of endothelium in the adenosine-induced coronary arteriolar dilatation was evaluated by comparing the response before and after endothelial removal. A nonionic detergent, CHAPS (0.4%), was perfused into the vessel for 1 to 2 minutes to remove endothelial cells. To ensure that the vascular smooth muscle function was not compromised by CHAPS treatment, dose-dependent dilation of the vessel in response to sodium nitroprusside (1 mmol/L to 10 μmol/L) was examined before and after denudation. Only vessels that exhibited normal vasodilation to endothelium-dependent vasodilator bradykinin (1 mmol/L), and showed unaltered vasodilation to sodium nitroprusside after endothelial removal were accepted for data analysis.

The following studies were performed to elucidate the possible mechanisms involved in the coronary arterial dilation to adenosine. First, the involvement of NO and prostaglandins in adenosine-induced dilatation was examined by extraluminal incubation (30 minutes) of isolated arterioles with the specific inhibitors L-NAME (10 μmol/L) and indomethacin (10 μmol/L), respectively. Second, the possible involvement of endothelial K_{ATP} channels in adenosine-induced vasodilation was examined by intraluminal incubation of vessels with glibenclamide (5 μmol/L) for 10 minutes. This technique has been shown to specifically inhibit endothelial K_{ATP} channel function in our previous studies. The role of vascular smooth muscle potassium channels was evaluated in denuded vessels treated with a non-specific potassium channel inhibitor, tetraethylammonium (TEA, 10 and 20 mmol/L, extraluminal). The involvement of K_{ATP} and calcium-activated potassium (K_{Ca}) channels in vasodilation to adenosine was examined by extraluminal incubation of intact or denuded vessels with the specific inhibitors glibenclamide (5 μmol/L, 30 minutes) and ibetiroxin (0.1 μmol/L, 60 minutes), respectively. Third, the role of G proteins in adenosine-induced dilatation was examined by extraluminal incubation (60 minutes) of the vessels with PTX (100 ng/mL) or cholera toxin (CTX, 2 μg/mL). Fourth, the dilation of denuded vessel to adenosine was performed in the presence of a dilated vessel in the presence of a dilated vessel.
depressing PSS solution containing 40 mmol/L KCl. The isotonic depressor solution was prepared by substituting 35 mmol/L NaCl with an equimolar amount of KCl. Finally, to examine the contribution of cAMP and cGMP, the vessels were treated with the cAMP-competitive antagonist Rp-8-Br-cAMPS (Biolog) and the soluble guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3,-d]quinoxalin-1-one (ODQ, 0.1 µmol/L; Research Biochemicals International), respectively. The vasodilation to adenosine was examined before and after extraluminal exposure of the vessels to each inhibitor. The efficacy of cAMP and cGMP inhibition was verified by the absence of vasodilation to 8-Br-cAMP (Research Biochemicals International) and sodium nitroprusside, respectively. It should be noted that glibenclamide has been shown to bind strongly, >99%, to albumin4,35; we performed studies with this K<sub>ATP</sub> channel blocker in the PSS without albumin.

**Measurement of Adenosine-Induced NO Release from Coronary Microvessels**

The porcine coronary arterioles (10 segments, 1 to 2 mm in length, 70 to 100 µm in situ diameter) were isolated and placed in a microcentrifuge tube containing 100 µL PSS at 37°C. After a 30-minute initial incubation, adenosine (10 µL, final concentration 1 µmol/L) was added to the bath vessel. The bathing solution was then collected for NO measurement after a 25-minute incubation with adenosine. The production of NO was evaluated by measuring nitrite using a chemiluminescence NO analyzer (Siever Instruments). Basically, the collected sample was injected into a reflux chamber containing glacial acetic acid and 1% potassium iodide at room temperature. Under these conditions, nitrite is quantitatively converted to NO. The NO gas was then purged into the chemiluminescence NO analyzer and quantified by reference to NaN<sub>3</sub> standards. Another series of experiments was performed in the presence of NO synthase inhibitor. The vessels were treated with L-NAME (10 µmol/L) for 30 minutes during the initial incubation period. Then, adenosine (1 µmol/L final concentration) was added to the vessel bath and NO production was assayed after 25 minutes of incubation. In addition, a separate experiment was run as a control by adding vehicle solution (PSS) instead of adenosine and L-NAME. The background level of nitrite in the solution was measured from the tube containing PSS only, and this value was subsequently subtracted from the sampled solution to obtain NO production. The protein levels in each vessel tube were quantified by bicinchoninic acid protein assay (Pierce) and were used as a basis to normalize the protein levels in each vessel tube were quantified by bicinchoninic acid protein assay (Pierce) and were used as a basis to normalize the production of NO. The NO production (Figure 2). The increased NO production by adenosine (1 µmol/L) (Figure 2). There was no statistical difference multiple-range test. Differences in resting diameter before and after pharmacological interventions and vasodilations to bradykinin and 8-Br-cAMP before and after intervention were compared by paired Student t tests. Significance was accepted at P<0.05.

**Chemicals**

Drugs were obtained from Sigma, except as specifically stated. Adenosine, bradykinin, ibiotoxin, indomethacin, L-NAME, CTX, PDX, sodium nitroprusside, 8-Br-cAMP, Rp-8-Br-cAMPS, and TEA were dissolved in PSS. Pinacidil was dissolved in ethanol. ODQ and glibenclamide were dissolved in DMSO as stock solutions (10 mmol/L). Subsequent concentrations of pinacidil, ODQ, and glibenclamide were diluted in PSS. The final concentrations of ethanol and DMSO in the vessel bath were 0.1 and 0.03%, respectively. Vehicle control studies indicated that these final concentrations of ethanol and DMSO had no effect on the arteriolar function.

**Data Analysis**

At the end of each experiment, the vessel was relaxed with sodium nitroprusside 100 µmol/L to obtain its maximal diameter at 60 cm H<sub>2</sub>O intraluminal pressure. We have previously shown that this concentration of sodium nitroprusside produced maximal relaxation of isolated vessels.36 Therefore, all diameter changes in response to agonists were normalized to the vasodilation in response to 100 µmol/L sodium nitroprusside and expressed as a percentage of maximal dilation. All data are presented as mean±SEM. Statistical comparisons of vasomotor responses and NO production under various treatments were performed with 1- or 2-way ANOVA when appropriate and tested with the Fisher protected least significant difference multiple-range test. Differences in resting diameter before and after pharmacological interventions and vasodilations to bradykinin and 8-Br-cAMP before and after intervention were compared by paired Student t tests. Significance was accepted at P<0.05.

**Results**

**Role of Endothelium-Derived Factors in Vasodilation to Adenosine**

In this study, all vessels developed a similar level of basal tone (68±1% of maximal diameter) at 36°C to 37°C bath temperature with 60 cm H<sub>2</sub>O intraluminal pressure. The average resting and maximal diameters of the vessel were 84±3 and 125±3 µm, respectively. Indomethacin (10 µmol/L) did not alter vasodilation elicited by adenosine, but L-NAME (10 µmol/L) significantly inhibited the vasodilatory response (Figure 1). The inhibitory effect produced by L-NAME was identical to that produced by soluble guanylyl cyclase blocker ODQ (0.1 µmol/L, Figure 1). Incubation of these vessels with L-NAME or ODQ did not significantly affect resting diameter.

**Measurement of Adenosine-Induced NO Release From Coronary Arterioles**

In the absence of adenosine, the NO production from coronary arterioles was 1.4±0.7 nmol/g protein. Adding adenosine (1 µmol/L) to the vessels produced an 8-fold increase in NO production (Figure 2). The increased NO production by adenosine (1 µmol/L) was not seen in the vessels treated with L-NAME (10 µmol/L) (Figure 2). There was no statistical difference in the NO production between control and L-NAME-treated vessels.

**Role of Endothelial K<sub>ATP</sub> Channels in Adenosine-Induced Vasodilation**

Intraluminal incubation of vessels with glibenclamide (5 µmol/L, 10 minutes) did not alter resting diameter, but attenuated arteriolar dilation to adenosine (Figure 3). It is
worth noting that the extent of attenuation by intraluminal glibenclamide was similar to that by L-NAME or ODQ (Figure 1). Furthermore, the addition of ODQ (0.1 μmol/L, 30 minutes) to these intraluminal glibenclamide-treated vessels did not further attenuate vasodilation to adenosine (Figure 3). These results suggest that intraluminal glibenclamide and ODQ inhibit the same vasodilatory pathway.

**Role of G Proteins in Adenosine-Induced Vasodilation**

Inhibition of arteriolar Gi and Go proteins by extraluminal PTX (100 ng/mL, 60 minutes) did not alter resting vascular diameter or vasodilation to nitroprusside (Table) but significantly attenuated adenosine-induced vasodilation (Figure 4A). Removal of endothelium attenuated vasodilation to adenosine, and this attenuation was identical to that produced by PTX (Figure 4A). In these denuded vessels, PTX did not have an additional inhibitory effect on adenosine-induced vasodilation (Figure 4A). To evaluate the role of Gi proteins in vasodilation, the intact vessels were treated with extraluminal CTX (2 μg/mL) for 60 minutes, and its effect on adenosine-induced dilation was reexamined. Initially, CTX produced a slight vasodilation (17±5%) of coronary arterioles, but the vessels regained tone after a 15-minute exposure to CTX. In contrast to PTX, coronary arteriolar dilation to adenosine was not affected by CTX (Figure 4B).

**Role of Vascular Smooth Muscle K⁺ Channels and Membrane Hyperpolarization in Arteriolar Dilation to Adenosine**

The role of smooth muscle potassium channels and membrane hyperpolarization in vasodilation to adenosine is elucidated in Figures 5 and 6. In this series of studies, the endothelium was initially removed to eliminate its contribution to vasodilation. Disruption of endothelium attenuated adenosine-induced vasodilation, which is consistent with the data shown in Figure 4A. Extraluminal incubation of denuded vessels with a nonspecific potassium channel blocker TEA (10 mmol/L) attenuated vasodilation to adenosine (Figure 5). A higher concentration of TEA (20 mmol/L) almost completely blocked the vasodilation (10% of maximal dilation, Figure 5). It is noted that 10 mmol/L TEA did not alter resting vessel tone, whereas 20 mmol/L TEA increased vessel tone from 68% to 61% of its maximal diameter without affecting dilation to sodium nitroprusside (Table). To probe the role of KATP channels in adenosine-induced dilation, the denuded vessels were treated with extraluminal glibenclamide for 30 minutes. Glibenclamide (5 μmol/L) did not affect the resting diameter but completely blocked dilation of denuded vessels to adenosine (Figure 6). It should be noted that the dilation of these glibenclamide-treated vessels to sodium nitroprusside was not affected (data not shown). In the presence of a high concentration of extraluminal KCl (40 mmol/L), adenosine-induced dilation was significantly attenuated (Figure 6). This concentration of KCl had a

**Effect of Pharmacological Treatments on Dilation of Coronary Arterioles to Sodium Nitroprusside**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (log[mol/L])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=30)</td>
<td>5±1 20±2 63±3 90±1</td>
</tr>
<tr>
<td>Denudation (n=6)</td>
<td>6±1 21±3 66±7 92±2</td>
</tr>
<tr>
<td>Glibenclamide (n=4)</td>
<td>8±1 22±2 64±4 91±3</td>
</tr>
<tr>
<td>Iberiotoxin (n=4)</td>
<td>8±2 23±2 66±3 93±2</td>
</tr>
<tr>
<td>L-NAME (n=5)</td>
<td>5±1 22±2 59±11 87±4</td>
</tr>
<tr>
<td>KCl (n=5)</td>
<td>7±2 19±6 57±4 91±3</td>
</tr>
<tr>
<td>ODQ (n=7)</td>
<td>0±0* 0±0* 0±0* 34±6*</td>
</tr>
<tr>
<td>PTX (n=6)</td>
<td>6±2 21±4 62±7 92±1</td>
</tr>
<tr>
<td>Rp-8-Br-cAMPS (n=6)</td>
<td>6±1 22±4 63±3 89±2</td>
</tr>
<tr>
<td>20 mM TEA (n=3)</td>
<td>7±3 27±7 63±4 91±2</td>
</tr>
</tbody>
</table>

All values are mean±SEM expressed as percentage of maximal dilation. Dose-dependent dilation of isolated coronary arterioles to sodium nitroprusside was studied before and after pharmacological treatments. n=number of vessels.

*P<0.05 vs control.
of the KATP channel antagonist glibenclamide. Coronary nitroprusside was examined after extraluminal administration for cGMP production, and the vasodilation produced by 5\text{m}\textsuperscript{139}6\text{CTX (2\text{Dose-dependent vasodilation to adenosine was not altered by n\textsuperscript{9}) did not further reduce the adenosine-induced dilation. B, tration of PTX to denuded vessels (resting diameter, 86\text{m}\textsuperscript{6\text{6}}6\text{meter, 82\text{m}\textsuperscript{6\text{4}}4\text{m; n\textsuperscript{9}) or PTX (100 ng/mL; resting diam-
eter, 82\text{m}\textsuperscript{6\text{6}}6\text{m; n\textsuperscript{9}) did not further reduce the adenosine-induced dilation. B, Dose-dependent vasodilation to adenosine was not altered by CTX (2\mu g/mL; resting diameter, 99\text{m}\textsuperscript{3\mu m; maximal diameter, 139\text{m}\textsuperscript{5\mu m; n\textsuperscript{3})}.

tendency to increase vascular tone but not in a significant manner and did not alter sodium nitroprusside-induced dilation (Table). In contrast to TEA and glibenclamide, inhibition of K\textsubscript{ATP} channels by extraluminal iberiotoxin (0.1 \mu mol/L) did not alter the vasodilatory response to adenosine (n=4, data not shown).

**Role of cGMP in K\textsubscript{ATP} Channel-Mediated Vasodilation**

Because the cGMP pathway is involved in the adenosine-induced dilation (Figure 1), it is likely that the opening of smooth muscle K\textsubscript{ATP} channels is mediated by the activation of this pathway as shown in porcine pial arteries.\(^{37}\) To examine this possibility, the NO donor sodium nitroprusside (10 \mu mol/L to 10 \mu mol/L) was used to activate guanylyl cyclase for cGMP production, and the vasodilation produced by nitroprusside was examined after extraluminal administration of the K\textsubscript{ATP} channel antagonist glibenclamide. Coronary arteriolar dilation to sodium nitroprusside was not altered by glibenclamide (5 \mu mol/L, Table), but it was inhibited by the soluble guanylyl cyclase inhibitor ODQ (0.1 \mu mol/L, Table). These results indicate that activation of guanylyl cyclase does not lead to K\textsubscript{ATP} channel-mediated coronary arteriolar dilation.

**Role of cAMP in Adenosine-Induced Vasodilation**

The contribution of cAMP in coronary arteriolar dilation to adenosine was examined before and after extraluminal incubation of vessels with cAMP antagonist Rp-8-Br-cAMPS (10 \mu mol/L, 40 minutes). As shown in Figure 7A, Rp-8-Br-cAMPS abolished coronary arteriolar dilation in response to the direct increase in cAMP by a cell-permeable cAMP analog, 8-Br-cAMP (2 \mu mol/L); however, this cAMP antagonist failed to block adenosine-induced vasodilation (Figure 7B). To eliminate the possible confounding effects from endothelium, another series of experiments was performed in denuded vessels. Similarly, Rp-8-Br-cAMPS abolished dilation of denuded vessels to 8-Br-cAMP but failed to inhibit vasodilation to adenosine (n=3, data not shown). It is worth

**Figure 4.** Effect of PTX and CTX on coronary arteriolar dilation to adenosine. A, Adenosine produced dose-dependent vasodilation under control conditions (resting diameter, 84\text{m}\textsuperscript{6\text{6}}6\text{m; maximal diameter, 122\text{m}\textsuperscript{5\mu m; n\textsuperscript{17})}. The vasodilatory response was significantly inhibited by either endothelial denudation (resting diameter, 82\text{m}\textsuperscript{4\mu m; n\textsuperscript{9}) or PTX (100 ng/mL; resting diameter, 82\text{m}\textsuperscript{6\mu m; n\textsuperscript{8})}. *P<0.05 vs control. Subsequent administration of vessels with cAMP antagonist Rp-8-Br-cAMPS (2 \mu mol/L, 40 minutes). As shown in Figure 7A, Rp-8-Br-cAMPS abolished coronary arteriolar dilation in response to the direct increase in cAMP by a cell-permeable cAMP analog, 8-Br-cAMP (2 \mu mol/L); however, this cAMP antagonist failed to block adenosine-induced vasodilation (Figure 7B). To eliminate the possible confounding effects from endothelium, another series of experiments was performed in denuded vessels. Similarly, Rp-8-Br-cAMPS abolished dilation of denuded vessels to 8-Br-cAMP but failed to inhibit vasodilation to adenosine (n=3, data not shown). It is worth

**Figure 5.** Effect of TEA on coronary arteriolar dilation to adeno-
sine. Adenosine produced dose-dependent vasodilation under control conditions (resting diameter, 89\text{m}\textsuperscript{6\mu m; maximal diam-
eter, 131\text{m}\textsuperscript{7\mu m; n\textsuperscript{6})}. The vasodilatory response was signifi-
cantly inhibited by endothelial denudation (resting diameter, 85\text{m}\textsuperscript{6\mu m; n\textsuperscript{6})}. Adenosine-induced dilation of these denuded vessels was further inhibited by 10 mmol/L TEA (resting diameter, 84\text{m}\textsuperscript{10\mu m; maximal diameter, 129\text{m}\textsuperscript{13\mu m; n\textsuperscript{3}}) and was almost completely blocked by 20 mmol/L TEA (resting diameter, 82\text{m}\textsuperscript{8\mu m; maximal diameter, 134\text{m}\textsuperscript{10\mu m; n\textsuperscript{3})}. *P<0.05 vs control.

**Figure 6.** Effect of glibenclamide and KCl on coronary arteriolar dilation to adenosine. Adenosine produced dose-dependent vasodilation under control conditions (resting diameter, 85\text{m}\textsuperscript{5\mu m; maximal diameter, 131\text{m}\textsuperscript{5\mu m; n\textsuperscript{6})}. This vasodila-
tory response was inhibited by endothelial denudation (resting diameter, 86\text{m}\textsuperscript{7\mu m; n\textsuperscript{10})}. Subsequent administration of glib-
enclamide (GB) to the denuded vessels completely blocked the dilation in response to adenosine (resting diameter, 79\text{m}\textsuperscript{7\mu m; maximal diameter, 123\text{m}\textsuperscript{7\mu m; n\textsuperscript{4}}) and was significantly inhibited by 20 mmol/L KCl (resting diameter, 71\text{m}\textsuperscript{7\mu m; maximal diameter, 112\text{m}\textsuperscript{11\mu m; n\textsuperscript{6})}. *P<0.05 vs control.
Role of cAMP in K\textsubscript{ATP}

Channel-Mediated Vasodilation

To examine whether K\textsubscript{ATP} channels were involved in the cAMP-mediated dilation of coronary arterioles, the effect of glibenclamide (5 \textmu mol/L) on vasodilation to 8-Br-cAMP was investigated. 8-Br-cAMP (2 \textmu mol/L) produced a significant vasodilation by increasing diameter from a control level of 145\pm 5 \mu m; maximal diameter, 140\pm 6 \mu m; n=6), suggesting that porcine coronary arteriolar dilation to adenosine is cAMP independent.

Discussion

The role of the endothelium in dilation of coronary arteries to adenosine is controversial. For example, previous studies have shown that adenosine-induced dilation is independent, partially dependent, or exclusively dependent on the presence of an intact endothelium. Our previous studies demonstrated that the vasodilation to adenosine is partially dependent on the endothelium, given that the vasodilatory response was not abolished by endothelial removal (Figures 4A, 5, and 6). However, in some in vitro studies, the investigators did not observe the endothelial component of adenosine-induced dilation in large coronary arteries. This discrepancy may be derived from the difference in vascular preparations, ie, pressurized microvessel segment versus stretched vascular ring. In addition, the use of preconstrictors for large vessel preparations may have masked or interfered with the endothelial function. It is important to note that the development of basal tone in our coronary arterioles precluded the use of preconstrictors and thus eliminated these potential confounding effects.

The activation of endothelial adenosine receptors may stimulate the production and release of endothelium-derived vasodilators. Administration of indomethacin to the coronary arterioles, with a concentration (10 \textmu mol/L) sufficient to block prostaglandin synthesis in our previous study, did not alter adenosine responsiveness (Figure 1). This result indicates that adenosine-induced vasodilation is not mediated by the release of prostaglandins. Because adenosine-induced coronary arteriolar dilation was attenuated by the NO synthase inhibitor N\textsuperscript{\textdegree}-monomethyl-L-arginine (L-NMMA) in our previous study and by L-NAME in the present study (Figure 1) and was inhibited to the same extent by endothelial removal (Figures 4A, 5, and 6), it is believed that endothelial production/release of NO is partially responsible for the observed adenosine response. This contention is directly supported by the result of NO measurement, which shows that NO production was increased after incubation of the coronary arterioles with adenosine (Figure 2). However, another possible explanation for the inhibitory effect by L-NAME and denudation is that basal production of NO plays a permissive or amplifying role in the adenosine-induced response. If NO indeed plays a permissive/amplifying role, we should see a restoration or enhancement of vasodilation in the denuded vessels in response to adenosine in the presence of a threshold concentration of NO donor sodium nitroprusside. In fact, we found that sodium nitroprusside (10 \textmu mol/L) produced a slight dilation (5%; Table) of the denuded vessels but did not restore or enhance the adenosine response (data not shown). Collectively, our data indicate that NO is actively participating in the vasodilation rather than acting as a permissive or amplifying agent for the relaxing effect of adenosine.

Interestingly, our data showed that the endothelium was more sensitive than the vascular smooth muscle to adenosine for vasodilation, ie, dilation threshold 1 nmol/L and 0.1 \textmu mol/L for endothelium and smooth muscle, respectively (Figures 4A, 5, and 6). This may suggest a dose-dependent activation of the NO-dependent versus NO-independent pathways for adenosine-induced vasodilation. For instance, the vasodilation at lower doses (\approx 10^{-8} \textmu mol/L) of adenosine is exclusively mediated by the NO (eg, abolished by L-NAME and denudation). In contrast, the higher doses (\approx 10^{-7} \textmu mol/L) of adenosine activate both NO-dependent and NO-independent pathways (Figures 1 and 3), but the latter pathway becomes more obvious and masks the NO-dependent component. Therefore, the contribution of NO/endothelium to the observed vasodilation is diminished with increasing adenosine concentration. At the highest dose of...
Adenosine (10^{-5} mol/L), the dilation occurs independently of NO or endothelium (Figures 1 and 4). This might explain the seemingly conflicting results on NO dependency reported in vivo. For instance, flow responses to an intracoronary infusion (10 \mu g/min) or a bolus injection (3 \mu g total) of low doses of adenosine were attenuated by NO inhibitors. On the other hand, higher doses of adenosine (0.5 mg/min or 8 mg/min) that produced maximal coronary dilation were not affected by NO inhibition. Therefore, these in vivo and our in vitro data support the notion that the contribution of NO/endothelium to adenosine-induced dilation is inversely related to the adenosine concentration. The dose-dependent activation of adenosine vasodilatory pathways in endothelial and smooth muscle cells is schematically illustrated in Figure 8 and discussed below.

As discussed above, the released NO is an important mediator for vasodilation in response to the lower concentrations of adenosine (\leq 10^{-7} mol/L). In general, NO released from the endothelium subsequently activates soluble guanylyl cyclase in underlying vascular smooth muscle cells and thus produces vasodilation. However, a direct activation of vascular smooth muscle K_{ATP} and K_{Ca} channels by NO was also reported. Therefore, signal transduction mediating dilation of coronary arteries to adenosine downstream from NO remains unclear. In the present study, inhibition of soluble guanylyl cyclase by ODQ attenuated the vasomotor response to adenosine. In addition, ODQ attenuated adenosine-induced dilation similarly to that by L-NAME (Figure 1) and denudation (Figures 4A, 5, and 6). Furthermore, subsequent administration of ODQ to vessels pretreated with luminal glibenclamide did not further enhance the inhibitory effect (Figure 3), suggesting that these inhibitors act on the same pathway for vasodilation. Because luminal glibenclamide did not affect vasodilation to NO donor sodium nitroprusside, the inhibition of adenosine-induced dilation by luminal glibenclamide is believed to be upstream of the NO-cGMP pathway. Altogether, these results suggest that the opening of endothelial K_{ATP} channels leads to membrane hyperpolarization for calcium influx and thus produces vasodilation through subsequent activation of NO synthase for NO production.

The opening of endothelial K_{ATP} channels by adenosine may involve activation of G proteins as suggested by patch-clamp studies in cardiac myocytes. It has been shown that adenosine receptors can be coupled to both G_{i} and G_{s} proteins in various types of tissues. In the present study, we found that G_{i} proteins do not play a role in coronary arteriolar dilation to adenosine, because CTX did not alter the vasodilatory response. In contrast, PTX attenuated adenosine-induced dilation to the same extent as L-NAME, ODQ, intraluminal glibenclamide, or denudation. It appears that the component of PTX-sensitive dilation resides in the endothelial guanylyl cyclase/cGMP pathway in this vasodilatory process. On the other hand, glibenclamide and iberiotoxin did not affect vasodilation to NO donor sodium nitroprusside (Table), indicating that adenosine-induced dilation associated with NO is not mediated by the opening of K_{ATP} and K_{Ca} channels in our preparation. In addition, ODQ attenuated adenosine-induced dilation in a manner identical to that by L-NAME (Figure 1) and denudation (Figures 4A, 5, and 6), and combined administration of these 2 inhibitors did not further enhance the inhibition (data not shown). It appears that activation of the NO/guanylyl cyclase pathway is responsible for the endothelium-dependent component of vasodilation to adenosine (Figure 8).

It has been shown that the membrane hyperpolarization leading to the influx of calcium into endothelial cells is necessary for the NO production from constitutive NO synthase during agonist stimulation. Interestingly, adenosine has been shown to produce a sustained hyperpolarization of cultured coronary endothelial cells. Recently, Kuo and Chancellor demonstrated that prevention of endothelial hyperpolarization by a high luminal KCl solution attenuated dilation of isolated porcine coronary arterioles to adenosine. This inhibitory effect is consistent with that elicited by L-NMMA, indicating that hyperpolarization of the endothelium by adenosine might be responsible for the release of NO (Figure 8). In our previous study, we speculated that the membrane hyperpolarization might be a result of opening of endothelial K_{ATP} channels, given that specific inhibition of endothelial K_{ATP} channel and prevention of endothelial hyperpolarization by intraluminal administration of glibenclamide and KCl, respectively, produce identical inhibition of coronary arteriolar dilation to adenosine. In the present study, inhibition of endothelial K_{ATP} channels by luminal glibenclamide attenuated adenosine-induced vasodilation in a manner identical to that produced by L-NAME (Figure 1), ODQ (Figure 1), and endothelial removal (Figures 4A, 5, and 6). Furthermore, subsequent administration of ODQ to vessels pretreated with luminal glibenclamide did not further enhance the inhibitory effect (Figure 3), suggesting that these inhibitors act on the same pathway for vasodilation. Because luminal glibenclamide did not affect vasodilation to NO donor nitroprusside, the inhibition of adenosine-induced dilation by luminal glibenclamide is believed to be upstream of the NO-cGMP pathway. Altogether, these results suggest that the opening of endothelial K_{ATP} channels leads to membrane hyperpolarization for calcium influx and thus produces vasodilation through subsequent activation of NO synthase for NO production.

Figure 8. Schematic illustration of proposed signaling mechanisms involved in coronary arteriolar dilation to adenosine. At lower concentrations of adenosine (\leq 10^{-7} mol/L), activation of endothelial adenosine receptors is coupled to PTX-sensitive G proteins and subsequent opening of endothelial K_{ATP} channels. Opening of these channels elicits a change in membrane potential (\Delta E_{m}; ie, hyperpolarization), leading to an influx of calcium for NO production by NO synthase (NOS). The endothelium-released NO diffuses to the underlying smooth muscle and stimulates guanylyl cyclase to produce cGMP for relaxation. At the higher concentrations of adenosine (\geq 0.1 \mu mol/L), the vasodilatory action is more dependent on the smooth muscle K_{ATP} channels that take over the role of NO for vasodilation, even though the release of NO is still present under this condition. The opening of the smooth K_{ATP} channels elicits membrane hyperpolarization and subsequent relaxation. Inhibition of these signaling pathways by their respective inhibitors is indicated by a minus sign.
ium, because the adenosine-induced response was not inhibited by PTX in denuded vessels (Figure 4A). Furthermore, PTX did not further enhance the inhibitory effect produced by L-NAME, ODQ, or luminal glibenclamide (n=3, data not shown). Collectively, our present results support the contention that the activation of endothelial PTX-sensitive K<sub>ATP</sub> channels for membrane hyperpolarization and subsequently leads to vasodilation through the release of NO (Figure 8).

At the higher concentrations of adenosine (>0.1 μmol/L), the contribution of endothelium-released NO to vasodilation is diminished, and the direct action of adenosine on smooth muscle predominates. Because potassium channels have been shown to play an important role in smooth muscle relaxation, in the present study we examined the possible involvement of these channels in adenosine-induced dilation of denuded coronary arterioles. The role of potassium channels in the vasodilation to adenosine was evident because the adenosine response was inhibited by a nonspecific potassium channel blocker, TEA. Although the concentrations of TEA (10 and 20 mmol/L) are likely to inhibit both K<sub>ATP</sub> and K<sub>Ca</sub> channels, the role of K<sub>Ca</sub> channels is not supported, because the dilation was not altered by ibetrixin. In contrast, inhibition of K<sub>ATP</sub> channels by glibenclamide abolished the adenosine response, suggesting that opening of K<sub>ATP</sub> channels in vascular smooth muscle is responsible for the endothelium-independent response to adenosine (Figure 6). It is likely that vascular hyperpolarization after the opening of smooth muscle K<sub>ATP</sub> channels results in vasodilation, because administration of a depolarizing agent, KCl (40 mmol/L), significantly attenuated adenosine-induced dilation (Figure 6). A higher concentration of KCl (60 mmol/L) nearly abolished the vasodilatory response (7% dilation, data not shown). However, the latter data are difficult to interpret, because the resting vessel diameter was significantly decreased, from 63% to 44% of its maximal diameter, by 60 mmol/L KCl. Nevertheless, these results suggest that the activation of smooth muscle K<sub>ATP</sub> channels leading to vascular hyperpolarization is an essential pathway for vasodilation to adenosine (Figure 8). It is important to note that adenosine-induced dilation of large porcine coronary arteries was unaffected by glibenclamide, indicating that heterogeneous vasodilatory mechanisms might exist in the coronary circulation. On the other hand, it should be noted that this disparate result might be related to the vessel size, different vascular preparations, and the use of preconstrictors in these large vessels.

It is generally believed or assumed that stimulation of adenosine receptors activates adenylyl cyclase in vascular smooth muscle to produce cAMP, which may in turn relax coronary arteries by activating cAMP-dependent protein kinase A. However, there is no direct evidence to support this contention in the coronary microcirculation. In fact, there are some doubts as to the role of cAMP pathway in adenosine-induced dilation of coronary arteries, because adenosine and adenosine receptor agonists seem not to increase intracellular levels of cAMP in coronary arteries in vitro. In the present study, we found that the dilation elicited by adenosine was not affected by cAMP antagonist Rp-8-Br-cAMPS, suggesting a minimal role of this pathway, if any, in vasodilation. The concentration of cAMP antagonist used in the present study was effective and selective, given that vasodilation to cAMP analog 8-Br-cAMP (Figure 7A) was abolished, and the cGMP-mediated dilation in response to sodium nitroprusside (Table) remained intact. The present study also ruled out the possibility of activation of K<sub>ATP</sub> channels by the cAMP–protein kinase A pathway, because vasodilation to cAMP agonist was not altered by glibenclamide. Collectively, the ability of glibenclamide but not of Rp-8-Br-cAMPS to inhibit vasodilation indicates that the activation of K<sub>ATP</sub> channels by adenosine is not mediated by the cAMP pathway. Furthermore, our data do not support the idea that activation of K<sub>ATP</sub> channels by adenosine is through the cGMP pathway, because vasodilation in response to sodium nitroprusside was not affected by glibenclamide (Table). Unlike the endothelium, the K<sub>ATP</sub> channel activation in smooth muscle appears to be independent of G<sub>Gi</sub>/G<sub>i</sub> proteins, because PTX (100 ng/mL, 60-minute incubation) had no effect on adenosine-induced dilation in the denuded vessels. In our previous study, we have shown that the same PTX treatment produced an effective inhibition of acidosis-induced dilation mediated by the opening of smooth muscle K<sub>ATP</sub> channels. Therefore, the failure of inhibiting adenosine-induced dilation of denuded vessels by PTX in the present study is unlikely the result of the ineffectiveness of this drug. Nevertheless, these findings highlight the involvement of different signaling pathways in the activation of smooth muscle K<sub>ATP</sub> channel by adenosine (PTX insensitive) and by acidosis (PTX sensitive).

In conclusion, porcine coronary arteriolar dilation to adenosine could be explained by at least 2 mechanisms. First, adenosine, at lower concentrations (eg, mmol/L range), selectively opens endothelial K<sub>ATP</sub> channels through activation of PTX-sensitive G proteins. This signaling leads to the production and release of NO, which subsequently activates smooth muscle soluble guanylyl cyclase for vasodilation. Second, at higher concentrations of adenosine (μmol/L range), the opening of smooth muscle K<sub>ATP</sub> channels associated with membrane hyperpolarization takes over the role of NO for vasodilation. It appears that the latter vasodilatory pathway is independent of G proteins and of cAMP/cGMP pathways. Currently, the detailed mechanism for the inverse relationship between adenosine concentration and the involvement of endothelium in vasodilation is unclear. It is possible that the G proteins in endothelium might serve as an amplifier for the cellular signaling linked to K<sub>ATP</sub> channels and thus might contribute to the high sensitivity of endothelium to adenosine. Therefore, a low concentration of adenosine is sufficient to activate the endothelial K<sub>ATP</sub> channel pathway for vasodilation. In contrast, a higher concentration of adenosine is required to activate smooth muscle K<sub>ATP</sub> channels, because the PTX-sensitive G protein is not involved in this vasodilatory pathway. This might explain the differential sensitivity of the adenosine response, in terms of endothelium versus smooth muscle pathway for vasodilation.

Acknowledgments

This study was supported by National Heart, Lung, and Blood Institute (Bethesda, Md) grants HL-48179 and K02 HL-03693.
References


37. Luckhoff A, Busse R. Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. Pflügers Arch. 1990;416:305–311.


45. Luckhoff A, Busse R. Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. Pflügers Arch. 1990;416:305–311.


47. vanDaller K, Hamprecht B. Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. J Neurochem. 1979;33:999–1005.


cAMP-Independent Dilation of Coronary Arterioles to Adenosine: Role of Nitric Oxide, G Proteins, and KATP Channels

Travis W. Hein and Lih Kuo

Circ Res. 1999;85:634-642
doi: 10.1161/01.RES.85.7.634

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/85/7/634

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org//subscriptions/