Transmural difference in coronary arteriolar dilation to adenosine: effect of luminal pressure and $K_{ATP}$ channels

CUIHUA ZHANG, TRAVIS W. HEIN, AND LIH KUO

Department of Medical Physiology, Cardiovascular Research Institute, The Texas A&M University System Health Science Center, College Station, Texas 77843-1114

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Zhang, Cuihua, Travis W. Hein, and Lih Kuo. Transmural difference in coronary arteriolar dilation to adenosine: effect of luminal pressure and $K_{ATP}$ channels. Am J Physiol Heart Circ Physiol 279: H2612–H2619, 2000.—Coronary blood flow in the subendocardium is preferentially increased by adenosine but is redistributed to the subepicardium during ischemia in association with coronary pressure reduction. The mechanism for this flow redistribution remains unclear. Since adenosine is released during ischemia, it is possible that the coronary microcirculation exhibits a transmural difference in vasomotor responsiveness to adenosine at various intraluminal pressures. Although the $K_{ATP}$-sensitive $K^+$ channel has been shown to be involved in coronary arteriolar dilation to adenosine, its role in the transmural adenosine response remains elusive. To address these issues, pig subepicardial and subendocardial arterioles (60–120 μm) were isolated, cannulated, and pressurized to 20, 40, 60, or 80 cmH$_2$O without flow for in vitro study. At each of these pressures, vessels developed basal tone and dilated concentration dependently to adenosine and the $K_{ATP}$ channel opener pinacidil. Subepicardial and subendocardial arterioles dilated equally to adenosine and pinacidil at 60 and 80 cmH$_2$O luminal pressure. At lower luminal pressures (i.e., 20 and 40 cmH$_2$O), vasodilation in both vessel types was enhanced. Enhanced vasodilatory responses were not affected by removal of endothelium but were abolished by the $K_{ATP}$ channel inhibitor glibenclamide. In a manner similar to reducing pressure, a subthreshold dose of pinacidil potentiated vasodilation to adenosine. In contrast to adenosine, dilation of coronary arterioles to sodium nitroprusside was independent of pressure changes. These results indicate that coronary microvascular dilation to adenosine is enhanced at lower intraluminal pressures by selective activation of smooth muscle $K_{ATP}$ channels. Since microvascular pressure has been shown to be consistently lower in the subendocardium than in the subepicardium, it is likely that the inherent pressure gradient in the coronary microcirculation across the ventricular wall may be an important determinant of transmural flow in vivo during resting conditions or under metabolic stress with adenosine release.

Address for reprint requests and other correspondence: L. Kuo, Dept. of Medical Physiology, Cardiovascular Research Institute, The Texas A&M Univ. System Health Science Center, TAMU 1114, College Station, TX 77843-1114 (E-mail: lkuo@tamu.edu).

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difference of adenosine response in vivo (32). Whereas our previous studies indicate that opening of ATP-sensitive potassium (K<sub>ATP</sub>) channels mediates coronary arteriolar dilation to adenosine (14, 18) through receptor activation (13), it has not been determined whether subepicardial and subendocardial microvessels exhibit differential responsiveness to K<sub>ATP</sub> channel activation. Therefore, in the present study, we examined the vasomotor responsiveness of subepicardial and subendocardial arterioles (60–120 μm) to adenosine and to K<sub>ATP</sub> channel activation at various intraluminal pressures. These studies were performed in vitro to eliminate the possible confounding influences from neurohumoral activation and from local hemodynamic and metabolic changes.

**MATERIALS AND METHODS**

**General preparation.** Pigs (8–12 wk old of either gender) were sedated with an intramuscular injection of 1:1 tiletamine-zolazepam (Telazol, 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 (1,000 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, excised, and immediately placed in cold (5°C) saline solution.

**Isolation and cannulation of microvessels.** The techniques for identification and isolation of porcine coronary microvessels were described previously (19). Briefly, a mixture of India ink and gelatin in physiological salt solution (PSS), containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl<sub>2</sub>, 1.17 MgSO<sub>4</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 MOPS, was perfused into the left anterior descending artery (0.3 ml) and the circumflex artery (0.4 ml) to enable visualization of coronary microvessels. Left ventricular subepicardial arterioles (60–120 μm in situ internal diameter and 0.6–1.0 mm long without branches) from the left anterior descending arterial distribution and the subendocardial arterioles of similar lengths and diameters from the surface of left ventricular papillary muscles were carefully dissected free from the surrounding cardiac tissue under cold (5°C) PSS containing BSA (1%; Amersham, Arlington Heights, IL) at pH 7.4. Each isolated arteriole was then transferred for cannulation to a Lucite vessel chamber containing PSS-albumin equilibrated with room air at ambient temperature. One end of the microvessel was cannulated with a glass micropipette (40 μm tip diameter) filled with filtered PSS-albumin, and the microvessel was securely tied to the pipette with 11-0 ophthalmic suture (Alcon, Fort Worth, TX). The ink-gelatin solution inside the vessel was flushed out at a low perfusion pressure (~20 cmH<sub>2</sub>O). The other end of the vessel was then cannulated with a second micropipette and tied with a suture. We previously showed that the ink-gelatin solution has no detectable detrimental effect on endothelial or vascular smooth muscle function (19).

**Instrumentation.** After cannulation of a blood vessel, the chamber was transferred to the stage of an inverted microscope (Diaphot 300, Nikon, Melville, NY) coupled to a charge-coupled device camera (model TM-34KC, Pulnix) and video micrometer (Cardiovascular Research Institute, Texas A&M University System Health Science Center). Internal diameters of the vessel were measured throughout the experiment using video microscopic techniques (21). The micropipettes were connected to independent reservoir systems, and intraluminal pressures were measured through sidearms of the two reservoir lines by low-volume-displacement strain-gauge transducers (Statham P23 Db, Gould, Cleveland, OH). The isolated vessels were pressurized without flow by setting both reservoirs at the same hydrostatic level. Leaks were detected by differences between reservoir pressure and intraluminal pressure. Preparations with leaks were excluded from further study.

**Experimental protocols.** Since the distribution of microvascular pressures is different in the subepicardial and subendocardial arterioles of the left ventricle (7), the present study compared the transmural vascular reactivity to adenosine and pinacidil at various pressures. The cannulated vessels were bathed in PSS-albumin solution at 36–37°C to allow development of basal tone at 20, 40, 60, or 80 cmH<sub>2</sub>O intraluminal pressure without flow for subepicardial and subendocardial arterioles. After vessels developed a stable basal tone (~40–60 min), the concentration-dependent vasodilations to adenosine (0.1 nM–10 μM), pinacidil (K<sub>ATP</sub> channel activator, 1 nM–1 μM), and sodium nitroprusside (endothelium-independent vasodilator, 1 nM–10 μM) were established at each of the four randomly selected intraluminal pressures.

The role of endothelium in the vasodilation to adenosine at different pressures was evaluated by comparing the response before and after endothelial removal. A nonionic detergent, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (0.4%), was perfused into the vessel for 1–2 min to remove endothelial cells (16). To ensure that the vascular smooth muscle function was not compromised by 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate treatment, concentration-dependent dilation of the vessel in response to sodium nitroprusside (1 nM–10 μM) was examined before and after denudation. Only vessels that exhibited normal basal tone, showed no vasodilation to the endothelium-dependent vasodilator bradykinin (1 nM) (16), and showed unaltered vasodilation to sodium nitroprusside after endothelial removal were accepted for data analysis. The selective inhibitor glibenclamide (0.1 μM) was used to evaluate the involvement of K<sub>ATP</sub> channels in the vasomotor responses of subendocardial or subepicardial microvessels at the various pressures. To further support the role of K<sub>ATP</sub> channels in altering adenosine responsiveness, coronary arteriolar dilation to adenosine was examined in the presence of a threshold concentration (0.1 μM) of the K<sub>ATP</sub> channel activator pinacidil.

**Chemicals.** Drugs were obtained from Sigma Chemical except as specifically stated. Adenosine and sodium nitroprusside were dissolved in PSS. Pinacidil was dissolved in alcohol, and glibenclamide was dissolved in DMSO. These drugs were then diluted in PSS to obtain the desired final concentration. The final concentrations of alcohol and DMSO in the tissue bath were 0.1 and 0.03%, respectively. Vehicle control studies indicated that these final concentrations of solvent had no effect on the arteriolar function.

**Data analysis.** At the end of each experiment, the vessel was relaxed with 100 μM sodium nitroprusside to obtain its maximal diameter at various intraluminal pressures described above. All diameter changes in response to agonists were normalized to the vasodilation in response to 100 μM nitroprusside and expressed as a percentage of maximal dilation. Values are means ± SE. Statistical comparisons of vasomotor responses under various treatments were performed with two-way ANOVA when appropriate and tested with Fisher’s protected least significant difference multiple range test. The EC<sub>50</sub> values (concentrations of adenosine or pinacidil that cause 50% of maximal dilation) were averaged from individual concentration-response curves. The pEC<sub>50</sub>
values were then calculated as the negative logarithm of mean values of EC_{50}. Differences in pEC_{50} values and in resting and maximal diameters at various pressures were detected with Student’s paired t-tests. Significance was accepted at P < 0.05.

RESULTS

Responsiveness of subepicardial and subendocardial arterioles to adenosine at various intraluminal pressures. After cannulation, subepicardial and subendocardial arterioles developed basal tone within 60 min at 20, 40, 60, or 80 cmH_{2}O intraluminal pressure and 37°C bath temperature. The basal tone developed by the isolated coronary arterioles is sensitive (i.e., loss of basal tone) to repeated stimulation with pharmacological agonists. Therefore, the effect of various pressures on adenosine responsiveness in the same vessel was basically studied only at two or three intraluminal pressures. However, five independent successful experiments were performed at all four pressures in the same vessels, and the resting and maximal diameters, as well as the vascular tone of these subepicardial and subendocardial arterioles, are presented in Table 1. The resting and maximal diameters and the vascular tone for both vessel types were not significantly different at the four intraluminal pressures. Although the resting diameter in the subepicardial vessels remained relatively constant as the pressure was reduced from 80 to 20 cmH_{2}O, the resting diameter in the subendocardial vessels consistently decreased at each pressure drop. Figure 1 shows that adenosine dilated the vessels in a concentration-dependent manner at all four pressures studied. Adenosine (10 \mu M) caused near-maximal dilation in all vessels; there was no significant difference in the maximal response among vessel type at various intraluminal pressures. The sensitivity of subepicardial (Epi) or subendocardial (Endo) microvessels to adenosine (Fig. 1) was similar at 80 cmH_{2}O [pEC_{50} = 6.30 ± 0.11 (Epi) and 6.39 ± 0.03 (Endo), n = 5] and 60 cmH_{2}O [pEC_{50} = 6.32 ± 0.04 (Epi) and 6.44 ± 0.11 (Endo), n = 5] luminal pressure, but it was significantly increased when the pressure was reduced to 40 cmH_{2}O [pEC_{50} = 7.45 ± 0.11 (Epi) and 7.14 ± 0.17 (Endo), n = 5] or 20 cmH_{2}O [pEC_{50} = 7.10 ± 0.14 (Epi) and 7.06 ± 0.20 (Endo), n = 5; Fig. 1]. Under the conditions of identical intraluminal pressure, there was no significant difference in the sensitivity of subepicardial and subendocardial arteriolar dilation in response to adenosine.

It is important to note that the coronary arteriolar pressure in the porcine heart has been reported to be 20 cmH_{2}O greater in the subepicardium than in the subendocardium (7). For example, in coronary arterioles of the same size, 20, 40, and 60 cmH_{2}O pressure in the subendocardium corresponds to 40, 60, and 80 cmH_{2}O in the subepicardium. Taking this pressure gradient into consideration, we plotted a ratio of Endo to Epi sensitivity to adenosine (i.e., ratio of pEC_{50} values) at various sets of intraluminal pressures (i.e., Endo/Epi = 60/80, 40/60, and 20/40 cmH_{2}O). Values >1.0 indicate a greater sensitivity of subendocardial arterioles to adenosine at various intraluminal pressures. After cannulation, subepicardial and subendocardial arterioles developed basal tone within 60 min

![Graph showing influence of intraluminal pressure on coronary arteriolar dilation to adenosine.](http://ajpheart.physiology.org/)

**Table 1.** Resting diameter, maximal diameter, and tone of porcine subepicardial and subendocardial arterioles at various intraluminal pressures

<table>
<thead>
<tr>
<th>Intraluminal Pressure, cmH_{2}O</th>
<th>Resting Diameter, ( \mu m )</th>
<th>Maximal Diameter, ( \mu m )</th>
<th>Tone, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subepicardium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>85 ± 5</td>
<td>137 ± 10</td>
<td>62 ± 2</td>
</tr>
<tr>
<td>60</td>
<td>84 ± 5</td>
<td>135 ± 10</td>
<td>62 ± 2</td>
</tr>
<tr>
<td>40</td>
<td>84 ± 6</td>
<td>133 ± 9</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>20</td>
<td>81 ± 6</td>
<td>123 ± 9</td>
<td>65 ± 2</td>
</tr>
<tr>
<td><strong>Subendocardium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>88 ± 10</td>
<td>134 ± 12</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>60</td>
<td>87 ± 10</td>
<td>132 ± 12</td>
<td>65 ± 2</td>
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<tr>
<td>40</td>
<td>84 ± 10</td>
<td>130 ± 11</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>20</td>
<td>77 ± 10</td>
<td>117 ± 11</td>
<td>65 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE derived from 5 vessels in which all 4 pressures were examined. Resting diameters were normalized by maximal diameters at their respective intraluminal pressures and expressed as tone.
vessels to adenosine; values <1.0 indicate a greater sensitivity of subepicardial vessels to adenosine. In Fig. 2, the adenosine pEC_{50} ratio at Endo/Epi pressure of 60/80 cmH_2O was 1.03. The adenosine pEC_{50} ratio increased significantly to 1.13 when the pressure was reduced to 40/60 cmH_2O. However, a further reduction in pressure to 20/40 cmH_2O decreased the pEC_{50} ratio to 0.95. In contrast to adenosine, the concentration-dependent dilations of subepicardial and subendocardial arterioles to serotonin (n = 5, data not shown) and to sodium nitroprusside (n = 5, data not shown) were identical and not affected by pressure variations.

**Role of endothelium in pressure-dependent dilation of coronary arterioles to adenosine.** To investigate whether the enhanced vasodilation to adenosine at lower intraluminal pressure (e.g., 40 cmH_2O) was dependent on an intact endothelium, arteriolar dilation to adenosine at 40 and 60 cmH_2O was examined after endothelial denudation. The resting vascular tone and the vasodilation to sodium nitroprusside were not altered by endothelial removal, but the vasodilation to bradykinin (1 nM) was abolished (i.e., 82 ± 3 and 2 ± 1% dilation before and after denudation, respectively). Dilation of these denuded subepicardial and subendocardial arterioles to adenosine was still significantly greater at 40 cmH_2O [pEC_{50} = 6.24 ± 0.14 (Epi) and 6.06 ± 0.11 (Endo), n = 5] than at 60 cmH_2O [pEC_{50} = 5.81 ± 0.11 (Epi) and 5.66 ± 0.08 (Endo), n = 5; Fig. 3].

**Role of smooth muscle K_ATP channels in enhanced dilation of coronary arterioles to adenosine.** Since we previously documented that coronary arteriolar dilation to adenosine is mediated by the activation of K_ATP channels (14, 18), it was speculated that the vasodilation mediated by this potassium channel may be also enhanced by the lower intraluminal pressures. To test this hypothesis, the dilation of subepicardial and subendocardial arterioles to the K_ATP channel activator pinacidil at 60 and 40 cmH_2O was evaluated. Figure 4 shows that pinacidil dilated the subepicardial (pEC_{50} = 6.35 ± 0.08, n = 5) and subendocardial (pEC_{50} = 6.24 ± 0.09, n = 5) vessels in a concentration-dependent manner at 60 cmH_2O intraluminal pressure. The dilations of these vessels to pinacidil were significantly increased when the pressure was reduced to 40 cmH_2O [pEC_{50} = 6.68 ± 0.04 (Epi) and 6.58 ± 0.11 (Endo), n = 5], suggesting that the K_ATP channel-mediated coronary arteriolar dilation is influenced by intraluminal pressure. To demonstrate the role of K_ATP channels in the enhanced vasodilation to pinacidil and adenosine at low intraluminal pressure, coronary arteriolar response to pinacidil and adenosine was examined in the presence of a subthreshold concentration of the K_ATP channel inhibitor glibenclamide (0.1 μM). This low concentration of glibenclamide did not affect vasodilations of subepicardial and subendocardial arterioles to pinacidil (n = 6 and 4 for Epi and Endo, respectively; Fig. 4) and adenosine (n = 5 for Epi and Endo; Fig. 5) at 60 cmH_2O intraluminal pressure, but
the enhanced vasodilations at 40 cmH\textsubscript{2}O luminal pressure were abolished. The role of K\textsubscript{ATP} channels in the enhancement of subepicardial arteriolar dilation to adenosine was further studied in the presence of a threshold concentration of pinacidil (0.1 \textmu M). Adenosine-induced dilation of subepicardial arterioles at 60 cmH\textsubscript{2}O intraluminal pressure was significantly enhanced by pinacidil, and this enhancement was eliminated by a subthreshold concentration of the K\textsubscript{ATP} channel inhibitor glibenclamide (\textit{n} = 5; Fig. 6). Incubation of the vessels with a subthreshold concentration of sodium nitroprusside (10 nM) did not potentiate vasodilation to adenosine (\textit{n} = 5; data not shown). Furthermore, pinacidil (0.1 \mu M) did not potentiate sodium nitroprusside-induced dilation of subepicardial arterioles (\textit{n} = 2; data not shown).

**DISCUSSION**

The present study demonstrates that there is no obvious difference in adenosine responsiveness between subepicardial and subendocardial arterioles under conditions with identical intraluminal pressure. However, the dilation of these arterioles to adenosine is potentiated at lower intraluminal pressures (e.g., 20 or 40 cmH\textsubscript{2}O compared with 60 or 80 cmH\textsubscript{2}O). Lowering intraluminal pressure also enhances the vascular dilation in response to the K\textsubscript{ATP} channel activator pinacidil. It appears that the enhanced vascular responsiveness to adenosine at lower pressure is a result of the increased activity of K\textsubscript{ATP} channels, because this enhancement can be mimicked in the presence of a threshold concentration of pinacidil and eliminated by a subthreshold concentration of the K\textsubscript{ATP} channel inhibitor glibenclamide. Furthermore, endothelial denudation does not affect this pressure-dependent vascular response, suggesting that activation of the smooth
vasodilation in the absence but not in the presence of the K\(_{\text{ATP}}\) channel inhibitor glibenclamide (0.1 \(\mu\)M). *\(P < 0.05\) vs. control.

Fig. 6. Effect of a threshold concentration of pinacidil on coronary arteriolar dilation to adenosine. Isolated coronary arterioles dilated to adenosine in a concentration-dependent manner. A threshold concentration of pinacidil (0.1 \(\mu\)M) enhanced the adenosine-induced vasodilation in the absence but not in the presence of the K\(_{\text{ATP}}\) channel inhibitor glibenclamide (0.1 \(\mu\)M). *\(P < 0.05\) vs. control.

muscle K\(_{\text{ATP}}\) channel is responsible for the observed phenomenon. Since coronary microvascular pressure is not uniformly distributed between the subepicardial and subendocardial myocardium, this pressure-dependent coronary microvascular response to adenosine might contribute to the redistribution of blood flow across the ventricular wall during metabolic stress resulting from coronary hypoperfusion. This implication is discussed below in reference to the previous findings in transmural difference of flow distribution and adenosine response in the coronary circulation.

In comparison to our findings, previous studies using the isolated porcine coronary microvessels have shown conflicting results in regard to subepicardial vs. subendocardial vasodilatory responses to adenosine. Piana et al. (27) reported no transmural differences in coronary microvascular dilation to adenosine (27). In contrast, Quillen and Harrison (28) demonstrated a greater adenosine response of subendocardial arterioles. These disparate results may be related to experimental protocols. For example, in the former study the vessels were pressurized at 40 mmHg (54 cmH\(_2\)O) and preconstricted with ACh (27); in the latter study the vessels were pressurized to 20 mmHg (~27 cmH\(_2\)O) and preconstricted with the thromboxane analog U-46619 (28). Our vasodilatory response to adenosine was similar to that reported by Piana et al., with a threshold concentration of 1 nM for subepicardial and subendocardial microvessels. In the vessels preconstricted with U-46619, the adenosine response curve was shifted to the right with a threshold concentration near 0.1 \(\mu\)M for both microvessel subtypes (28). Because vasoconstrictor agents have the potential to influence the adenosine response by activating and superimposing different contractile mechanisms (11, 18, 31), U-46619 may have a heterogeneous interference with the vasodilation to adenosine in this previous study (28). It is worth noting that the development of basal tone in our coronary arterioles precluded the use of preconstrictors and thus eliminated these potential confounding effects. Regardless of the preconstrictor issue, neither of these studies evaluated the adenosine responsiveness at different levels of intraluminal pressure. Thus, in our isolated-vessel preparation with the development of basal tone, it is clear that, under the same intraluminal pressure, subepicardial and subendocardial dilations to adenosine are not different.

Although there was no transmural difference in the sensitivity to adenosine at similar pressure levels in our present study, this may not be a physiologically relevant comparison, since microvascular pressures for subendocardial vessels have been reported to be consistently lower than those for subepicardial vessels in vivo (27). Taking this pressure distribution into account, it was found that when the microvessels were pressurized to the reported physiological levels, e.g., 60 cmH\(_2\)O for subepicardium and 40 cmH\(_2\)O for subendocardium (27), the sensitivity of subendocardial vessels to adenosine was greater than that of the subepicardial vessels (Fig. 2). This may explain the in vivo findings that the subendocardial arterioles are more responsive to adenosine than the subepicardial arterioles of identical size (32). It appears that the subendocardial arterioles are normally at the optimal pressure conditions for adenosine response due to the heterogeneous pressure distribution across the ventricular wall. Therefore, it is likely that the observed preferential increase in subendocardial flow by adenosine under normal perfusion (32) may be the result of greater responsiveness of subendocardial microvessels to adenosine at lower intraluminal pressure, as shown in the present study.

Interestingly, previous in vivo preparations in dogs have demonstrated that exogenous adenosine induces a greater blood flow in the subepicardium than in the subendocardium during hypoperfusion (i.e., reduction of coronary pressure to 40 mmHg) (1, 9, 12). However, the reason for this preferential flow distribution toward the subepicardium during hypoperfusion remains unclear. In the present study, using an isolated-vessel preparation, we were able to directly examine the response of subepicardial and subendocardial microvessels to adenosine at various pressures without the potential confounding influences of neurohumoral and local control mechanisms. As described above, the gradual decrease in luminal pressure across the ventricular wall (e.g., 60 cmH\(_2\)O at subepicardium and 40 cmH\(_2\)O at subendocardium) results in a greater microvascular response to adenosine in the subendocardium under resting conditions. However, a reduction in coronary perfusion pressure to 40 mmHg (i.e., ~54 cmH\(_2\)O), which leads to a decrease in subepicardial arteriolar pressure (e.g., from 60 to 40 cmH\(_2\)O), could potentiate the dilation of these microvessels to adenosine (Fig. 2). In contrast, the adenosine response in the subendocardial region remains unchanged, since a reduction of pressure from 40 to 20 cmH\(_2\)O did not alter adenosine responsiveness in these microvessels (Fig. 2). Therefore, a redistribution of blood flow to the
subepicardium by adenosine is likely to occur during moderate hypoperfusion.

In vivo study of the coronary circulation also showed that a reduction in coronary microvascular pressure, as a result of a proximal coronary stenosis, causes a redistribution of perfusion with a greater blood flow in the subepicardium than in the subendocardium (3, 9). It has been suggested that the variations in coronary flow reserve (6), increased wall stress (6), and reduced collateral flow (29) may be involved in the decreased subendocardial-to-subepicardial flow ratios. In addition to these potential mechanisms, our present findings suggest that the intrinsic variation of vascular responsiveness, in a pressure-dependent manner, across the ventricular wall may contribute to the redistribution of transmural flow during a reduction in coronary perfusion pressure. Because adenosine release may be enhanced during coronary ischemia and the subendocardial-to-subepicardial adenosine pEC20 ratio in these coronary microvessels is significantly decreased at lower coronary arteriolar pressure (Fig. 2), it is speculated that the greater intrinsic responsiveness of the subepicardial arterioles to adenosine may contribute to the redistribution of blood flow to this region.

The KATP channel opener pinacidil has also been shown to elicit redistribution of blood flow from the subendocardium to the subepicardium during coronary stenosis (2) or hypotension (10) in vivo. These findings implicate the involvement of KATP channels in the redistribution of transmural blood flow during hypoperfusion, but it is not known whether this flow response is also related to the pressure gradient across the ventricular wall. Consistent with the adenosine results, the pressure-dependent vascular responsiveness was also observed for pinacidil in the present study. Interestingly, the potentiation of vasodilation to adenosine by a lower pressure can be mimicked by pretreating the vessels with a threshold concentration (i.e., without altering basal tone) of pinacidil. This effect appeared to be specific for KATP channel activation, because a threshold concentration of sodium nitroprusside did not alter the dilation of these vessels to pinacidil or to adenosine, and pinacidil also did not enhance vasodilation to sodium nitroprusside. Furthermore, a subthreshold concentration of the KATP channel inhibitor glibenclamide (0.1 μM) abolished the potentiating effect of pinacidil and also the pressure-dependent vascular responsiveness to adenosine and to pinacidil. On the basis of these observations, it is suggested that the initial recruitment of KATP channels, by a threshold concentration of pinacidil or by lowering intraluminal pressure, can facilitate the subsequent activation of these channels for vasodilation. Since coronary arterioles exhibit myogenic responses (23), the alteration of KATP channels by luminal pressure may influence vascular tone and thus subsequently alter the vascular responsiveness to KATP channel agonists. However, as shown in Table 1, the resting vascular tone was not significantly altered by luminal pressure in subepicardial and subendocardial arterioles, and the inhibition of KATP channels affected neither the resting vascular diameter nor the pressure-diameter relationship of the microvessels (unpublished observations). It appears that the KATP channel activity is not involved in the determination of myogenic tone or myogenic response. Interestingly, it is worth noting that KATP channel activity in the vascular smooth muscle is inversely regulated by protein kinase C (5). This signaling molecule has been shown to be activated by increasing intraluminal pressure in the microvessels (15, 25). Therefore, the reduction of intraluminal pressure may facilitate the activation of KATP channels by reducing protein kinase C activity. This effect seems to be specific for the KATP channel agonists (i.e., adenosine or pinacidil), since lowering pressure did not affect vasodilation to the KATP channel-independent vasodilators serotonin and sodium nitroprusside. In addition, our results indicate that endothelial cells do not play a role in this activation, since the enhanced vasodilation at lower pressure was not affected by endothelial removal.

In conclusion, the present study provides evidence that coronary subepicardial and subendocardial arterioles exhibit identical vascular responsiveness to the KATP channel activators adenosine and pinacidil. However, by activating smooth muscle KATP channels, vascular dilation to these agonists is enhanced at lower intraluminal pressures. Since coronary microvascular pressure is not homogeneously distributed across the ventricular wall, the lower intraluminal pressure at the subepicardium would potentially recruit blood flow to this region by adenosine. However, during coronary arterial stenosis or occlusion, the release of adenosine associated with the reduction of arteriolar pressure in the subepicardium could redistribute blood flow in this area. Therefore, the inherent distribution of coronary microvascular pressure in the ventricular wall may be an important determinant of transmural flow in vivo during resting conditions or under metabolic stress.

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