Microvascular Sites and Mechanisms Responsible for Reactive Hyperemia in the Coronary Circulation of the Beating Canine Heart

Hiroshi Kanatsuka, Nobuyo Sekiguchi, Kouichi Sato, Kenjiro Akai, Yan Wang, Tatsuya Komaru, Kouichi Ashikawa, and Tamotsu Takishima

Our aim was to elucidate the site and mechanism responsible for reactive hyperemia in coronary circulation. In in vivo beating canine hearts, microvessels of the left anterior descending coronary artery (LAD) were observed through a microscope equipped with a floating objective. Flow velocity of the LAD was measured with a suction-type Doppler probe. The LAD was occluded for 20 or 30 seconds and then released, and reactive hyperemia was observed before and after 8-phenyltheophylline (7.5 mg/kg i.v.) or glibenclamide (200 μg/kg into the LAD) infusion. During the occlusion, only arterial microvessels smaller than 100 μm in diameter dilated. Dilation of those vessels was partially attenuated by 8-phenyltheophylline and completely abolished with glibenclamide. In the early phase of reactive hyperemia, all arterial microvessels dilated, and the magnitude of peak dilation was greater in vessels smaller than 100 μm compared with those larger than 100 μm. Vasodilation during reactive hyperemia ceased within 60 seconds in vessels smaller than 100 μm but was sustained for more than 120 seconds in those larger than 100 μm. 8-Phenyltheophylline did not change peak dilation of arterial microvessels but reduced dilation after the peak. Glibenclamide remarkably attenuated dilation of all arterial microvessels in the whole phase of reactive hyperemia. These results indicate that all arterial microvessels are responsible for reactive hyperemia after coronary artery occlusions of 20–30 seconds, but there is greater participation of vessels smaller than 100 μm in the early phase of reactive hyperemia. Dilation of vessels larger than 100 μm assumes an important role in the later phase. ATP-sensitive K+ channels mediate dilation of arterial microvessels both in brief ischemia and reactive hyperemia. (Circulation Research 1992;71:912–922)

KEY WORDS • coronary microcirculation • floating objective • ATP-sensitive K+ channel • adenosine

Reactive hyperemia is a ubiquitous phenomenon in many organs and in the coronary circulation.1–3 This phenomenon has been used to estimate coronary flow reserve in experimental4,5 and clinical6,7 studies. Nevertheless, the site and mechanism regulating reactive hyperemia are still unknown. We previously reported that the site responsible for autoregulation and active hyperemia differ in the coronary microcirculation.8,9 In those studies, only coronary arterial microvessels smaller than 100 μm in diameter dilated when perfusion pressure was reduced, and arterial microvessels smaller than 380 μm dilated when metabolic demand was increased. Because the site responsible for these two dominant local coronary controls differs, it is important to know the site responsible for reactive hyperemia to evaluate coronary flow reserve by using this phenomenon. In addition, several diseases, such as hypertension, atherosclerosis, and diabetes, are known to cause disorders of the specific mechanism of coronary regulation.10–13 Therefore, elucidation of the mechanism of reactive hyperemia will result in information important for the understanding of coronary regulation.

Accordingly, the purposes of this study were to clarify the site responsible for reactive hyperemia and to examine the role of two postulated mediators of coronary flow regulation, i.e., adenosine14,15 and ATP-sensitive K+ channels,16,17 in reactive hyperemia.

Materials and Methods

Microscope System and Image Analysis

Complete details of the microscope system with a floating objective have been reported elsewhere.18,19 Briefly, a floating objective consisting of a pair of convex lenses aligned on a common optical axis transmits to a fixed position a real image of epimyocardial microvessels in a beating left ventricle. The convex lens facing the heart can move in unison with the cardiac motion without touching the cardiac surface. The image of an object on the front focus of this lens is transmitted to the

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back focus of the second convex lens with parallel light. Consequently, changes in distance between these two convex lenses do not affect the position and magnification of the transmitted real image. The transmitted real image is then observed with a standard microscope. The convex lens facing the heart is mounted in a thin aluminum tube (floating lens) to reduce its total weight (16 g). The aluminum tube is supported by six low-resistance ball bearings and is suspended by a weight-adjusting coil spring.

The epimyocardium of the left ventricle (left anterior descending coronary artery [LAD] area) was transilluminated using an optical fiber and a xenon arc lamp. The optical fiber (0.6 mm in diameter), which was mounted in the lumen of a 20-gauge stainless-steel needle, was introduced into the midmyocardium. The needle was attached to a needle holder that allowed its tip to move up and down in unison with the cardiac motion. To adjust the focal distance between the heart and the floating lens, the floating lens was lifted just above the surface of the heart by an arm connected to the needle holder.

The microscope objective used for this study was Leitz model PL-fl (×10; N.A., 0.30). Images obtained with the microscope were stored on a magnetic tape with a high-speed video system (model MHS-200, Nac Inc., Tokyo) at 200 frames per second. The time of each frame was recorded on each video frame for correlation with hemodynamics. These stored images were then analyzed on a video monitor (model V-44, Nac) by using an X-Y coordinator (model MHS-200, Nac) and/or on printed images by a video-graphic printer (model UP-811, Sony, Tokyo). The sites of vessels where we measured the diameters were identified by using reference points such as the branching point of vessels.

**General Preparation**

Mongrel dogs (n=36) of either sex weighing 2.8–7.7 kg were sedated with ketamine (20 mg/kg i.m.) and then anesthetized with α-chloralose (50 mg/kg α-chloralose plus 50 mg/kg sodium borate i.v.). Additional doses, if necessary, were given to maintain anesthesia. Ventilation was controlled by a positive-pressure respirator (model NSH-34RH, Harvard Apparatus, South Natick, Mass.) at an end-expiratory pressure of 3–5 cm H2O. Arterial blood gases were maintained within the physiological range by adjusting the rate and volume of the respirator and/or using oxygen-enriched air. Body temperature was maintained at 37–38°C by means of a homeothermic blanket system. A balloon catheter was introduced into the right femoral vein for the infusion of drugs and fluids. A catheter was introduced into the aortic root via the right carotid artery and connected to a strain-gauge pressure transducer (model MPU 0.5, Toyo Sokki, Tokyo) to measure aortic pressure. A left thoracotomy was performed in the fifth intercostal space. The pericardium was opened, and the heart was suspended in a pericardial cradle. The sinus node was suppressed by a local injection of 10% buffered formaldehyde (0.3–0.5 ml), and the heart rate was then kept constant at 140 beats per minute throughout the experiments by atrial pacing. A snare was placed around the descending thoracic aorta, and the tip of the balloon catheter was placed in the inferior vena cava to control aortic pressure. The exposed cardiac surface was kept moist by a continuous drip of warm physiological solution containing (mM) NaCl 118.2, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, calcium disodium EDTA 0.026, and glucose 5.5, maintained at 37°C and pH 7.40. A 2–3-mm section of the proximal portion of the LAD was carefully dissected free, and a snare of monofilament nylon thread was placed around it. In 12 dogs, a 24-gauge cannula (0.67 mm o.d., Surflo, Terumo, Tokyo) was retrogradely inserted into the LAD by the direct puncture method20 for intracoronary drug infusion. The cannula was inserted at a site distal to the branching point of the artery that perfused the area of interest, and its tip was placed proximal to the branching point. To reduce excessive cardiac movement, two 24-gauge steel needles were inserted horizontally (5–7 mm apart) through the midmyocardium of the left ventricle. Both ends of each needle were fixed to a needle holder held with coil springs. This apparatus allowed the heart to move perpendicularly but limited excessive horizontal movement. The transilluminated area was thereby held in the microscopic field of view. Flow velocity of the

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**TABLE 1. Blood Gases, pH, and Aortic Pressure**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>8-Phenythiopephylene</th>
<th>Glibenclamide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>pH</td>
<td>7.42±0.01</td>
<td>7.41±0.03</td>
<td>7.43±0.02</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>93.2±3.2</td>
<td>89.0±4.0</td>
<td>98.9±6.3</td>
</tr>
<tr>
<td>PacO2 (mm Hg)</td>
<td>35.0±0.9</td>
<td>36.9±1.7</td>
<td>33.2±1.1</td>
</tr>
<tr>
<td>20-Second OCC</td>
<td>26</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
<td>115±2</td>
<td>119±3</td>
<td>119±4</td>
</tr>
<tr>
<td>DAP (mm Hg)</td>
<td>86±3</td>
<td>90±4</td>
<td>91±3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>81±5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>84±6</td>
</tr>
<tr>
<td>30-Second OCC</td>
<td>...</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
<td>...</td>
<td>114±3</td>
<td>117±3</td>
</tr>
<tr>
<td>DAP (mm Hg)</td>
<td>...</td>
<td>84±6</td>
<td>79±5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80±7</td>
<td>83±4</td>
</tr>
</tbody>
</table>

Before, before 8-phenythiopephylene or glibenclamide infusion; after, after 8-phenythiopephylene or glibenclamide infusion; OCC, occlusion of the left anterior descending coronary artery; SAP, systolic aortic pressure; DAP, diastolic aortic pressure. Values are mean±SEM.
LAD was measured with a 20-MHz small suction-type Doppler probe\(^2\) that was placed on the site distal to the snare and proximal to the area of interest and connected to a pulsed Doppler flowmeter (model 110, Triton Technology Inc., San Diego, Calif.). The positions of the Doppler probe and the range gate were adjusted to obtain the maximum frequency shift with the least noise. Aortic pressure and phasic and mean voltage output from the velocimeter were recorded on a rectigraph (model 8K, San-Ei Sokki, Tokyo).

**Experimental Protocol**

In three dogs, the LAD was occluded for 20 seconds and then released, and the reactive hyperemic response was observed.

In 16 dogs, the role of adenosine in reactive hyperemia was examined. In 11 dogs of this series, a reactive hyperemic response after 20 seconds of LAD occlusion was observed, and adenosine (1.0 mg/kg per minute i.v., Sigma Chemical Co., St. Louis, Mo.) was then infused for approximately 5 minutes until hemodynamic param-

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**FIGURE 1.** Bar graphs showing effect of 8-phenyltheophylline (8-PT) or glibenclamide (GC) on basal flow or on the excess flow caused by adenosine (AD), dipyridamole (DP), or BRL38227 (BRL). RH, reactive hyperemia. Left anterior descending coronary artery (LAD) flow was expressed as the percent change of control. Panel a: 8-PT (7.5 mg/kg i.v.) did not change basal flow but clearly attenuated the effect of AD (1.0 mg/kg per minute i.v.) and DP (1.0 mg/kg i.v.). Panel b: GC (200 μg/kg into the LAD) decreased basal flow by 14%, but the excess flow caused by AD was not attenuated at the end of the experiment. GC significantly attenuated the excess flow caused by BRL (0.1 μg/kg per minute into the LAD).

**FIGURE 2.** Tracings showing phasic aortic pressure and coronary flow during left anterior descending coronary artery occlusion and reperfusion before (control) and after the infusion of 8-phenyltheophylline. 8-Phenyltheophylline did not alter the peak flow but reduced the duration of reactive hyperemia.
etters stabilized. After these procedures, 8-phenyltheophylline (7.5 mg/kg i.v., Sigma) was administered, and the reactive hyperemic response was again observed. Before (n=8) and/or after (n=10) reactive hyperemia, adenosine was also infused to confirm the effect of 8-phenyltheophylline. In three dogs, dipyridamole (1.0 mg/kg i.v.) was infused at the end of the experiments. In the remaining five dogs, the reactive hyperemic response after 30 seconds of LAD occlusion was observed before and after the administration of 8-phenyltheophylline. Between each procedure, 10 minutes was allowed for hemodynamic parameters to return to the control values.

In 17 dogs, the role of ATP-sensitive K+ channels in reactive hyperemia was examined. In 12 dogs of this series, the reactive hyperemic response after 20 seconds of LAD occlusion was observed, and then adenosine (1.0 mg/kg per minute i.v., n=7) was infused. Ten minutes after stopping adenosine infusion, or after the release of the snare, glibenclamide (200 μg/kg, Sigma) was slowly infused into the LAD. Four minutes after the glibenclamide infusion, the LAD was occluded for 20 seconds and then released, and the reactive hyperemic response was again observed. In seven dogs, adenosine (1.0 mg/kg per minute i.v.) was infused at the end of the experiments (approximately 25 minutes after the infusion of glibenclamide). In five dogs, plasma glucose was measured before and 10 minutes after the infusion of glibenclamide. In the remaining five dogs, the reactive hyperemic response after 30 seconds of LAD occlusion was observed before and after the administration of glibenclamide.

An additional 20 dogs underwent the same operation, and the potency of glibenclamide as an ATP-sensitive K+ channel blocker was examined using BRL38227 (Smith Kline Beecham). BRL38227 is the (−) enantiomer of the racemate (cromakalim) and has a pharmacological potency 100–200 times greater than the (+) enantiomer. Flow velocity of the LAD was measured during the infusion of BRL38227 (0.1 μg/kg per minute into the LAD) with (n=8) or without (n=12) the pretreatment of glibenclamide (200 μg/kg into the LAD).

**Figure 3.** Left anterior descending coronary artery (LAD) flow and diameter change of coronary arterial microvessels during 20 seconds of ischemia and reactive hyperemia (RH). ID, internal diameter. Vessels were divided into three groups: small (<100 μm), medium (100–200 μm), and large (>200 μm), according to control diameters. LAD flow and diameters were expressed as the percent change of each value before 20 seconds of flow arrest, respectively. Panel a: Graph shows that LAD flow during RH was 407±26% of control at the peak and was sustained for more than 120 seconds. Panel b: Bar graphs show that, during 20 seconds of ischemia, diameters of all vessels were initially reduced, and then only the small vessels dilated. After release of the snare, all vessels dilated, and the magnitude of peak dilation was greater in small vessels than in others. Diameters of small vessels returned to the control value within 60 seconds, but the dilation of medium and large vessels was sustained for more than 120 seconds.
FIGURE 4. Images of arterial microvessels on a monitor screen at control (top panel) and 6 seconds after the release of the occluder (bottom panel). Left anterior descending coronary artery occlusion was started at 5 seconds and released at 25 seconds, according to the timer on the monitor screen. An arteriole and a small artery clearly dilated after the release of the occluder.

Data Analysis

For the comparison of paired samples in each group, Student's t test for paired samples was used when appropriate. For multiple comparison of data, analysis of variance was used to assess the statistical significance. When significant values were obtained, an unpaired t test (corrected for multiple comparisons with the Bonferroni inequality adjustment) was used to determine which measurements differed significantly from one another. All data are expressed as mean±SEM, and p<0.05 was used as the probability level for statistical significance.

Results

Blood Gases, Hemodynamics, and Plasma Glucose

Blood gases and pH were maintained within physiological ranges during the experiments (Table 1). Aortic pressure in each procedure was within the physiological range at control and was held nearly constant throughout the experiment (Table 1). Plasma glucose was 96.6±15.6 and 75.4±12.2 mg/dl before and after glibenclamide infusion, respectively (n=5, p<0.05).

Potency of 8-Phenyltheophylline as Adenosine Receptor Blocker and Glibenclamide as ATP-Sensitive K" Channel Blocker

Adenosine increased the LAD flow by 267±40% and 248±29% before 8-phenyltheophylline and glibenclamide infusion, respectively (Figure 1). Ten minutes after the infusion of 8-phenyltheophylline, LAD flow returned to almost the control value, and the effect of adenosine on LAD flow was significantly attenuated. Dipyridamole sufficient to cause submaximal vasodilation slightly increased LAD flow in the presence of 8-phenyltheophylline (Figure 1a). Four minutes after the administration of glibenclamide, LAD flow reached
a stable value that was less than the control value. Adenosine similarly increased LAD flow before and approximately 25 minutes after the administration of glibenclamide. Glibenclamide significantly attenuated the effect of BRL38227 on LAD flow (Figure 1b).

Responses of LAD Flow and Coronary Arterial Microvessels During 20 Seconds of LAD Occlusion and Reactive Hyperemia

During reactive hyperemia after 20 seconds of LAD occlusion, peak LAD flow was observed approximately 5 seconds after the release of the snare (Figure 2). LAD flow then gradually declined but was sustained for more than 120 seconds (Figure 3a). Coronary arterial microvessels of all dogs (n=26) that received 20 seconds of LAD occlusion were divided into three sizes according to control diameters: small (67±4 μm in diameter; range, 38–98 μm; n=22 vessels), medium (133±7 μm in diameter; range, 100–199 μm; n=16 vessels), and large (250±13 μm in diameter; range, 205–313 μm; n=8 vessels). Diameters of all vessels decreased 4 seconds after the start of occlusion, and 18 seconds after the start of occlusion, only the small vessels dilated (Figure 3b). When the snare was released, all vessels quickly dilated, and peak dilation was observed 3–7 seconds later (Figure 4). The magnitude of peak dilation was greater in the small vessels than in the others. Diameters of small vessels returned to the control value within 60 seconds, but dilation of medium and large vessels was sustained for more than 120 seconds (Figure 3b).

Effect of 8-Phenyltheophylline on Responses of LAD Flow and Coronary Arterial Microvessels During 20 or 30 Seconds of LAD Occlusion and Reactive Hyperemia

During reactive hyperemia, 8-phenyltheophylline did not change peak LAD flow in either of the groups that received 20 or 30 seconds of LAD occlusion but did reduce LAD flow at 15 and 30 seconds in the group that received 20 seconds of LAD occlusion and at 15, 30, and 60 seconds in the group that received 30 seconds of LAD occlusion (Figures 2, 5a, and 6a). Because re-
sponses of medium and large arterial microvessels were similar, the vessels were divided into two sizes: small and medium+large, i.e., small (68±5 μm in diameter; range, 50–89 μm; n=9 vessels) and medium+large (158±18 μm in diameter; range, 100–275 μm; n=11 vessels) in the group that received 20 seconds of LAD occlusion (n=11 dogs) and small (65±8 μm in diameter; range, 41–90 μm; n=6 vessels) and medium+large (177±30 μm in diameter; range, 118–256 μm; n=4 vessels) in the group that received 30 seconds of LAD occlusion (n=5 dogs). During LAD occlusion, 8-phenyltheophylline attenuated the dilation of small vessels at 18 seconds in both groups but failed to attenuate it at 28 seconds in the group that received 30 seconds of LAD occlusion (Figures 5b and 6b). During reactive hyperemia, 8-phenyltheophylline did not change the peak dilation of vessels of any size in either group. Between 15 and 60 seconds after release of the snare, 8-phenyltheophylline attenuated the dilation of small vessels (at 15 and 30 seconds) and of medium+large vessels (at 30 and 60 seconds) in both groups (Figures 5b and 6b).

**Effect of Glibenclamide on Responses of LAD Flow and Coronary Arterial Microvessels During 20 or 30 Seconds of LAD Occlusion and Reactive Hyperemia**

During reactive hyperemia, glibenclamide did not change peak LAD flow but clearly attenuated LAD flow at 15, 30, 60, and 120 seconds in both groups that received 20 or 30 seconds of LAD occlusion (Figures 7a and 8a). Arterial microvessels were also divided into two sizes: small and medium+large, i.e., small (62±6 μm in diameter; range, 38–84 μm; n=8 vessels) and medium+large (180±20 μm in diameter; range, 106–313 μm; n=12 vessels) in the group that received 20 seconds of LAD occlusion (n=12 dogs) and small (71±6 μm in diameter; range, 45–87 μm; n=7 vessels) and medium+large (208±39 μm in diameter; range, 142–400 μm; n=6 vessels) in the group that received 30 seconds of LAD occlusion (n=5 dogs). In both groups, glibenclamide almost abolished the dilation of small vessels during LAD occlusion and significantly attenuated peak dilation of vessels of all sizes during reactive hyperemia (Figures 7b and 8b). At 15, 30, and 60

**FIGURE 6.** Effect of 8-phenyltheophylline (8-PT) on responses of left anterior descending coronary artery (LAD) flow and coronary arterial microvessels during 30 seconds of ischemia and reactive hyperemia (RH). ID, internal diameter. Vessels were divided as described in the legend of Figure 5. LAD flow and diameters were expressed as described in the legend of Figure 3. Panel a: Graph shows that 8-PT did not change peak flow but significantly reduced repayment area. Panel b: Bar graphs show that 8-PT attenuated the ischemic vasodilation at 18 seconds but did not alter it at 28 seconds and that 8-PT did not affect peak dilation during RH in vessels of any size. The effects of 8-PT on LAD flow and response of vessels were similar to the vessels that received 20 seconds of ischemia, except for the magnitude of reduction in repayment area.
seconds during reactive hyperemia, dilation of vessels of all sizes was significantly attenuated or almost abolished in the presence of glibenclamide in both groups (Figures 7b and 8b).

**Discussion**

This study further clarified the sites and the mechanisms responsible for reactive hyperemia in the coronary circulation. Accordingly, there are several important observations. Within 20–30 seconds of flow arrest, arterial microvessels smaller than 100 μm in diameter dilate. The dilation is partially attenuated by 8-phenyltheophylline in the early phase and almost abolished by glibenclamide in the whole phase. During reactive hyperemia, arterial microvessels of all sizes dilate, but greater peak dilation occurs in small vessels (<100 μm) than in medium+large vessels (>100 μm). The duration of dilation is longer in medium+large vessels than in small vessels. 8-Phenyltheophylline does not change peak dilation but attenuates the dilation after the peak. Glibenclamide strikingly suppresses the dilation of all arterial microvessels in the whole phase of reactive hyperemia.

**Critique of Methods**

In the present study, a floating objective was used for the observation of the coronary microcirculation. In this method, mechanical factors that may affect the coronary microcirculation were eliminated to the extent possible. However, the light-conducting needle inserted into the myocardium may cause microtrauma and a change in intramyocardial pressure. However, other investigators have suggested that this point is of little importance. Previous reports from our laboratory have also demonstrated that the responses of microvessels to dilazep, which potentiates endogenous adenosine, to nicorandil, which opens the ATP-sensitive K+ channel, and to other compounds are well preserved in this preparation. A validation study for the effect of an intracoronary cannula was previously reported, suggesting that it does not affect the perfusion pressure in the area of interest.

In several protocols,
the effect of the duration of ischemia on the following reactive hyperemia was examined. To make the duration of LAD occlusion discrete, monofilament nylon thread was used. Because this material has elastic recoil, quick release of the occlusion was possible. Finally, it is important to recognize that the current study examined mechanisms of reactive hyperemia after coronary artery occlusions of 20–30 seconds. It is possible that other mechanisms may be involved with reactive hyperemia after shorter or longer coronary artery occlusions.

**Potency of 8-Pheny theophylline as Adenosine Receptor Blocker and Glibenclamide as ATP-Sensitive K⁺ Channel Blocker**

Methylxanthines are competitive adenosine receptor blockers, and they also have phosphodiesterase-blocking properties. Consequently, they increase heart rate and cardiac contractility, thereby increasing coronary flow. In the present study, 8-phenyleth-ophylline was used to block adenosine receptors. This compound is a potent blocker of the adenosine recep-

**FIGURE 8.** Effect of glibenclamide (GC) on responses of left anterior descending coronary artery (LAD) flow and coronary arterial microvessels during 30 seconds of ischemia and reactive hyperemia (RH). ID, internal diameter. Vessels were divided as described in the legend of Figure 5. LAD flow and diameters were expressed as described in the legend of Figure 3. Panel a: Graph shows that LAD flow during RH was not altered at the peak but was strikingly reduced at 15, 30, 60, and 120 seconds by GC. Panel b: Bar graphs show that GC abolished the ischemic vasodilatation of small vessels at 18 and 28 seconds and that the effect of GC on LAD flow and responses of vessels during RH was almost similar to the vessels that received 20 seconds of ischemia.
stimuli. The response of coronary flow to adenosine was similar before and after glibenclamide infusion, suggesting that there was no fixed disorder of the relaxation mechanism in the vascular smooth muscle. However, this result does not preclude the possibility that adenosine can open ATP-sensitive K⁺ channels.17 Because, in the present study, glibenclamide was infused as a bolus into the LAD and adenosine was examined at the end of the experiment (25 minutes later), the effect of glibenclamide may have been reduced when adenosine was infused.

**Role of Adenosine and ATP-Sensitive K⁺ Channels in the Responses of Coronary Arterial Microvessels to Brief Ischemia**

During LAD occlusion, arterial microvessels smaller than 100 μm in diameter dilated within 18 seconds. Glibenclamide almost completely abolished the dilation at 18 and 28 seconds, and 8-phenyltheophylline partially attenuated the dilation at only 18 seconds. These results suggest that the dilation of small vessels during ischemia was mostly mediated by ATP-sensitive K⁺ channels and that adenosine may dilate those vessels in part via ATP-sensitive K⁺ channels only at the early phase. It is uncertain whether the opening of ATP-sensitive K⁺ channels is regulated by the reduced ATP levels in smooth muscle. It has been reported that the ATP level of the myocardium, which has high energy consumption, did not change during 24 seconds of coronary occlusion.34 Therefore, the ATP level in smooth muscle may not decrease during 20–30 seconds of ischemia. One possible explanation could be the existence of compartmentalized pools of intracellular ATP governed by anaerobic metabolism—using key glycolytic enzymes.35 Another possibility is that the opening probability of ATP-sensitive K⁺ channels may relate to the change in distending pressure. In isolated coronary arterial microvessels, potent myogenic response has been observed in vessels smaller than 100 μm in diameter but was absent in vessels larger than 200 μm.36 Therefore, it is conceivable that the dilation of small vessels relates to the lowered transmural pressure. In addition, a proportional depolarization of vascular smooth muscle to increased transmural pressure has been observed in the small renal arteries.38

**Responses of Coronary Arterial Microvessels During Reactive Hyperemia and the Role of Adenosine and ATP-Sensitive K⁺ Channels**

The present study indicates that dilation of vessels of all sizes is responsible for reactive hyperemia. Dilation of small vessels assumes a more important role in the earlier phase of reactive hyperemia, and dilation of large vessels assumes an important role in the late phase. In both groups that received 20 and 30 seconds of ischemia, 8-phenyltheophylline did not alter the flow and vasodilation at the peak but attenuated both at 15, 30, and 60 seconds. As shown in Figures 5 and 6, reduction in the repayment area by 8-phenyltheophylline was greater in the group that received 30 seconds of ischemia. These results were consistent with previous reports.9,40 When ATP-sensitive K⁺ channels were blocked with glibenclamide, dilation of arterial mi-

crovessels was strikingly suppressed throughout the whole phase of reactive hyperemia. Coronary flow was also significantly attenuated except for the peak flow. The discrepancy between flow and diameter change at the peak may be explained by the effect of coronary capacitance; i.e., coronary flow just after release of the snare may be caused by the blood that fills the collapsed vessels. Such an effect of capacitance may also explain why aminophylline failed to suppress the repayment area in reactive hyperemia after very short ischemia.41 The increased blood flow after very short ischemia may be caused by the change in capacitance and not by vasodilation. Present data suggest that peak vasodilation was not mediated by adenosine but mostly by ATP-sensitive K⁺ channels. ATP-sensitive K⁺ channels govern most responses of coronary arterial microvessels throughout the whole phase of reactive hyperemia, and adenosine also plays some role in the phase following the peak of reactive hyperemia. The present data may also explain why hypercapnic acidosis enhances the reactive hyperemic response after long periods of ischemia,42 because a decrease in intracellular pH reduces the inhibitory effect of ATP on ATP-sensitive K⁺ channels in the skeletal muscle.43 Changes in intracellular pH may also have a similar effect on these channels in the coronary circulation.

**References**


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