Beating myocardium counteracts myogenic tone of coronary microvessels:
involvement of ATP-sensitive potassium channels

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Abstract

Beating myocardium counteracts myogenic tone of coronary microvessels: involvement of ATP-sensitive potassium channels. Am J Physiol Heart Circ Physiol 291: H3050–H3057, 2006. First published July 21, 2006; doi:10.1152/ajpheart.00039.2006.—Myogenic tone is intrinsic to vascular tissue and plays an important role in determining basal coronary resistance. However, the effect of the beating heart on myogenic tone is unknown. We investigated the effects of myocardium-derived vasoactive factors on the myogenic tone of coronary microvessels in the resting condition and during increased metabolism. Pressurized isolated coronary vessels (detector vessel, DV) of rabbits (n = 33, maximal inner diameter 201 ± 8 μm) were gently placed on beating hearts of anesthetized dogs and observed with an intravitral microscope equipped with a floating objective. To shut off the myocardium-derived vasoactive signals, we placed plastic film between DV and the heart. The intravascular pressure was changed from 120 to 60 cmH2O, and pressure-diameter curves were obtained with and without the contact of DV and the myocardium. The direct contact shifted the pressure-diameter curve upward (P < 0.05 vs. without contact), and myogenic tone was reduced by ~40%. When endothelium of DV was denuded, the shift persisted, but the degree of shift was reduced to 10% (P < 0.05 vs. with endothelium). The shift was abolished by glibenclamide, an ATP-sensitive potassium (KATP) channel blocker. A similar upward shift was induced by rapid pacing, but the shift was not blocked by glibenclamide. We conclude that the beating myocardium counteracts myogenic tone by releasing transferable vasoactive signals that affect the endothelium and the vascular smooth muscle, and that the signals are solely mediated by the activation of KATP channels, unlike the rapid pacing-induced vasoactive factors.

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MATERIALS AND METHODS

In the present study, we hypothesized that the beating heart affects the myogenic coronary vascular tone by releasing transferable vasoactive factors during a normal perfusion state. To test the hypothesis, we used a bioassay method that we developed to evaluate the myocardium-derived vasoactive factors. This system enables us to separately control the metabolic state of the beating heart and the condition of the coronary microvessels and to minimize the effect of other confounding vasoactive factors (30, 31, 33). We also investigated the involvement of ATP-sensitive potassium (KATP) channels, because these channels are considered to couple the cell metabolism to the electrical activity of the vascular tissue, thereby affecting the coronary vascular tone (8).

Preparation of Detector Vessels

Male Japanese White rabbits (n = 33, age 10–13 wk, body weight 2.19 ± 0.03 kg) were anesthetized with pentobarbital sodium (50 mg/kg iv) and heparinized (1,000 units iv). After the rabbits were killed by bleeding from the carotid artery, the hearts were excised and immediately immersed in chilled Krebs solution (in mmol/l: 120.0 NaCl, 4.7 KCl, 25.0 NaHCO3, 2.0 CaCl2, 1.17 MgSO4, 1.2 NaH2PO4, 0.02 calcium disodium EDTA, and 5.0 glucose, bubbled with 95% O2 and 5% CO2).

From the ventricles of the excised hearts, coronary microvessels (n = 33, maximal inner diameter 201 ± 8 μm, 91–296 μm) were carefully isolated and used as detector vessels in the bioassay. One
end of the vessel was cannulated to a polyethylene micropipette filled with filtered Krebs solution, the tip of which was tapered (~120 μm) and tied with a monofilament of silk suture thread (diameter ~10 μm). The micropipette was connected to a pressure reservoir. The other end of the vessel was ligated completely, and the intravascular pressure was monitored with a pressure transducer (Uniflow; Baxter, Deerfield, IL) to detect vascular leaks. Vessels with any leakage were not used for further experiments. The detector vessel was incubated in warm Krebs solution (38°C) until use.

In nine experiments, the endothelium of the detector vessel was removed as previously described (16). Briefly, 2 ml of air were carefully injected into the lumen of the vessel via the micropipette, and the vessels were slowly perfused with Krebs solution at 40 cmH2O for 10 min to clear the debris before the ligation.

**Beating Heart Preparation**

Mongrel or beagle dogs of either sex (n = 33, body weight 5.8 ± 0.3 kg) were used and prepared as described in previous studies in our laboratory (1, 31). Briefly, the dogs were anesthetized with ketamine (10 mg/kg sc) and α-chloralose (60 mg/kg iv; Wako, Osaka, Japan) and artificially ventilated. Thereafter, the heart surface was exposed through thoracotomy and pericardiotomy. The heart rate was kept constant at 120 beats/min by left atrial pacing after sinoatrial block with formaldehyde injection (~0.5 ml). The right jugular vein and the carotid artery were cannulated for fluid infusion and for aortic pressure monitoring, respectively.

Two 16-gauge stainless steel needles were inserted horizontally (5–7 mm apart) into the midmyocardium of the left ventricle. Both ends of each needle were fixed to a needle holder to limit the excessive cardiac motion and to keep the region of interest within the observation field.

**Bioassay Method**

The incubated detector vessel was gently placed on the beating left ventricle of the dog. Only one detector vessel was used for each experiment. The detector vessel was pressurized to 60 cmH2O and was kept moist throughout the experiment by continuous superfusion of warmed Krebs solution (38°C) at a rate of 1.67 ml/min.

For visualization of the detector vessel, an intravital microscope equipped with a floating objective was used. This optical system was originally developed for visualization of coronary microvessels of the beating heart in vivo in our laboratory (1).

The microscope objective was a Leitz model PL-8 (×10; numeric aperture 0.30), and the spatial resolution of the optical system was 2 μm. An objective lifter was used to carefully adjust the distance between the objective and the vessel so as not to compress the detector vessel. Epi-illumination with a mercury lamp was applied to obtain the images of the detector vessels with a charge-coupled device (CCD) video camera (HV-D28S; Hitachi Kokusai Electric, Tokyo, Japan). The vascular images were recorded on a digital videocassette recorder (DSR-20; Sony, Tokyo, Japan). After the experiments, the vascular images were captured in a personal computer. The inner diameters of the detector vessels at end-diastolic phase were measured three times using Scion Image beta 4.0.2 (Scion, Frederick, MD). To evaluate the diameter changes following each intervention, we measured the diameters at the same point by using the polyethylene micropipette as a reference point.

**In Vitro Experiments**

To examine the myogenic response per se, we performed in vitro experiments as previously described (32). Briefly, coronary arterial microvessels were isolated from rabbit hearts and cannulated with dual glass micropipettes in a vessel chamber (CH/2/M; Living Systems Instrumentation, Burlington, VT) containing Krebs solution. They were pressurized to 60 cmH2O without flow throughout the experiment and bathed at 38°C by the continuous circulation of warmed Krebs solution through the vessel chamber. Vessels with any leaks were not used for further experiments. The microvessel chamber was transferred to the stage of an inverted microscope (DIAPHOT TM-O; Nikon) equipped with the CCD camera. The images of the vessels were captured on a personal computer monitor, and the inner diameters were measured.

**Experimental Protocols**

Experiments were performed ~60 min after the detector vessels were placed on the beating hearts, when all monitored variables had become stable and the intrinsic tone of the detector vessels had been established.

**Protocol 1. Vehicle Group.** The effects of the vasoactive factors derived from the beating heart on the myogenic tone in the resting condition were investigated (n = 9). The intravascular pressure was sequentially decreased from 120 to 60 cmH2O in a stepwise fashion (120, 100, 80, and 60 cmH2O) by changing the water level of the pressure reservoir. At 5 min after each pressure change, the detector vessel diameter was measured and the intravascular pressure-diameter relationship was established. The measurements were sequentially repeated with and without direct contact of the detector vessel with the beating heart. To shut off the direct contact, we placed a plastic film between the vessel and the heart. The order of the absence and presence of the plastic film was randomly assigned. At the end of each experiment, sodium nitroprusside (100 μmol/l; Wako) was superfused and the intravascular pressure was again changed.

**Glibenclamide Group.** In eight experiments, the involvement of K_{ATP} channels was investigated. Glibenclamide (5 μmol/l; Wako), which potently blocks K_{ATP} channels by binding to their sulfonylurea receptors, was superfused onto the detector vessel, and the diameter changes were observed in the same manner as described above. In this experiment, the diameters in the presence of the plastic film were measured first, because we had confirmed that the order of the experiments did not affect the results in the above-described protocol.

**Endothelial Denudation Group.** In nine experiments, the involvement of endothelium was investigated. The endothelium of the detector vessel was denuded as mentioned above, and the same protocol used for the vehicle group was performed.

**Protocol 2.** The effect of rapid pacing on the myogenic tone was investigated (n = 5). The intravascular pressure-diameter relationship was obtained at the canine heart rates of 120 and 240 beats/min. The intravascular pressure changes were the same as for protocol 1. Glibenclamide (5 μmol/l; Wako), which potently blocks K_{ATP} channels by binding to their sulfonylurea receptors, was superfused onto the detector vessel to investigate the involvement of K_{ATP} channels (n = 5). In three cases, the experiments of protocol 2 were conducted sequentially after those of protocol 1.

**In vitro experiment.** After the inner diameters of the isolated microvessels were reduced by 20–50% by the development of spontaneous tone in the vessel chamber, intravascular pressure was changed in a stepwise manner (120, 100, 80, and 60 cmH2O; 10 min for each pressure). After the pressure-diameter curve was obtained, 5 μmol/l glibenclamide was applied to the vessel chamber, and the intravascular pressure was again sequentially changed. At the end of experiments, 100 μmol/l nitroprusside was applied to cause the maximal dilation.

**Drugs**

Glibenclamide was dissolved with DMSO and then diluted with Krebs solution to the target concentration. The final concentration of DMSO was 0.005 vol%. Nitroprusside was freshly dissolved in Krebs solution.

**Data Analysis**

All variables are expressed as means ± SE. The inner diameters of the detector vessels are expressed as the relative diameters normalized...
to the maximal diameter (100 μmol/l nitroprusside, intravascular pressure 60 cmH2O).

Myogenic tone was defined as percent reduction in diameter produced by the development of the intrinsic tone from the maximal diameter. Myogenic responses are expressed as the myogenic index, calculated as $100 \times [(D_i - D_{i-1})/(P_{i-1} - P_i)]$, where the subscripts $i$ and $f$ refer to the initial and final values for vascular diameter ($D$) and intravascular pressure ($P$).

The paired samples for blood gas analysis, hemodynamic variables, and detector vessel diameters and responses were statistically analyzed using Student’s $t$-test for paired data when appropriate. The pressure-dependent changes of the detector vessel diameters were analyzed using one-way analysis of variance for repeated measures, and Dunnett’s multiple comparison test was performed to detect which pressure changes produced significant diameter changes. The effects of the myocardium-derived metabolic factors on the pressure-diameter curves were statistically analyzed using two-way analysis of variance for repeated measures. Regression analysis was performed to statistically analyze the correlation between the vascular size and the myogenic tone or the myogenic responses. All of the statistical analyses were performed using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, CA). At $P < 0.05$, the differences were accepted as significant.

RESULTS

Myocardium-Derived Vasodilator Signals at Rest

The hemodynamic data and blood gas analysis of the dogs during protocol 1 are shown in Table 1. There were no significant changes in canine blood pressure throughout the experiments, and the arterial blood pH and blood gas data were kept within physiological ranges.

Representative images of the detector vessels in protocol 1 are shown in Fig. 1. When intravascular pressure was applied to the detector vessel right after its placement on the beating heart, the vessel passively dilated (Fig. 1A). Thereafter, the vessel gradually contracted and the intrinsic tone was established (Fig. 1B). When direct contact of the detector vessel with beating heart was shut off with a plastic film, the microvessel contracted further (Fig. 1C). In response to nitroprusside, the vessel dilated (Fig. 1D).

The group data of protocol 1 are shown in Fig. 2. The detector vessel constricted to ~75% of the maximal diameter when the detector vessel was not in contact with the heart (Fig. 2A). When the detector vessel was in contact with the beating heart, the pressure-diameter curve was shifted upward (Fig. 2A, $P < 0.05$ for groups, $P < 0.05$ for intravascular pressure, $P = $ NS for interaction). However, the diameters were still smaller than the maximal diameters at every pressure level, which means that the myogenic tone still remained during the contact.

In the presence of glibenclamide, the pressure-diameter curves with and without the vessel-myocardium contact were identical (Fig. 2B, $P =$ NS for groups, $P < 0.01$ for intravascular pressure, $P =$ NS for interaction). Superfusion of glibenclamide did not change the diameters of the detector vessels (before superfusion, 159 ± 7 μm; after superfusion, 158 ± 7 μm). When the endothelium of the detector vessel was denuded, the upward shift of the pressure-diameter curve by the myocardial contact persisted ($P < 0.05$ for groups, $P < 0.01$ for intravascular pressure, $P =$ NS for interaction), but the magnitude of the shift was significantly reduced compared with the vehicle group (Fig. 2, C and D).

The myogenic vascular tone of the detector vessel (intravascular pressure 60 cmH2O, without myocardial contact) was variable but significantly correlated with the vessel sizes (Fig. 3A). The myogenic index, which represents the magnitude of myogenic responses, was slightly but significantly correlated with the vessel sizes (Fig. 3B). There were no statistical differences in the detector vessel sizes among the three groups (vehicle group: $n = 9$, 179 ± 18 μm; glibenclamide group: $n = 8$, 195 ± 15 μm; endothelial denudation group: $n = 9$, 207 ± 12 μm).

The results of the in vitro experiments in the vessel chamber are shown in Fig. 3C. The pressure-diameter curves in the presence and absence of the glibenclamide were almost iden-

Table 1. Hemodynamic data and blood gas analysis of dogs

<table>
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<tr>
<th></th>
<th>Systolic BP, mmHg</th>
<th>Diastolic BP, mmHg</th>
<th>Mean BP, mmHg</th>
<th>Double Product, $\times 10^2$ mmHg/min</th>
<th>Arterial Blood pH</th>
<th>Arterial PCO2, mmHg</th>
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<td><strong>Protocol 1 (n = 26)</strong></td>
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<td>Contact (+)</td>
<td>107±10</td>
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<td>7.39±0.01</td>
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<td>HR 120 beats/min</td>
<td>115±5</td>
<td>82±7</td>
<td>102±5</td>
<td></td>
<td>7.40±0.02</td>
<td>37±1</td>
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<td>HR 240 beats/min</td>
<td>112±5</td>
<td>85±5</td>
<td>98±5</td>
<td></td>
<td>235±13†</td>
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<td>Glibenclamide (n = 5)</td>
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<td>HR 120 beats/min</td>
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<td>98±10</td>
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<td>242±23†</td>
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Values are means ± SE. BP, blood pressure; contact (+), in contact with myocardium; contact (−), not in contact with myocardium; HR, heart rate. *$P < 0.05$ vs. contact (+); †$P < 0.05$ vs. HR 120 beats/min.
Fig. 1. Representative images of detector vessels in protocol 1. A: a detector vessel passively dilated right after its placement on a beating canine heart and the application of intravascular pressure (60 cmH2O). B: intrinsic myogenic tone was developed in 1 h (the vessel was in contact with the beating heart). C: when the contact between the detector vessel and the beating heart was shut off, the vessel contracted further. D: superfusion of sodium nitroprusside (100 μmol/l) dilated the vessel. Scale bars, 100 μm.

Fig. 2. Pressure-diameter curves of detector vessels in the presence (+) or absence (−) of contact between the vessels and myocardium of the beating heart for vehicle (A; n = 9), glibenclamide (B; 5 μmol/l, n = 8), or endothelial denudation of the detector vessel (C). *P < 0.05 among intravascular pressure changes. **P < 0.05, with vs. without contact. D: effect of endothelial denudation on percent reduction in myogenic tone by vessel-myocardium contact. *P < 0.05 vs. with endothelium.
tical, demonstrating that glibenclamide did not affect the vascular myogenic tone or the myogenic responses.

**Detection of Vasodilator Signals Produced by Increased Myocardial Metabolism**

In protocol 2, the blood pressure did not change by rapid pacing and the double product was almost doubled (Table 1). There were no differences in the maximal diameters between vehicle (n = 5, 217 ± 16 μm) and glibenclamide groups (n = 5, 221 ± 23 μm).

The pressure-diameter curve was significantly shifted upward by tachycardia (Fig. 4A, P < 0.01 for groups, P = NS for intravascular pressure, P = NS for interaction). Unlike in protocol 1, however, the upward shift by the increased myocardial metabolism was not blocked by glibenclamide (Fig. 4B, P < 0.05 for groups, P < 0.01 for intravascular pressure, P = NS for interaction).

**DISCUSSION**

In the present study, we found the following: 1) the beating myocardium releases transferable vasoactive signals that attenuate the myogenic tone of coronary microvessels in the resting condition; 2) the attenuation of myogenic tone is partly mediated by the microvascular endothelium; and 3) the activation of $K_{ATP}$ channels accounts for the dilation in response to signals derived from the beating heart in the basal condition but not for that in response to signals from the heart with increased metabolism.

**Methodology**

In our bioassay system, we could control the conditions of the coronary microvessels and those of the myocardium independently. The effect of shear stress could be excluded because the flow in the detector vessel was zero. Because the detector vessels were separated from the blood stream and nervous control, the effects of neurohumoral factors could also be considered to be minimal. Thus we could focus on the myocardium-derived metabolic factors that act on the detector vessel.

The detector vessels were large microvessels (~200 μm in mean value) because of methodological limitations. The vascular walls of small microvessels are too thin to be detected with our optical system. There could be an argument whether vessels of this size play a role in determining the vascular resistance. However, Chilian et al. (5) demonstrated in cat hearts that 20% of the vascular resistance resides in coronary microvessels >200 μm by measuring the microvascular luminal pressure. Nellis et al. (24) showed in rabbit beating hearts that 20% of the pressure drop takes place in coronary arterial microvessels >140 μm, although the exact diameter data were...
not provided. Accordingly, we believe that the detector vessels we used bore appreciable vascular resistance.

With the assumption that the distending pressure of arterial microvessels of 200 μm in situ is 20% less than the coronary perfusion pressure, we estimate that the 60–120 cmH₂O of distending pressure applied to the detector vessels represented 55–110 mmHg of coronary perfusion pressure, which covers the physiological range of the mean coronary perfusion pressure.

There have been some reports focusing on the myocardium-modulated vascular control. Tiefenbacher et al. (34) put a supernatant of enzymatically dissolved myocytes onto isolated coronary vessels. They demonstrated that vasoactive factors are released from myocytes to coronary microvessels. However, our method is more physiological and direct than theirs in terms of using beating hearts instead of isolated myocytes.

To keep the detector vessel viable, we superfused warmed Krebs solution at the rate of 1.67 ml/min throughout the experiment. It is possible that the myocardium-derived metabolic factors were therefore diluted by this superfusion. Thus we may have underestimated the effect of the myocardium-derived vasoactive factors. In other words, the effect of the metabolic factors remained potent even after dilution.

Myogenic Tone and Vasoactive Signals From Myocardium

In the present study, the detector vessel diameters with myogenic tone were 70–80% of the maximal diameters when the vessels were isolated from the myocardium-derived vasoactive factors. This is consistent with a previous report by Liao and Kuo (20) for this size class of vessels. The magnitude of the myogenic tone in vessels >200 μm is controversial. Nakayama et al. (23) reported that this size class of vessels does not exhibit myogenic tone in pigs, whereas Rajagopalan et al. (27) demonstrated very potent myogenic tone in pig arteries >200 μm. These discrepancies can be explained by great heterogeneity of the coronary microvascular tone as evidenced by the profound spatial heterogeneity of the coronary flow reserve (2).

In our experiments, the myogenic tone and the myogenic responses were variable but were correlated with the detector vessel size with statistical significance. The size dependency of the myogenic vascular controls may affect the shape of the pressure-diameter curves, especially the slopes, in the present studies. For example, the apparent difference in the slopes of the pressure-diameter curves between Fig. 2, A and B, may have been caused by the tendency that the detector vessels in the vehicle group were smaller than in the glibenclamide group, although the difference did not attain statistical significance. The primary interest of the present studies, however, was the shift of the pressure-diameter curves caused by the vessel-myocardium contact, rather than myogenic control per se, and we successfully detected the vasodilator signals from the beating hearts in normal conditions.

We elucidated for the first time that the beating myocardium inhibits the myogenic tone by 30–50% at any distending pressure level. The attenuation of the myogenic tone may likely account for the matching of the coronary microvascular tone with the myocardial metabolic state; that is to say, it was a manifestation of metabolic factors.

Glibenclamide abolished the attenuation of the myogenic tone. It is unlikely that KATP channels are involved in the myogenic tone per se, because glibenclamide did not change the diameters of the detector vessels when the effect of myocardium-derived factors was shut off. Furthermore, our in vitro study clearly demonstrated that glibenclamide does not affect the vascular intrinsic tone per se. These findings are consistent with earlier studies (9). Therefore, the KATP channel activation mediates the attenuation of the myogenic tone via myocardium-derived dilator signals. Because KATP channels are known to couple the cellular energy metabolism to the membrane excitability (25), it is noteworthy that the myocardial metabolic factors counteracted the myogenic tone by modulating this channel for matching the coronary vascular tone with the myocardial metabolism. The present data are consistent with earlier studies showing that KATP channels play a pivotal role.
in determining the resting coronary flow in animals and human (11, 28).

When the endothelium of the detector vessel was denuded, the shift of the pressure-diameter curve was reduced, demonstrating that the reduction in the myogenic tone by the myocardium-derived factors is partly dependent on the endothelium. The endothelial K\textsubscript{ATP} channels have been known to play a role in the regulation of the coronary microvascular tone (14). Therefore, it is possible that the metabolic factors from the myocardium activate K\textsubscript{ATP} channels in both the endothelium and the vascular smooth muscle. Another possibility is that the myocardium-derived factor releases endothelium-derived vasoactive factor, which activates the vascular smooth muscle K\textsubscript{ATP} channels. Recently, it was demonstrated that in skeletal muscle arterioles, vasoactive effects of hydrogen peroxide, one of the possible endothelium-derived hyperpolarizing factors, are mediated by K\textsubscript{ATP} channels (6).

Investigators in our laboratory have previously shown that K\textsubscript{ATP} channels function in all size classes of coronary microvessels and that K\textsubscript{ATP} channels and pertussis toxin-sensitive G proteins are functionally linked in both small and large coronary microvessels (18, 29). It is possible that the myocardium-derived vasoactive signals activate the G proteins in coronary microvessels, leading to K\textsubscript{ATP} channel activation.

The attenuation of the myogenic tone by metabolic factors should affect the myogenic vascular control in vivo. Perfusion pressure changes produce coronary microvascular diameter changes only in small vessels <100 \mu m in diameter in vivo (15), whereas in vitro studies have shown myogenic regulation in all sizes of coronary microvessels (20). Although it is difficult to evaluate the myogenic responsiveness in vivo in the heart because there are many confounding factors that come into play for the regulation of the vascular tone (17), it is possible that the myocardial metabolic factors mask the myogenic responsiveness in large coronary microvessels.

We have shown that myocardium-derived signals attenuating the myogenic tone at rest are different from those released during increased metabolism. Glibenclamide did not inhibit the effect of metabolic factors released by rapid pacing. The signaling network between the myocardium and coronary microvessels may be much more redundant during increased metabolism than in resting conditions.

**Clinical Implication**

It is possible that in diseased conditions in which the K\textsubscript{ATP} channel function is impaired, the signal transduction from the myocardium to coronary vascular bed is impaired. Miura et al. (22) recently demonstrated that the activity of K\textsubscript{ATP} channels is reduced in coronary arterioles from patients with diabetes mellitus. Furthermore, glibenclamide, which we used in the present study, is widely used in the treatment of type 2 diabetes mellitus. Given that the sensitivity of pancreatic \beta\text{-}cells and vascular smooth muscle to glibenclamide is similar as evidenced by an animal study (7), this agent has a potentially deleterious effect on the functional link between the myocardium and coronary vessels in the resting condition. Notably, some previous studies have reported an adverse effect of glibenclamide on cardiovascular events (13). The involvement of K\textsubscript{ATP} channel dysfunction and the effect of sulfonylurea in coronary flow regulation in diabetes mellitus deserve further investigation.

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