Activation of inward rectifier K\(^+\) channels by hypoxia in rabbit coronary arterial smooth muscle cells

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Activation of inward rectifier K\(^+\) channels by hypoxia in rabbit coronary arterial smooth muscle cells. Am J Physiol Heart Circ Physiol 289: H2461–H2467, 2005; doi:10.1152/ajpheart.00331.2005. We examined the effects of acute hypoxia on Ba\(^{2+}\)-sensitive inward rectifier K\(^+\) (K\(_{IR}\)) current in rabbit coronary arterial smooth muscle cells. The amplitudes of K\(_{IR}\) current were definitely higher in the cells from small-diameter (<100 \(\mu\)m) coronary arterial smooth muscle cells (SCASMC, -12.8 ± 1.3 pA/pF at -140 mV) than those in large-diameter coronary arterial smooth muscle cells (>200 \(\mu\)m, LCASMC, -1.5 ± 0.1 pA/pF). Western blot analysis confirmed that Kir2.1 protein was expressed in SCASMC but not LCASMC. Hypoxia activated much more K\(_{IR}\) currents in symmetrical 140 K\(^+\). This effect was blocked by the adenyl cyclase inhibitor SQ-22536 (10 \(\mu\)M) and mimicked by forskolin (10 \(\mu\)M) and dibutyryl-cAMP (500 \(\mu\)M). The production of cAMP in SCASMC increased 5.7-fold after 6 min of hypoxia. Hypoxia-induced increase in K\(_{IR}\) currents was abolished by the PKA inhibitors, Rp-8-(4-chlorophenylthio)-cAMPS (10 \(\mu\)M) and KT-5720 (1 \(\mu\)M). The inhibition of G protein with GDP\(\beta\)S (1 mM) partially reduced (~50%) the hypoxia-induced increase in K\(_{IR}\) currents. In Langendorff-perfused rabbit hearts, hypoxia increased coronary blood flow, an effect that was inhibited by Ba\(^{2+}\). In summary, hypoxia augments the K\(_{IR}\) currents in SCASMC via cAMP- and PKA-dependent signaling cascades, which might, at least partly, explain the hypoxia-induced coronary vasodilation.

Coronary vascular smooth muscle in guinea pig, rabbit, and rat hearts relaxes in response to acute hypoxia or a decrease in P\(_{O_2}\) (7, 27, 30, 37). Vasodilation in response to hypoxia in coronary vascular smooth muscle is likely a protective response to increase blood flow to areas of low P\(_{O_2}\). For many years, hypoxic vasodilation in the peripheral vasculature has been recognized as an important regulatory response, but the mechanisms for this response remain controversial. The mechanisms proposed to explain coronary hypoxic vasodilation include the release of vasodilator substances, such as nitric oxide or prostaglandins, from endothelial cells and erythrocytes or the production of vasodilator metabolites, such as adenosine, by the cardiac myocytes (2, 5, 11, 26, 35, 36). Another postulation is that hypoxia directly activates ATP-sensitive K\(^+\) (K\(_{ATP}\)) channels in arterial smooth muscle cell membranes, resulting in vascular hyperpolarization and relaxation (4, 7). Clearly, these routes are not mutually exclusive, and each could exert a partial or complete vasodilatory effect.

Previous studies (16, 19, 20) have also suggested that the mechanism of hypoxic vasodilation in large coronary arteries is different from small coronary arteries. Although the different responses to hypoxia in large and small coronary arteries are unclear, several studies using isolated hearts have suggested that the activation of K\(_{ATP}\) channels in smooth muscle is a major mechanism of hypoxic vasodilation in both small and large arteries. Some reports (3, 18, 28, 29) have identified inward rectifier K\(^+\) (K\(_{IR}\)) channels in small-diameter coronary and cerebral arteries. The activation of K\(_{IR}\) channels also underlies the vasodilation of small arteries. K\(_{IR}\) channels provide the dominant K\(^+\) conductance around the resting membrane potential (23) and may modulate basal tone of small coronary and cerebral arteries (14, 18).

Based on this knowledge, it was tempting to examine whether a hypoxic stimulation modulates the K\(_{IR}\) channels in coronary arterial smooth muscle cells, which could explain the more prominent response to hypoxia in smaller arteries than in larger arteries. In support of this hypothesis, we first investigated in this study whether hypoxia activated K\(_{IR}\) channels in cells isolated from small-diameter coronary arterial smooth muscle in the absence of substances normally released by endothelial or cardiac cells. In addition, we also investigated the components of the signaling pathways between hypoxia and the activation of K\(_{IR}\) channel.

MATERIALS AND METHODS

Cell preparation. Twenty-nine New Zealand White rabbits (~1.5 to 2.0 kg) of either sex were simultaneously anesthetized with pentobarbital sodium (50 mg/kg) and injected with heparin (100 U/kg) into the ear vein. The hearts were removed immediately and immersed in normal Tyrode solution. The left anterior descending coronary arteries were dissected out and cleaned of blood and connective tissue. Before the enzymatic treatment, the diameter of the freshly dissected artery was measured by using a video edge detector (Crescent Electronics, Sandy, UT). Single cells were obtained by using an enzymatic procedure. The arteries were transferred to 1 ml of Ca\(^{2+}\)-free normal Tyrode solution that contained (in mg/ml) 1.0 papain, 1.5 BSA, and 1.0 DTT. After incubation for ~25 min, the arteries were transferred to 1 ml of Ca\(^{2+}\)-free normal Tyrode solution containing collagenase.
(2.8 mg/ml), BSA, and DTTO for ~20 min. After enzyme treatment, the artery was rinsed in Kraftbruhe (KB) medium. Single cells were dispersed in this solution by trituration of the tissue through a fire-polished glass pipette. The isolated cells were also stored in KB solution at 4°C and used on the day of preparation.

**Solution.** Normal Tyrode solution contained (in mM) 135 NaCl, 5.4 KCl, 0.33 NaH2PO4, 1.8 CaCl2, 0.5 MgCl2, 5 HEPES, and 16.6 glucose, adjusted to pH 7.4 with NaOH. External solutions (140 K⁺) were made by substituting NaCl for KCl in the normal Tyrode solution. To obtain hypoxic flow solutions, the 140 K⁺ solutions were continuously bubbled with 100% N2 in glass reservoirs for at least 40 min. The oxygen partial pressure (Po2) of these solutions in the experimental chamber, measured by using an oxygen electrode (oxygen meter, World Precision Instruments), was found to be in the range of 15–30 mmHg. In contrast, the normoxic control solution had a Po2 in the range of 120–140 mmHg. The pipette-filling solution contained (in mM) 115 K-aspartate, 25 KCl, 5 NaCl, 5 Mg-ATP, 1 EGTA, and 10 HEPES, adjusted to pH 7.2 with KOH. KB solution contained (in mM) 70 KOH, 50 L-glutamate, 20 KH2PO4, 55 KCl, 20 taurine, 5 MgCl2, 20 glucose, 10 HEPES, and 0.5 EGTA, adjusted to pH 7.3 with KOH.

**Drugs.** All pharmacological compounds were prepared as stock solution in water or DMSO at >1,000 times the concentration used during the experiment. Forskolin, SQ-22536, GDPβS, KTS-5720, and glibenclamide were purchased from Sigma (St. Louis, MO). Dibutyryl-cAMPs were purchased from Tocris (Ellisville, MO). Rp-8-(4-chlorophenylthio)-cAMPs (Rp-8-CPT-cAMPs) were purchased from Tocris (Ellisville, MO). Rp-8-(4-chlorophenylthio)-cAMPs (Rp-8-CPT-cAMPs) were purchased from Tocris (Ellisville, MO).

**Measurement of coronary blood flow.** The measurement of coronary blood flow (CBF) rate from whole heart was performed by a fraction collector (Pharmacia model 18–1003-64, Uppsala, Sweden). The flow volume per 1 min was gravimetrically determined. Measurement of coronary blood flow was performed by using a digital interface (Digidata 1200, Axon Instruments), which was coupled to an IBM-compatible microcomputer. The voltage and current signals were filtered at 1 kHz and sampled at a rate of 2–3 kHz. The patch pipettes were pulled from borosilicate capillaries (Clark Electromedical Instruments, Pangbourne, UK) using the Narishige PP-83 puller (Narishige Scientific Instrument, Tokyo, Japan). We used patch pipettes that had a resistance of 3–4 MΩ when filled with the pipette solutions listed in Solutions. The single smooth muscle cells had a mean membrane capacitance of 14.3 ± 1.0 pF (n = 45 cells) in small-diameter and 16.3 ± 0.8 pF (p = 32 cells) in large-diameter coronary arteries, respectively.

**Measurement of coronary blood flow.** The measurement of coronary blood flow (CBF) rate from whole heart was performed by a modified Langendorff method. The heart was quickly removed and placed in ice-cold Krebs-Henseleit (KH) solution containing (in mM) 120 NaCl, 4.7 KCl, 1.8 CaCl2, 1.2 MgSO4, 1.18 KH2PO4, 25 NaHCO3, and 16.5 glucose, bubbled with 95% O2-5% CO2. The heart was cannulated by inserting a silicone tube into the coronary artery via the aorta, and the heart perfused retrogradely at a constant pressure of 70 mmHg with oxygenated KH solution, according to the method of Langendorff. The coronary effluent was collected at 1-min intervals by using a fraction collector (Pharmacia model 18–1003-64, Uppsala, Sweden). The flow volume per 1 min was gravimetrically determined. During coronary perfusion, all perfusates were maintained at 37°C. Hypoxic KH solution was obtained by bubbling 95% N2-5% CO2.

**Western blot analysis.** Membrane proteins were extracted from isolated small-diameter coronary arterial cells. Cells were centrifuged at 1,000 g for 5 min. The supernatant was discarded and the cells were resuspended in lysis buffer. The suspension was homogenized and centrifuged at 1,000 g to pellet cellular debris, and then the remaining supernatant was centrifuged at 45,000 g at 4°C for 20 min. The pellet was resuspended in resuspension buffer.

Proteins (10 μg each) from coronary arteries were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gels were transferred to Immobilon-P membranes (Millipore, Billerica, MA), which were blocked overnight in Tris-buffered or phosphate-buffered saline containing 5% nonfat dry milk and then probed with the GAPDH antibody at a dilution of 1:1,000 and antiserum for Kir2.1, Kir2.3 (Santa Cruz Biotechnology, Santa Cruz, CA), and Kir2.2 (Chemicon International, Temecula, CA) at a dilution of 1:500 for 1 h at room temperature. Membranes were incubated with secondary antibodies, a goat anti-mouse IgG for GAPDH (Santa Cruz), a mouse anti-goat IgG for Kir2.1 and Kir2.3 (Santa Cruz), and a goat anti-rabbit IgG for Kir2.2 (Vector). These secondary antibodies were conjugated to horseradish peroxidase at a dilution of 1:3,000 for 1 h at room temperature. Immunoreactivity was visualized by using enhanced chemiluminescence (Amersham ECL Western blotting detection kit, Amersham Biosciences, Piscataway, NJ).

**RESULTS**

**Ba2⁺-sensitive Kir currents in rabbit coronary arterial smooth muscle cells.** Smooth muscle cells were isolated from small-diameter coronary arterial smooth muscle cells (<100 μm, SCASMC) and large-diameter coronary arterial smooth muscle cells (>200 μm, LCASMC). Currents were recorded in response to a depolarizing voltage ramp from −140 to +20 mV at 0.5 V/s. Figure 1A illustrates representative cases of Ba2⁺-sensitive Kir currents in LCASMC (Fig. 1A, left) and SCASMC (Fig. 1A, right). Both extracellular and intracellular K⁺ concentrations were 140 mM to increase the size of Kir currents. The contribution of KATP channels to measured current was minimized by inclusion of ATP (5 mM) in the pipette solution. In contrast to LCASMC, Ba2⁺-sensitive Kir current was present in SCASMC. As expected for inward rectifier, the current is larger in the inward direction than in the outward direction and reverses close to the equilibrium potential (Ek) of 0 mV. The current density of Ba2⁺-sensitive currents at −140 mV is summarized in Fig. 1B (LCASMC, −1.5 ± 0.1 pA/pF; and SCASMC, −2.7 ± 0.1 pA/pF, respectively).

To further identify the subtype of Kir channels, Western blot analysis was used to examine the expression of Kir receptor proteins in native LCASMC and SCASMC using antibodies that were specific to Kir2.1, Kir2.2, or Kir2.3. As shown in Fig. 1C, Kir2.2 and Kir2.3 were not detected in either type of cell, whereas Kir2.1 was detected only in SCASMC (the band at ~48 kDa). Similar results were obtained from four different experiments. We confirmed that these antibodies are working properly by detecting Kir2.1, Kir2.2, and Kir2.3 in rabbit ventricle or hippocampus expressing these channels (data not shown). Thus we concluded that Kir2.1, but not Kir2.2 and Kir2.3, is expressed in native SCASMC.
Hypoxia activates KIR currents. Figure 2A shows the effect of acute hypoxia on the Ba$^{2+}$-sensitive KIR currents recorded from LCASMC (Fig. 2A, left) and SCASMC (Fig. 2A, right). Acute hypoxia was induced by changing the perfusate from a normoxic control to the N$_2$-bubbled one (see MATERIALS AND METHODS). LCASMC, which do not have KIR channel, were unaffected by the hypoxic solution, whereas the same solution increased the Ba$^{2+}$-sensitive KIR currents in SCASMC. The hypoxic effect began to develop ~2 min after the switch to hypoxic solution and reached a maximum within 5 min. Washout of the hypoxia with normoxic solution usually restored the current to >80% of control value (Fig. 2A). Although inclusion of 5 mM ATP may block the K$_{ATP}$ channel, we have considered the possibility that K$_{ATP}$ currents were still activated by hypoxia. We, therefore, examined the effect of glibenclamide (10 µM), a specific K$_{ATP}$ channel blocker, on the hypoxic-
induced increase in $K_{IR}$ currents. However, as shown in Fig. 2A, glibenclamide did not affect the hypoxic-induced increase in $K_{IR}$ currents. The effect of hypoxia on $Ba^{2+}$-sensitive currents recorded at $-140$ mV is summarized in Fig. 2C (LCASMC, $-1.3 \pm 0.2$ and $-1.5 \pm 0.3$ pA/pF in normoxia and hypoxia, respectively; and SCASMC, $-12.9 \pm 0.6$ and $-18.3 \pm 1.2$ pA/pF in normoxia and hypoxia, respectively).

**Activation of adenylyl cyclase increases $K_{IR}$ currents.** To test a hypothesis that the hypoxia-induced increase in $K_{IR}$ currents is mediated by cAMP, SCASMC were treated with SQ-22536 (10 $\mu$M), an activator of adenylyl cyclase, on $K_{IR}$ currents. As shown in Fig. 3A and B, SQ-22536 inhibited the hypoxia-induced increase in $K_{IR}$ currents to control levels (normoxia, $-12.5 \pm 1.0$ pA/pF; hypoxia, $-20.5 \pm 1.7$ pA/pF; and hypoxia + SQ-22536, $-13.7 \pm 0.9$ pA/pF, respectively). Similarly, the hypoxia-induced increase in $K_{IR}$ currents was completely blocked after the pretreatment of cells with SQ-22536 (normoxia, $-11.5 \pm 1.1$ pA/pF; SQ-22536, $-11.0 \pm 0.8$ pA/pF; and SQ-22536 + hypoxia, $-11.6 \pm 0.8$ pA/pF, respectively; data not shown).

To confirm that adenylyl cyclase mediates the effects of hypoxia on $K_{IR}$ currents in SCASMC, we examined whether the activation of adenylyl cyclase by forskolin, which is known to activate adenylyl cyclase in many cellular systems, would activate $K_{IR}$ channels. As shown in Fig. 3C and D, 10 $\mu$M forskolin increased $K_{IR}$ currents, and the subsequent exposure to hypoxic solution showed no further effect (normoxia, $-13.6 \pm 1.5$ pA/pF; forskolin, $-18.6 \pm 2.5$ pA/pF; and forskolin + hypoxia, $-19.0 \pm 2.2$ pA/pF, respectively). We also tested the effect of dibutyryl-cAMP, a cell-permeable analog of cAMP, on the $K_{IR}$ channels. As expected, 500 $\mu$M dibutyryl-cAMP caused further activation of $K_{IR}$ currents, and in the presence of dibutyryl-cAMP, hypoxia did not further increase $K_{IR}$ currents (normoxia, $-12.4 \pm 1.3$ pA/pF; dibutyryl-cAMP, $-16.0 \pm 1.2$ pA/pF; and dibutyryl-cAMP + hypoxia, $-16.1 \pm 1.0$ pA/pF, respectively; data not shown).

**Hypoxia increases production of cAMP.** To detect the hypoxia-induced increase in cAMP production directly, we measured cAMP concentrations using ELISA after preincubation of cells with a nonspecific phosphodiesterase inhibitor, IBMX (100 $\mu$M), which inhibits the degradation of the cyclic nucleotide. The effect of acute hypoxia on the production of cAMP is shown in Fig. 4. Within 2 min of hypoxia, there was no detectable change in cAMP. However, after 2 min, a slow but pronounced increase in cAMP was visible. After 4 min of hypoxia, the production of cAMP was increased more dramatically. When expressed as a percentage of the forskolin-induced maximum effects, cAMP production increased 5.7-
fold in 6 min of treatment with hypoxic solution compared with the control in normoxic solution (12.8 ± 2.6% of normoxic solution and 73.0 ± 8.3% of hypoxic solution, respectively).

Inhibition of hypoxia-induced currents by inhibitors of cAMP-dependent kinase. Increased adenylyl cyclase activity and the consequent increase in intracellular cAMP have been shown to activate PKA in coronary arteries (12). If hypoxia causes K<sub>IR</sub> channel activation by such a pathway, it should be possible to inhibit the effect of hypoxia using agents that block PKA. Thus we used the cAMP analogs Rp-8-CPT-cAMPs (10 μM) and KT-5720 (1 μM) to inhibit PKA. Rp-8-CPT-cAMPs alone had no significant effect on the Ba<sup>2+</sup>-sensitive K<sub>IR</sub> currents. However, as shown in Fig. 5, A and B, bath application of Rp-8-CPT-cAMPs substantially reduced the hypoxia-induced increase in K<sub>IR</sub> currents (normoxia, −14.5 ± 1.3 pA/pF; hypoxia, −19.0 ± 1.9 pA/pF; and hypoxia + Rp-8-CPT-cAMPs, −14.4 ± 1.5 pA/pF, respectively). The application of KT-5720, another PKA inhibitor, also reversed the hypoxia-induced increase in K<sub>IR</sub> currents (Fig. 5B). A further indication of the involvement of PKA in the application of hypoxic solution had no effect on K<sub>IR</sub> currents under the pretreatment with Rp-8-CPT-cAMPs (normoxia, −13.9 ± 1.1 pA/pF; Rp-8-CPT-cAMPs, −13.5 ± 1.2 pA/pF; and Rp-8-CPT-cAMPs + hypoxia, −14.0 ± 1.4 pA/pF, respectively; data not shown).

Hypoxia increases K<sub>IR</sub> currents through activation of G protein. As a next step, we tested whether the hypoxia directly stimulates PKA or activates G proteins governing PKA. Figure 6 shows that the inclusion of GDP<sub>β</sub>S (1 mM) in the pipette attenuated the hypoxia-induced increase in K<sub>IR</sub> currents by ~50% (normoxia, −13.2 ± 1.2 pA/pF; hypoxia in the presence of GDP<sub>β</sub>S, −16.3 ± 1.2 pA/pF; normoxia, −12.9 ± 1.4 pA/pF; and hypoxia in the absence of GDP<sub>β</sub>S, −19.1 ± 1.3 pA/pF). A higher concentration of GDP<sub>β</sub>S (10 mM) did not result in a further block of the effect of hypoxia on the K<sub>IR</sub> currents (−16.1 ± 1.7 pA/pF, n = 7 cells; data not shown). This result suggested that hypoxia activated adenylyl cyclase by both G protein-dependent and -independent mechanisms.

Hypoxia contribute to regulation of coronary blood flow by activation of K<sub>IR</sub> channel. We examined the physiological role of hypoxia-sensitive K<sub>IR</sub> channels in relation to vascular tone and blood flow in rabbit heart. To measure CBF, we used a modified Langendorff apparatus as described in MATERIALS AND METHODS. The CBF became stable at 4.8 ± 0.3 ml/min (n = 6 hearts) after 30 min of perfusion with KH solution. To block K<sub>ATP</sub> channels (which are activated by hypoxia), recordings were performed after pretreatment of the heart with glibenclamide (10 μM). In the glibenclamide-pretreated heart, CBF was ~36% less than that in the untreated heart. CBF in the glibenclamide-pretreated heart during hypoxia was ~27% greater (glibenclamide-pretreated heart, 2.7 ± 0.2 vs. hypoxia + glibenclamide-pretreated heart, 3.5 ± 0.2 ml/min, n = 4 hearts), and this increase was blocked by the application of Ba<sup>2+</sup> (50 μM, Fig. 7A). To further evaluate the hypoxia-induced increase in CBF, we pretreated glibenclamide-exposed heart with Ba<sup>2+</sup>. The application of Ba<sup>2+</sup> alone decreased CBF by ~32%. The application of Ba<sup>2+</sup> and glibenclamide reduced CBF additively (Fig. 7B), which suggested that these two agents blocked separate targets. As shown in Fig. 7B, the hypoxia-induced changes in CBF suppressed by pretreatment with Ba<sup>2+</sup> in the presence of glibenclamide (n = 4 hearts). These results indicated that K<sub>IR</sub> channel opening is apparently activated during hypoxia.

**DISCUSSION**

The major findings of this study were that 1) the density of K<sub>IR</sub> current was greater in cells isolated from small-diameter coronary arteries than in cells from larger arteries; 2) hypoxia caused an increase in K<sub>IR</sub> current in SCASMC; 3) the hypoxia-induced increase in K<sub>IR</sub> currents was attenuated by the inhibition of adenylyl cyclase and PKA and was mimicked by the activation of adenylyl cyclase; 4) the hypoxia-induced increase in K<sub>IR</sub> currents was mediated partially by the activation of G
The activation of KIR channels by hypoxia.

The density of KIR currents was greater in cells from smaller coronary arteries than in cells from larger coronary arteries. The activation of the KIR channel has been proposed to underlie K⁺-induced vasodilation of small coronary and cerebral arteries. In small coronary arteries, K⁺ efflux from cardiac myocytes, particularly under ischemic conditions, can cause extracellular K⁺ concentrations to rise as high as 15 mM. Therefore, alterations in extracellular K⁺ concentrations might affect the metabolic regulation of CBF. It is thought that large-diameter arteries (which we found in the present study to have a low density of KIR currents) do not regulate local blood flow and therefore need not be responsive to local metabolites such as K⁺.

In the cerebral circulation, the extracellular concentration of K⁺ increases from 3 mM to values >10 mM during cerebral hypoxia, hypoglycemia, ischemia, or physiological changes in neuronal activity. This rise in the extracellular concentration of K⁺ induces increases in local cerebral blood flow when cerebral activity is elevated. Modest increases in extracellular K⁺ concentrations cause the vasodilation of small-diameter cerebral and coronary vessels and might thereby cause a selective increase in the perfusion of metabolically active tissue. Additionally, our results provide evidence that acute hypoxia can cause the activation of KIR channel through a signal transduction pathway that involves the stimulation of adenylyl cyclase, increased production and accumulation of cAMP, and activation of PKA; each of these mechanisms is independent of extracellular K⁺ concentration. Therefore, during hypoxia, the efflux of K⁺ from cardiac myocytes as well as the activation of PKA may activate KIR channels in small coronary vascular smooth muscle. The activation of KIR channel would dilate the arteries by relaxing vascular smooth muscle, which increased blood flow in the heart.

It has previously been demonstrated that challenge with chronic and acute hypoxia triggers an increase in the level of cAMP in vascular smooth muscle cells. Basal adenylyl cyclase activity in pulmonary arteries did not change with prolonged hypoxia, whereas in systemic arteries (thoracic aorta) it increased 3.5- and 5.3-fold after 3 and 7 days of chronic hypoxia, respectively. Also, isoproterenol-, sodium fluoride-, and forskolin-stimulated adenylyl cyclase activity in pulmonary arteries did not change in chronic hypoxia, whereas enzyme activity in systemic arteries is markedly increased.

In resistance arteries of skeletal muscle and cerebral vascular beds, prostacyclin released from the endothelium in response to acute reduced PO₂ is a crucial mediator of hypoxic dilation occurring as a result of the production of cAMP and the opening of KATP channels. However, there were no reports that acute hypoxia directly increased production of cAMP. Our data first suggested that acute hypoxia increased the activity of adenylyl cyclase and the consequent increase in cAMP (Figs. 3 and 4), which activates PKA. This response would lead to the activation of KATP and KIR channels. Previous reports suggested that KATP-channel activation is an important mechanism underlying hypoxic vasodilation in the coronary circulation. The most obvious mechanism by which a reduction in PO₂ could lead to the activation of KATP channels is to produce a change in the energy metabolism of coronary smooth muscle cells, which would cause KATP channel to open in response to a fall in submembrane concentrations of ATP. In the coronary and middle cerebral artery, hypoxic vasodilation induced by lowering PO₂ is eliminated by glibenclamide, which implies that KATP channels are activated at least during short periods of hypoxia. However, some reports suggested that the inhibition of KATP channel was ineffective in attenuating hypoxia-induced vasodilation in rabbit and porcine coronary arteries. Also, the contribution of vasodilators and endothelium dependency varies depending on the vascular bed, species, vessel size (conduit or resistance arteries), and experimental preparation. For example, in isolated large coronary arteries, hypoxia caused direct relaxation of coronary vascular smooth muscle. Endothelial cells did not contribute to this hypoxic relaxation but instead caused a transient hypoxic constriction that was mediated by a decrease in the activity of endothelium-derived nitric oxide (NO).

In our present study, we showed that hypoxia increased KIR currents in small-diameter coronary arteries by activating PKA in the absence of endothelium and cardiac myocytes. Therefore, small coronary arterial smooth muscle are more active in the response to hypoxia by virtue of the activation of KATP and KIR currents. This suggests that the hypoxia-induced increase in
KIR currents is a crucial part of the explanation of why arteries of different sizes respond differently to hypoxia.

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REFERENCES