Diabetes Mellitus Impairs Vasodilation to Hypoxia in Human Coronary Arterioles

Reduced Activity of ATP-Sensitive Potassium Channels

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Abstract—ATP-sensitive K⁺ channels (K_{ATP}) contribute to vasomotor regulation in some species. It is not fully understood the extent to which K_{ATP} participate in regulating vasomotor tone under physiological and pathophysiological conditions in the human heart. Arterioles dissected from right atrial appendage were studied with video microscopy, membrane potential recordings, reverse transcription–polymerase chain reaction, and immunohistochemistry. Hypoxia produced endothelium-independent vasodilation and membrane hyperpolarization of vascular smooth muscle cells, both of which were attenuated by glibenclamide. Aprikalim, a selective K_{ATP} opener, also induced a potent endothelium-independent and glibenclamide-sensitive vasodilation with membrane hyperpolarization. Reverse transcription–polymerase chain reaction detected mRNA expression for K_{ATP} subunits, and immunohistochemistry confirmed the localization of the inwardly rectifying Kir6.1 protein in the vasculature. In patients with type 1 or type 2 diabetes mellitus (DM), vasodilation was reduced to both aprikalim (maximum dilation, DM(+) 90±2% versus DM(−) 96±1%, P<0.05) and hypoxia (maximum dilation, DM(+) 56±8% versus DM(−) 85±5%, P<0.01) but was not altered to sodium nitroprusside or bradykinin. This reduction in K_{ATP} function could contribute to the greater cardiovascular mortality and morbidity in DM. (Circ Res 2003;92:151-158.)

Key Words: human ▪ coronary microcirculation ▪ ATP-sensitive potassium channels ▪ hypoxia ▪ diabetes mellitus

ATP-sensitive K⁺ channels (K_{ATP}) are present in a variety of tissues. K_{ATP} participate in vasodilation to hypoxia and ischemia. K_{ATP} function is altered in the presence of diabetes mellitus (DM). In some species, vasodilation to K_{ATP} openers is impaired by DM, whereas in other species and vascular beds, the vasodilation may be augmented. We examined whether K_{ATP} opening elicits coronary arteriolar dilation in humans and whether K_{ATP} are responsible for hypoxia-induced vasodilation. We also determined whether K_{ATP}-mediated vasodilation is impaired in coronary vessels from subjects with DM. This could have important implications for patients with DM, who may suffer from reduced metabolic vasodilation.

Materials and Methods

Materials

Aprikalim (RP52891, Rhone-Poulenc Rorer, France) was prepared in saline with 1% BSA. All other chemicals were obtained from Sigma Chemical Co and dissolved in distilled water, except indomethacin, which was dissolved in distilled water with 1 N NaOH, and the pH was adjusted to 7.4 with 0.1 N HCl. All concentrations represent the final molar concentrations (mol/L) in the organ chambers.

General Preparation

Fresh specimens of right atrial appendage were obtained from 200 patients undergoing cardiac surgery. All protocols and procedures were approved by the appropriate institutional review boards. After surgical removal, the atrial appendage was placed in cold oxygenated Krebs solution, as described previously. Human coronary arterioles (HCAs) were isolated and transferred to an organ chamber containing oxygenated warmed (37°C) Krebs solution, where they were connected via glass micropipettes to a hydrostatic reservoir. The preparation was transferred to the stage of an inverted microscope (CK2, Olympus) for continuous measurement of internal diameter. All pharmacological agents were added to the external bathing solution. One vessel was used from each subject.

HCAs that failed to constrict by >30% of expected passive diameter to 75 mmol/L KCl were discarded. All HCAs dilated to

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adenosine diphosphate (ADP, 10⁻⁴ mol/L), confirming integrity of endothelial function. Endothelin-1 (≈10⁻⁴ mol/L) or acetylcholine (≈5×10⁻⁷ mol/L) was, if needed, added to adjust basal tone to a consistent level between 30% to 60% of passive diameter.

**Aprikalim Treatment**

After constriction, vasodilation to cumulative increases in the concentration of aprikalim (10⁻¹⁰ to 10⁻³ mol/L, a selective K<sub>ATP</sub> opener) was performed in the presence and absence of N<sup>ω</sup>-nitro-L-arginine methyl ester (L-NAME) (10⁻⁴ mol/L, an NO synthase inhibitor), indomethacin (10⁻³ mol/L, a cyclooxygenase inhibitor), glibenclamide (10⁻⁴ mol/L, a selective K<sub>ATP</sub> blocker), or mechanical endothelial denudation. Reversibility of dilation to aprikalim was tested after a 30-minute exposure to and after washout of glibenclamide, Bradykinin (BK) (10⁻¹² to 10⁻⁶ mol/L), an endothelium-dependent vasodilator, was tested in the presence of L-NAME and indomethacin. Vasodilation to sodium nitroprusside (SNP) (10⁻⁴ to 10⁻⁴ mol/L) or papaverine (10⁻⁴ mol/L), endothelium-independent dilators, was also tested. In some vessels, diazoxide (10⁻⁴ to 10⁻⁴ mol/L, another K<sub>ATP</sub> opener) was also used.

**Hypoxia Induction**

Hypoxic conditions were produced by bubbling Krebs solution with 5% CO₂ and 95% N₂ gas in a covered vessel chamber. Vascular responses to two consecutive 15-minute periods of hypoxia separated by 60 minutes of intervening normoxia were examined in the absence and presence of inhibitors or denudation. Denudation was confirmed by a reduction in dilation to ADP (10⁻³ mol/L) to <10% (after 4±2% versus before 85±6%, P<0.05) and preserved dilation to SNP (10⁻⁴ mol/L; after 97±2% versus before 99±1%, P=NS). Responses to aprikalim were also studied before and after hypoxia.

**Measurement of Vascular Smooth Muscle Membrane Potential**

We measured resting membrane potential (E<sub>m</sub>) of vascular smooth muscle cells (VSMCs) and changes in E<sub>m</sub> to aprikalim or hypoxia, as described previously. Briefly, pressurized HCAs were impaled from the adventitial surface with a glass microelectrode (40 to 90 MΩ impedance, filled with 3 mol/L KCl) and connected to a high-impedance biological amplifier (Axoclamp, Axon Instruments).

**Reverse Transcription-Polymerase Chain Reaction (RT-PCR)**

From each subject, 5 to 10 HCAs of 1 to 2 mm in length were isolated. Extraction and isolation of mRNA was achieved with oligo(dT<sub>15</sub>)-linked magnetic beads (Dynal), and first-strand cDNA was synthesized from mRNA using Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences) according to established protocols. Reverse transcription was initiated by addition of 0.5 μg Oligo(dT)₁₅ Primer (Promega) at 37°C for 75 minutes. Protocols were performed using 1 μL of the cDNA in 30 μL reaction containing 0.2 μmol/L of each primer specific for K<sub>ATP</sub>, 200 μmol/L of each dNTP, and 0.7 U of Taq polymerase (PCR Supermix, Life Technologies). Primers were used for detection of the inwardly rectifying Kir6.1 (expected product, 134 bp) and SUR1 (134 bp) and Kir6.2 (636 bp) and rectifying Kir6.1 (expected product, 737 bp) and Kir6.2 (636 bp) and SUR2A/2B (451/312 bp). Cycling conditions were as follows: for Kir6.1 and 6.2, 96°C, 3 minutes, 40 cycles (96°C, 15 seconds; 55°C, 30 seconds; 72°C, 15 seconds); for SUR1 and SUR2A/2B, 94°C, 3 minutes, 40 cycles (94°C, 30 seconds; 58.8°C, 1 minute; 72°C, 1 minute) and 72°C, 7 minutes. An aliquot (10 μL) of reverse transcription-polymerase chain reaction (RT-PCR) products was analyzed on 2% TEA agarose gels (Life Technologies). PCR products were visualized by staining with ethidium bromide with ultraviolet transillumination. In the experiments with no or faint expression, a second PCR was also conducted to confirm the results. Negative controls lacking cDNA or reverse transcriptase during cDNA synthesis did not amplify any products, indicating lack of contamination with genomic DNA.

**Immunohistochemistry**

Small pieces (~1 mm³) of pectinate muscle were fixed with 4% paraformaldehyde in PBS, infiltrated with 20% sucrose HEPES buffer solution, and frozen in OCT compound. Sections (8-μm thick) were immunolabeled with a polyclonal antibody against Kir6.1 protein (dilution 1:250, Santa Cruz Biotechnology, Inc). Immunostains were visualized by using avidin-biotin horseradish peroxidase visualization systems (Vectastain Universal Quick kit, Vector Laboratories). As a control for nonspecific binding, the primary antibody was omitted.

**Statistical Analysis**

Vasodilations to aprikalim, hypoxia, and BK are expressed as percent dilation, with 100% representing the change in diameter to SNP (10⁻⁴ mol/L). Student’s paired t test was used to compare responses to hypoxia, change in E<sub>m</sub>, maximal dilations, and ED₅₀ values (the molar concentration of dilator that produced a 50% maximal response). Dilation to aprikalim under different conditions was compared using 2-way repeated-measures ANOVA with a Bonferroni correction. Multivariate analysis assessed the influence of age, sex, and underlying diseases (Table 1) on vasodilations, resting E<sub>m</sub> and vascular tone. Regression models for all doses or time intervals isolated the confounding effects of the other diseases on dilator responses. Analysis of covariance (ANACOVA) was performed to adjust for contributions of each factor to an impaired response. SAS for Windows, version 8, was used for analyses. Significance was defined as P<0.05. Data are expressed as mean±SEM.

**Results**

Two hundred HCAs with a passive internal diameter of 100±3 μm (range, 30 to 271 μm) were dissected. Patient demographics are summarized in Table 1.
Mechanism of Hypoxic Vasodilation

Hypoxia produced a gradual but potent vasodilation in HCAs (Figure 1; 25±6, 56±7, and 75±5% at 5, 10, and 15 minutes, respectively; n=29). The reduction in PO2 was achieved within 5 minutes with minimal change in Pco2 or pH. On reoxygenation, vascular tone recovered rapidly to resting levels after transient peak dilation at 1 minute (79±5%). PO2, Pco2, and pH returned to original levels within 5 minutes. No change in diameter was observed during normoxia of similar duration (n=9). Vessels exposed to two consecutive 15-minute episodes of hypoxia (75% at 5, 10, and 15 minutes, respectively; n=15 years; coronary artery disease, 89%; male, 90%; age, 61±12 years; coronary artery disease, 89%; myocaridial infarction, 88%; myocardial infarction, 25%; hypertension, 33%; hypercholesterolemia, 44%; DM, 44%; congestive heart failure, 25%). The reduced response to hypoxia observed in the presence of glibenclamide could conceivably be the result of impaired KATP function or an inability of KATP to respond to a second episode of hypoxia. Dilation to aprikalim was similar before and after exposure to hypoxia (data not shown, P=NS, n=4). Thus, it is unlikely that hypoxia alters KATP activation during a subsequent period of hypoxia.

KATP-Mediated Vasodilation

We next tested whether KATP activation can dilate HCAs. Aprikalim produced potent vasodilation in a concentration-dependent manner with logED50 of 7.0±0.2 (vehicle produced no dilation; data not shown). Glibenclamide inhibited dilation to aprikalim (Figure 2B; maximum dilation, 22±6 versus control 90±4%, P<0.05, n=5) and attenuated diazoxide-induced dilation (maximum dilation, 13±5 versus control 44±4%, P<0.05, n=6) but had no effect on dilation to SNP (maximum dilation, 86±5 versus control 87±6%, P=NS, n=6) or papaverine (maximum dilation, 95±2 versus control 92±4%, P=NS, n=6). We previously showed that this dose of glibenclamide has no effect on the dilation to BK or shear stress.10 Aprikalim-induced dilation was unchanged by L-NAME (−logED50, 7.1±0.4 versus control 7.0±0.5, P=NS; maximum dilation, 93±3 versus control 93±3%, P=NS, n=5), indomethacin (−logED50, 6.9±0.4 versus control 7.0±0.5, P=NS; maximum dilation, 87±6 versus control 93±3%, P=NS, n=5), or endothelial denudation (−logED50, 6.4±0.3 versus control 6.2±0.3, P=NS; maximum dilation, 96±2 versus control 93±4%, P=NS, n=4). Thus, dilation to selective KATP opening with aprikalim is independent of the endothelium in HCAs.

Membrane Hyperpolarization to Aprikalim and Hypoxia

We measured the change in VSMCs Em to KATP activation. Figure 3A shows a typical example of the effect of aprikalim

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**Figure 1.** Vascular response to hypoxia and reoxygenation. Hypoxia produced a prominent and reversible vasodilation of HCAs (n=28; male, 80%; age, 61±15 years; coronary artery disease, 93%; myocardial infarction, 7%; hypertension, 27%; hypercholesterolemia, 17%; DM, 30%; congestive heart failure, 7%). After reoxygenation, the diameter returned to original levels. No change in vascular diameter was observed during normoxia of similar duration (n=9; male, 78%; age, 64±12 years; coronary artery disease, 89%; myocaridial infarction, 22%; hypertension, 33%; hypercholesterolemia, 44%; DM, 44%; congestive heart failure, 22%).

**Figure 2.** Role of KATP in dilation of HCAs to hypoxia and aprikalim. A, Glibenclamide (10−6 mol/L) reduced hypoxia-induced vasodilation (###P<0.005 vs control, n=12). Patient demographics: male, 100%; age, 67±9 years; coronary artery disease, 92%; myocardial infarction, 25%; hypertension, 58%; hypercholesterolemia, 17%; DM, 42%; congestive heart failure, 8%. B, Vessels dilated to aprikalim (10−10 to 10−5 mol/L) in a glibenclamide-sensitive (10−6 mol/L) manner (###P<0.05 vs control, n=5). Patient demographics: male, 60%; age, 51±25 years, coronary artery disease, 80%; myocardial infarction, 20%; hypertension, 0%; hypercholesterolemia, 0%; DM, 20%; congestive heart failure, 40%.
Figure 3. A, Changes in VSMC $E_m$ to aprikalim. Representative recording of $E_m$ in vessels at basal tone shows aprikalim-induced hyperpolarization. Arrows indicate time of drug application. Fifty-five-year-old man with coronary artery disease. B, Induced hyperpolarization. Arrows indicate time of drug application. Fifty-five-year-old man with coronary artery disease. C, After membrane depolarization and vasoconstriction, 33%; hypercholesterolemia, 33%; DM, 17%; congestive heart failure, 80%; myocardial infarction, 20%; hypertension, 60%; hypercholesterolemia, 0%; DM, 40%; congestive heart failure, 0%.

K<sub>A</sub>ATP Expression in the Coronary Microcirculation

In HCAs, RT-PCR detected transcripts for Kir6.1 and SUR2B from all subjects tested (Figures 4A and 4B), whereas mRNA for either Kir6.2, SUR1, or SUR2A was detected in only one subject.

Positive staining for Kir6.1 was observed by immunohistochemistry in HCAs (Figure 4C). VSMCs showed strong immunostaining for Kir6.1 protein, but endothelial cells were only faintly immunostained. This same pattern was observed in each of the three subjects.

Influence of Disease on K<sub>A</sub>ATP–Mediated Vasodilation

By multivariate analysis, only the presence of DM predicted the impaired vasodilation to hypoxia (Table 2). ANACOVA also indicated that DM was correlated with reduced dilation to hypoxia independent of other risk factors and conditions (Figure 5A; maximum dilation, DM(+) 56±8 versus DM(−) 85±5%, $P<0.01$, n=30).

DM was also identified by multivariate analysis and ANACOVA as the only independent predictor of reduced dilation to aprikalim (Table 2 and Figure 5B; $-\log[ED_{50}]$, DM(+) 6.3±0.3 versus DM(−) 7.3±0.2, $P<0.05$; maximum dilation, DM(+) 90±2 versus DM(−) 96±1%, $P<0.05$; n=34).

In contrast to hypoxia and aprikalim, no cardiovascular risk factors were associated with alterations in dilation to SNP or endothelium-derived hyperpolarizing factor–mediated vasodilation to BK by multivariate analysis and ANACOVA (SNP, Figure 5C; $-\log[ED_{50}]$, DM(+) 7.1±0.3 versus DM(−) 7.1±0.2, $P=NS$; maximum dilation, DM(+) 97±2 versus DM(−) 92±1%, $P=NS$, n=35 and (BK, Figure 5D; $-\log[ED_{50}]$, DM(+) 7.6±0.2 versus DM(−) 7.6±0.1, $P=NS$; maximum dilation, DM(+) 92±5 versus DM(−) 90±3%, $P=NS$, n=37). Therefore, it is unlikely that impaired dilation to aprikalim or hypoxia in patients with DM is attributable to a nonspecific reduction in dilation.

Resting $E_m$ from diabetic patients (n=14) and nondiabetic patients (n=35) was similar (DM $-47±2$ versus non-DM $-47±1$ mV, $P=NS$). No risk factor was predictive of an alteration in resting $E_m$ by either multivariate analysis or ANACOVA. Spontaneous vascular tone was likewise not correlated with disease (data not shown).

Although we were not able to obtain full medication information, it is likely that some diabetic patients were treated with sulfonylureas, which may have influenced dilation to aprikalim. When diabetic patients were divided

on $E_m$ of a VSMC in a cannulated and pressurized HCAs. Aprikalim produced hyperpolarization from a resting $E_m$ of $-52$ to $-54$, $-58$, and $-85$ mV at $10^{-5}$, $10^{-7}$, and $10^{-9}$ mol/L, respectively, in a dose-dependent fashion. BK did not additionally hyperpolarize the VSMC, indicating a near-maximal hyperpolarization. Increases in extraluminal KCl depolarized the VSMC. Summary data are shown in Figure 3B.

To link vasodilator and electrophysiological observations, we simultaneously measured changes in vessel diameter and $E_m$ during hypoxia. Figure 3C shows that hypoxia elicited dilation and hyperpolarization that were attenuated by glibenclamide (maximum dilation, 46±5 versus control 73±7%, $P<0.05$, n=5).

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DM could alter non-K<sub>ATP</sub>-mediated mechanisms of hypoxic dilation. Glibenclamide reduced hypoxic vasodilation less in arterioles from subjects with DM (maximum dilation, 27 ± 7 vs control 52 ± 10%, P < 0.05, n = 6) than from subjects without DM (maximum dilation, 26 ± 10 vs control 78 ± 7%, P < 0.05, n = 6). However, the residual dilation after glibenclamide was similar, suggesting that the major impairment in hypoxic dilation in DM is attributable to the K<sub>ATP</sub>-sensitive component.

**Discussion**

This study is the first to describe impaired vasodilation to hypoxia and K<sub>ATP</sub> activation in HCAs from patients with DM. The major new findings are 5-fold. First, hypoxia induces a

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**TABLE 2. Multivariate Analysis of the Influence of Underlying Diseases, Sex, and Age on HCA Dilations**

<table>
<thead>
<tr>
<th>Disease</th>
<th>%Max. Dilation (R&lt;sup&gt;2&lt;/sup&gt;=0.41)</th>
<th>%Max. Dilation (R&lt;sup&gt;2&lt;/sup&gt;=0.35)</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (R&lt;sup&gt;2&lt;/sup&gt;=0.40)</th>
<th>%Max. Dilation (R&lt;sup&gt;2&lt;/sup&gt;=0.21)</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (R&lt;sup&gt;2&lt;/sup&gt;=0.18)</th>
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<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>SE</td>
<td>P</td>
<td>Coefficient</td>
<td>SE</td>
</tr>
<tr>
<td>DM</td>
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<td>10.0</td>
<td>0.01*</td>
<td>-5.6</td>
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<td>0.10</td>
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</tr>
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<tr>
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<td>0.70</td>
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</tr>
<tr>
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<td>24.4</td>
<td>0.66</td>
<td>3.5</td>
<td>3.9</td>
</tr>
<tr>
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<td>20.3</td>
<td>0.17</td>
<td>2.8</td>
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<tr>
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<td>17.6</td>
<td>0.92</td>
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<tr>
<td>Age</td>
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<td>0.5</td>
<td>0.74</td>
<td>-0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

DM indicates diabetes mellitus; HTN, hypertension; HC, hypercholesterolemia; CHF, congestive heart failure; CAD, coronary artery disease; MI, myocardial infarction; SE, standard error; and Max., maximum. n indicates the number of patients studied. *P<0.05.
potent and endothelium-independent vasodilation. Second, K$_{ATP}$ activation dose-dependently hyperpolarizes VSMCs and induces vasodilation. Third, the mechanism of hypoxia-induced vasodilation involves opening of K$_{ATP}$. Fourth, vasodilation to both hypoxia and K$_{ATP}$ stimulation is impaired in both type 1 and type 2 DM. Finally, mRNA and protein for K$_{ATP}$ subunits are present in HCAs, K$_{ATP}$ possibly being comprised of SUR2B and Kir6.1. These findings indicate that in DM, vasodilator function associated with K$_{ATP}$ opening is reduced in the human coronary microcirculation.

**Vasodilation to Aprikalim**

Human vessels can dilate in response to K$_{ATP}$ openers,$^{17,18}$ however, the mechanism is not clear. Vasodilations to both aprikalim and diazoxide were attenuated by glibenclamide, but dilations to SNP, papaverine, BK,$^8$ and shear stress$^{10}$ were not affected. Patch-clamp analysis shows that glibenclamide blocks K$_{ATP}$ current in response to K$_{ATP}$ openers in VSMCs from human coronary arteries.$^{19}$ These findings indicate that K$_{ATP}$ openers dilate HCAs by selectively opening K$_{ATP}$. We demonstrated that aprikalim-induced vasodilation was not affected by inhibiting NO synthase or cyclooxygenase using doses of antagonists that are effective in human vessels.$^{8,10,11,20,21}$ This is similar to findings in rat cerebral and porcine coronary arterioles.$^{2,4}$ However, it contrasts with observations made in small cerebral arteries of diabetic rats.$^5$

**Hypoxia-Induced Vasodilation**

Several animal studies have reported that K$_{ATP}$ opening mediates hypoxia-induced vasodilation in conduit and resistance arteries.$^{1,2}$ However, inhibition of K$_{ATP}$ is reported to be ineffective in attenuating hypoxia-induced vasorelaxation in rabbit aorta and porcine coronary arteries.$^{22,23}$ In the present study, glibenclamide significantly reduced hypoxic vasodilation in HCAs, suggesting that K$_{ATP}$ are involved in transducing vascular signals to reduced tissue oxygen concentrations in the human heart.

In the present study, NO did not contribute to hypoxia-induced vasodilation. However, results from animal studies are varied, some indicating an important role for NO in isolated guinea pig hearts and rabbit coronary arteries$^{22,24}$ and others showing no effect in porcine cerebral arterioles and small coronary arteries.$^2,25$ The contribution of NO varies depending on the vascular bed, species, vessel size (conduit or resistance arteries), or experimental preparations. This highlights the importance of examining responses in vessels from human subjects.

An enhanced dilation to hypoxia at 5 minutes was observed in the presence of indomethacin. A similar augmentation has been described in porcine, monkey, and human coronary arteries.$^{20,26}$ Human endothelial cells showed the release of vasoconstrictor prostaglandins during the early phase of hypoxia.$^{21}$

Hypoxia-induced vasodilation is endothelium-dependent in porcine small coronary arteries ($\approx$170 $\mu$m diameter),$^2$ whereas it is endothelium-independent in porcine coronary arteries ($\approx$1 mm diameter).$^{27}$ In human conduit coronary arteries, Siegel et al$^{28}$ reported that the contribution of endothelium to hypoxia-induced vasodilation is 49%, whereas Toda et al$^{20}$ reported no alteration after endothelial dednation. Our study identifies a unique response in the coronary microcirculation. We observed a direct hyperpolarizing and vasorelaxing effect of hypoxia on coronary smooth muscle cells that is endothelium-independent.

**K$_{ATP}$ Expression in the Coronary Microcirculation**

The molecular profile of vascular K$_{ATP}$, especially in the coronary microcirculation, is not fully determined. Part of the
difficulty arises from the complex nature of the channel, in that \( K_{ATP} \) are octamers composed of four Kir6.0 subfamily subunits (Kir6.1 and 6.2) and four SUR subunits (SUR1 and SUR2), combined into a heteromultimeric complex. \( K_{ATP} \) in VSMCs are proposed to comprise SUR2B and Kir6.1, based on similar electrophysiological characteristics of the reconstituted channels to the native one. A recent study using a genetic mouse model lacking Kir6.1 showed the absence of \( K_{ATP} \) activity in VSMCs, suggesting a critical role of Kir6.1 for vascular \( K_{ATP} \) activity. It is also reported that microvascular endothelial cells in the guinea pig heart express \( K_{ATP} \) composed of SUR2B and Kir6.1 and/or Kir6.2 subunits. These observations are consistent with our results showing the mRNA expression of Kir6.1, Kir6.2, and SUR2B and the localization of Kir6.1 protein in HCAs. RT-PCR revealed the expression of SUR1 and SUR2A in only one of three vessels tested. This may be attributed to presence of inflammatory cells or perivascular cells, including myocytes and neurons, which express SUR1 and SUR2A, or a compensatory upregulation of this channel subunit. Additional studies should examine these possibilities.

**DM and Impairment of \( K_{ATP} \)-Mediated Vasodilation**

The mechanism of impaired vasodilation to \( K_{ATP} \) activation is not clear. We found impaired dilation to aprikalim and hypoxia but not to SNP and BK in HCAs from subjects with diabetes. Thus, the impairment seems to be specific for \( K_{ATP} \) mechanisms, because VSMC relaxation followed by decrease in intracellular \( Ca^{2+} \) concentration either by cGMP production attributable to NO (SNP) or by membrane hyperpolarization through \( Ca^{2+} \)-activated \( K^+ \) channel activation, as occurs with BK, was not reduced. As with other studies, we observed reduced vasodilation to \( K_{ATP} \) opening. In contrast to our observations, an enhanced vasodilation to aprikalim was seen in diabetic canine coronary arterioles. This discrepancy might be dependent on the disease duration (months to years in rat models and humans versus hours to days in canine models) and/or the different experimental methods (in vitro versus in vivo).

**Potential Problems**

Sulfonylureas are clinically prescribed \( K_{ATP} \) blockers. In clinically relevant concentrations, glibenclamide increases basal coronary resistance and attenuates vasodilation to hypoxia in the heart. Because of the nature of the IRB approval, we are unable to retrospectively identify medication use. Some diabetic patients may have been treated with sulfonylureas, and it is possible that effect of these medicines could be responsible for the reduced dilation to aprikalim and hypoxia. For two reasons, we believe that such an effect, if present, was small and did not influence our interpretation of the results. First, the effect of aprikalim is fully reversible, because rinsing vessels with Krebs solution eliminated the inhibitory effect of glibenclamide on aprikalim-induced vasodilation. Multiple rinses during dissection and before experimentation would be expected to remove previously administered sulfonylureas. Second, the impaired dilation to aprikalim or hypoxia was seen in both type 1 and type 2 DM. It is unlikely that type 1 diabetic patients were treated with sulfonylureas. These reasons make it unlikely that retained or chronic effects of diabetic medications contributed to the impaired dilation to hypoxia and aprikalim.

In the present study, glibenclamide did not completely abolish vasodilation to hypoxia. Other factors may be involved in hypoxic dilation, as reported in other tissues, including \( Ca^{2+} \)-activated \( K^+ \) channels and cytochrome P450 metabolites, decrease in tissue pH, adenosine receptor activation, or production of lactate. An insufficient dose of glibenclamide is not likely, because the same dose markedly inhibited dilation to aprikalim. It is unlikely that non-\( K_{ATP} \) mechanisms are responsible for the reduced response to hypoxia, because the residual dilation to hypoxia after glibenclamide is similar in both DM and non-DM subjects.

**Clinical Implications**

DM is associated with an increased risk of cardiovascular and cerebrovascular morbidity and mortality. In addition to the more accelerated conduit coronary atherosclerosis, DM is associated with diffuse microvascular disease. Even for the same degree of atherosclerosis, patients with DM suffer greater morbidity from ischemia. Clinical studies indicate that DM impairs myocardial perfusion. Our results dem-
onstrate impaired dilation to KATP opening in DM. This could impose vasodilator responses, especially during ischemia or hypoxia in patients with DM, possibly contributing to increased myocardial ischemic injury in these patients.

In summary, in HCA, hypoxia-induced vasodilation is mediated largely by activation of KATP. Vasodilations to hypoxia and direct KATP opening are impaired in DM. This coronary microcirculatory dysfunction may contribute to the higher cardiovascular mortality and morbidity in DM.

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