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} participates 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 transcriptase–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 transcriptase–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. Baseline myogenic tone and resting membrane potential were not affected by DM. We conclude that DM impairs human coronary arteriolar dilation to K_{ATP} opening, leading to reduced dilation to hypoxia. This reduction in K_{ATP} function could contribute to the greater cardiovascular mortality and morbidity in DM.

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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} participates 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} is 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) was prepared in 50% ethanol and distilled water. Endothelin-1 (Peninsula Laboratories, Inc.) 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^{-3} \text{ mol/L}), confirming integrity of endothelial function. Endothelin-1 (\approx 10^{-10} \text{ mol/L}) or acetylcholine (\approx 5 \times 10^{-7} \text{ 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} \text{ mol/L}, a selective K_{ATP} opener) was studied in the presence and absence of N^\circ-nitro-L-arginine methyl ester (L-NAME) (10^{-4} \text{ mol/L}, an NO synthase inhibitor), indomethacin (10^{-4} \text{ mol/L}, a cyclooxygenase inhibitor), glibenclamide (10^{-5} \text{ mol/L}, a selective K_{ATP} 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} \text{ mol/L}, an endothelium-dependent vasodilator, was tested in the presence of L-NAME and indomethacin. Vasodilation to sodium nitroprusside (SNP) (10^{-10} to 10^{-4} \text{ mol/L}) or papaverine (10^{-4} \text{ mol/L}), endothelium-independent dilators, was also tested. In some vessels, diazoxide (10^{-2} to 10^{-4} \text{ mol/L}, another K_{ATP} 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 tone was maintained at 90% of the maximal response to BK, and CO2 returned to original levels within 5 minutes with minimal change in P CO2 or pH. Reoxygenation, vascular tone recovered rapidly to resting E_m and vascular tone. Regression models for all dosing or time intervals isolated the confounding effects of the other diseases on dilator responses. Analysis of covariance (ANCOVA) 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 P O2 was achieved within 5 minutes with minimal change in P CO2 or pH. On reoxygenation, vascular tone recovered rapidly to resting levels after transient peak dilation at 1 minute (79±5%). P O2, P CO2, 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 periods of hypoxia separated by a 60-minute period of intervening normoxia demonstrated similar degrees of vasodilation (data not shown, P=NS, n=6).

Treatment with L-NAME did not alter vasodilation to hypoxia (75±7 versus control 87±9%, P=NS at 15 minutes, n=6). Indomethacin potentiated dilation only at 5 minutes (59±10 versus control 24±10%, P<0.05). Endothelial removal had no effect (68±10 versus control 75±14%, P=NS at 15 minutes, n=4). Thus, the endothelium is not critical for hypoxic vasodilation of HCAs.

Glibenclamide reduced hypoxia-induced vasodilation (Figure 2A; 27±6 versus control 65±7%, P<0.005 at 15 minutes, n=12). The reduced response to hypoxia observed in the presence of glibenclamide could conceivably be the result of impaired K_{ATP} function or an inability of K_{ATP} to respond to a

### Immunohistochemistry

Small pieces (\approx 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.

### Measurement of Vascular Smooth Muscle Membrane Potential

We measured resting membrane potential (E_m) of vascular smooth muscle cells (VSMCs) and changes in E_m 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). Measurement of vascular smooth muscle membrane potential (E_m) of pectinate muscle were fixed with 4% paraformaldehyde.
second episode of hypoxia. Dilation to aprikalim was similar before and after exposure to hypoxia (data not shown, \( P = \text{NS}, n = 8 \)). Therefore, it is unlikely that hypoxia alters \( K_{\text{ATP}} \) activation during a subsequent period of hypoxia.

**K\textsubscript{ATP}-Mediated Vasodilation**

We next tested whether \( K_{\text{ATP}} \) activation can dilate HCAs. Aprikalim produced potent vasodilation in a concentration-dependent manner with \( -\log[\text{ED}_{50}] \) value 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%, \( P = \text{NS}, n = 6 \)) or papaverine (maximum dilation, 95±2 versus control 92±4%, \( P = \text{NS}, n = 6 \)). We previously showed that this dose of glibenclamide has no effect on the dilation to BK\(^8 \) or shear stress.\(^{10} \) Aprikalim-induced dilation was unchanged by L-NAME (\( -\log[\text{ED}_{50}] \)), 7.1±0.4 versus control 7.0±0.5, \( P = \text{NS} \); maximum dilation, 93±3 versus control 93±3%, \( P = \text{NS}, n = 5 \), indomethacin (\( -\log[\text{ED}_{50}] \)), 6.9±0.4 versus control 7.0±0.5, \( P = \text{NS} \); maximum dilation, 87±6 versus control 93±3%, \( P = \text{NS}, n = 5 \), or endothelial denudation (\( -\log[\text{ED}_{50}] \)), 6.4±0.3 versus control 6.2±0.3, \( P = \text{NS} \); maximum dilation, 96±2 versus control 93±4%, \( P = \text{NS}, n = 4 \). Thus, dilation to selective \( K_{\text{ATP}} \) opening with aprikalim is independent of the endothelium in HCAs.

**TABLE 1. Demographics (n=200)**

<table>
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Data are mean±SD. \( n \) indicates the number of patients studied.

**Figure 1.** Vascular response to hypoxia and reoxygenation. Hypoxia produced a prominent and reversible vasodilation of HCAs (\( n = 29 \); male, 90%; 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 normoxic experiments of similar duration (\( n = 9 \); male, 78%; age, 64±12 years; coronary artery disease, 89%; myocardial infarction, 22%; hypertension, 33%; hypercholesterolemia, 44%; DM, 44%; congestive heart failure, 22%).
Membrane Hyperpolarization to Aprikalim and Hypoxia

We measured the change in $V_{SMC}$ to $K_{ATP}$ activation. Figure 3A shows a typical example of the effect of aprikalim on $E_m$ of a VSMC in a cannulated and pressurized HCA. Aprikalim produced hyperpolarization from a resting $E_m$ of $-52$, $-54$, $-58$, and $-85\text{ mV}$ at $10^{-9}$, $10^{-7}$, and $10^{-5}\text{ mol/L}$, 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 (dilation, $46\pm5$ versus control $73\pm7\%$, $P<0.05$, $n=5$).

$K_{ATP}$ 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 $K_{ATP}$–Mediated Vasodilation

By multivariate analysis, only the presence of DM predicted the impaired vasodilation to hypoxia (Table 2). ANCOVA also indicated that DM was correlated with reduced dilation to hypoxia independent of other risk factors and conditions (Figure 5A; maximum dilation, DM$^{+}$ $56\pm8$ versus DM$^{-}$ $85\pm5\%$, $P<0.01$, $n=30$).

DM was also identified by multivariate analysis and ANCOVA as the only independent predictor of reduced dilation to aprikalim (Table 2 and Figure 5B; $-\log[ED_{50}]$, DM$^{+}$ $6.3\pm0.3$ versus DM$^{-}$ $7.3\pm0.2$, $P<0.05$; maximum dilation, DM$^{+}$ $90\pm2$ versus DM$^{-}$ $96\pm1\%$, $P<0.05$; $n=34$).

In contrast to hypoxia and aprikalim, no cardiovascular risk factors were associated with alterations in dilation to SNP or in endothelium-derived hyperpolarizing factor–mediated vasodilation to BK except by multivariate analysis and ANCOVA (SNP, Figure 5C; $-\log[ED_{50}]$, DM$^{+}$ $7.1\pm0.3$ versus DM$^{-}$ $7.1\pm0.2$, $P=NS$; maximum dilation, DM$^{+}$ $97\pm2$ versus DM$^{-}$ $92\pm1\%$, $P=NS$, $n=35$) and (BK, Figure 5D; $-\log[ED_{50}]$, DM$^{+}$ $7.6\pm0.2$ versus DM$^{-}$ $7.6\pm0.1$, $P=NS$; maximum dilation, DM$^{+}$ $92\pm5$ versus DM$^{-}$ $90\pm3\%$, $P=NS$, $n=37$). 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 $-47\pm2$ versus non-DM $-50\pm2\%$, $P=NS$, $n=49$).

**Figure 2.** Role of $K_{ATP}$ in dilation of HCAs to hypoxia and aprikalim. A, Glibenclamide ($10^{-6}\text{ mol/L}$) 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 $10^{-5}\text{ mol/L}$) in a glibenclamide-sensitive ($10^{-6}\text{ mol/L}$) 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%.
−47±1 mV, P=NS). No risk factor was predictive of an alteration in resting $E_m$ by either multivariate analysis or ANCOVA. 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 according to subtype, hypoxia-induced vasodilation was similarly reduced in both type 1 (n=8) and type 2 (n=8) DM compared with non-DM controls (n=25) (Figure 6A; maximum dilation, type 1 21±3 and type 2 45±13 versus non-DM 81±5%, respectively, $P<0.05$). Vasodilation to aprikalim was similarly reduced in both type 1 (n=6) and type 2 (n=8) DM compared with the non-DM group (n=31) (Figure 6B; $-\log[ED_{50}]$, type 1 6.3±0.2 and type 2 6.1±0.2 versus...
non-DM 7.1±0.1, P<0.05 respectively; maximum dilation, type 1 84±6 and type 2 91±2 versus non-DM 95±1%, P=NS). In addition, aprikalim-induced dilation was unchanged before and after a 30-minute exposure and washout of glibenclamide (maximum dilation, after 97±2 versus before 99±0%; -log[ED50], after 7.4±0.3 versus before 7.3±0.3, P=NS, n=6), indicating reversible inhibition of glibenclamide.

DM could alter non-KATP-mediated mechanisms of hypoxic dilation. Glibenclamide reduced hypoxic vasodilation less in arterioles from subjects with DM (maximum dilation, 27±7 versus control 52±10%, P<0.05, n=6) than from subjects without DM (maximum dilation, 26±10 versus control 78±7%, P<0.05, n=6). However, the residual dilation after glibenclamide was similar, suggesting that the

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

<table>
<thead>
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<th>Hypoxia (n=30)</th>
<th>Aprikalim (n=34)</th>
<th>SNP (n=35)</th>
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<td>%Max. Dilation (R2=0.35)</td>
<td>ED50 (R2=0.40)</td>
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</tr>
<tr>
<td>Age</td>
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<td>0.74</td>
</tr>
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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.
major impairment in hypoxic dilation in DM is attributable to the K_ATP-sensitive component.

**Discussion**

This study is the first to describe impaired vasodilation to hypoxia and K_ATP activation in HCAs from patients with DM. The major new findings are 5-fold. First, hypoxia induces a 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; however, the mechanism is not clear. Vasodilations to both aprikalim and diazoxide were attenuated by glibenclamide, but dilations to SNP, papaverine, BK, and shear stress 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. 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. This is similar to findings in rat cerebral and porcine coronary arterioles. However, it contrasts with observations made in small cerebral arteries of diabetic rats.

**Hypoxia-Induced Vasodilation**

Several animal studies have reported that K_ATP opening mediates hypoxia-induced vasodilation in conduit and resistance arteries. However, inhibition of K_ATP is reported to be ineffective in attenuating hypoxia-induced vasorelaxation in rabbit aorta and porcine coronary arteries. In the present study, glibenclamide significantly reduced hypoxic vasodilation in HCAs, suggesting that K_ATP is 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 and others showing no effect in porcine cerebral arterioles and small coronary arteries. 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. Hypoxia-induced vasodilation is endothelium-dependent in porcine small coronary arteries (≈170 μm diameter), whereas it is endothelium-independent in porcine coronary arteries (≈1 mm diameter). In human conduit coronary arteries, Siegel et al reported that the contribution of endothelium to hypoxia-induced vasodilation is 49%, whereas Toda et al reported no alteration after endothelial denudation. 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\textsubscript{ATP} Expression in the Coronary Microcirculation

The molecular profile of vascular K\textsubscript{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\textsubscript{ATP} is an octamer 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\textsubscript{ATP} in VSMCs is 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\textsubscript{ATP} activity in VSMCs, suggesting a critical role of Kir6.1 for vascular K\textsubscript{ATP} activity. It is also reported that microvascular endothelial cells in the guinea pig heart express K\textsubscript{ATP} composed of SUR2B and Kir6.1 or Kir6.2 subunits. These observations are consistent with our results showing the mRNA expression of Kir6.1, Kir6.2, and SUR1 and SUR2 in HCAs. RT-PCR revealed the expression of SUR1 and SUR2 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 SUR2, or a compensatory upregulation of this channel subunit. Additional studies should examine these possibilities.

DM and Impairment of K\textsubscript{ATP}-Mediated Vasodilation

The mechanism of impaired vasodilation to K\textsubscript{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.$^8$ was not reduced. As with other studies,$^3$$^5$ 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.$^6$ This discrepancy might be dependent on the disease duration (months to years in rat models and humans versus hours to days in canine models) 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$^{53,34}$ and attenuates vasodilation to hypoxia in the heart.$^1,2$ 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,$^{25,35}$ decrease in tissue pH,$^{36}$ adenosine receptor activation,$^{37}$ or production of lactate.$^{38}$ 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.$^3$ Clinical studies indicate that DM impairs myocardial perfusion.$^{40,41}$ Our results demonstrate impaired dilation to $K_{ATP}$ opening in DM. This could impede vasodilator responses, especially during ischemia or hypoxia in patients with DM, possibly contributing to increased myocardial ischemic injury in these patients.

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

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Reduced Activity of ATP-Sensitive Potassium Channels
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