Endothelial ATP-sensitive potassium channels mediate coronary microvascular dilation to hyperosmolarity

HIROSHI ISHIZAKA AND LIH KUO
Department of Medical Physiology, Microcirculation Research Institute, Texas A&M University Health Science Center, College Station, Texas 77843–1114

Ishizaka, Hiroshi, and Lih Kuo. Endothelial ATP-sensitive potassium channels mediate coronary microvascular dilation to hyperosmolarity. *Am. J. Physiol.* 273 (*Heart Circ. Physiol.* 42): H104–H112, 1997.—Coronary arterial occlusion has been shown to increase osmolarity in the myocardial interstitium. Intracoronary injection of hyperosmolar solutions reduces coronary vascular resistance. However, the response of coronary microvessels to an abluminal increase in osmolarity is unclear, and the underlying mechanism for its vasomotor regulation has not been elucidated. In this regard, porcine coronary arterioles (81 ± 2 μm) were isolated, cannulated, and pressurized for in vitro study. Hyperosmolarity (300–345 mosM) was produced by adding D-glucose or D-sucrose to the extravascular solution. After the arterioles developed a stable vascular tone, a graded vasodilation was observed when glucose or sucrose was incrementally administered. This hyperosmotic vasodilation was abolished after endothelial removal. Intraluminal administration of KCl (80 mM) or the ATP-sensitive potassium (K_{ATP})-channel inhibitor glibenclamide (1 μM) to the intact vessels significantly attenuated the hyperosmotic vasodilation. Inhibition of inward rectifier potassium channels by a low concentration of $BaCl_2$ (10 μM) did not affect vasodilation. However, a high concentration of $BaCl_2$ (100 μM), which has been reported to inhibit K_{ATP} channels, attenuated the hyperosmotic vasodilation. Iberiotoxin (100 nM), a calcium-activated potassium (K_{Ca})-channel inhibitor, had no effect on hyperosmolarity-induced vasodilation. Inhibition of the synthesis of endothelial nitric oxide, prostaglandins, and arachidonic acid metabolites from cytochrome *P*-450 had no effect on hyperosmotic vasodilation. Furthermore, inhibition of vascular smooth muscle K_{ATP} channels and the large- and small-conductance K_{Ca} channels by extraluminal administration of glibenclamide, iberiotoxin, and apamin, respectively, did not alter vasodilation in response to hyperosmolarity. These results indicate that dilation of coronary arterioles in response to hyperosmotic stimulation requires an intact endothelium. However, the response is independent of the release of nitric oxide, prostaglandins, or cytochrome *P*-450-related endothelium-derived hyperpolarizing factor and is not a result of activation of K_{ATP} and K_{Ca} channels in vascular smooth muscle. It is suggested that the opening of K_{ATP} channels in vascular endothelium and subsequent hyperpolarization of that cell type mediate coronary microvascular dilation in response to hyperosmolarity.

microcirculation; nitric oxide; arterioles; endothelium-derived hyperpolarizing factor

CORONARY BLOOD FLOW is primarily determined by coronary microvascular resistance (7), which is normally closely matched to the metabolic demand of the heart. Oxygen, carbon dioxide, hydrogen ion, potassium, and adenosine have been suggested to be mediators responsible for metabolic control of coronary blood flow (30). In addition to these factors, an increase in interstitial osmolarity also has been implicated in this process (2). In vivo studies indicate that administration of hyperosmotic solutions to the coronary circulation reduces coronary resistance in direct proportion to the degree of hyperosmolarity (13, 38) as a result of relaxation of the coronary microvasculature (38). However, it is not clear whether coronary microvessels are able to dilate in response to an increase in interstitial osmolarity. In anesthetized dogs, a brief (15-s) coronary occlusion increases coronary sinus blood osmolarity (34). In addition, the interstitial osmolarity of the myocardium increases by 20 and 40 mosM within 10 and 50 min, respectively, after coronary occlusion in porcine hearts (37). Increased interstitial osmolarity has been proposed to be one of the factors responsible for the coronary hyperemia after release of occlusion (34). However, it is not clear whether coronary microvessels are responsive to these degrees of changes in osmolarity, and the extent of vascular dilation to hyperosmotic stimulation has not been characterized. Furthermore, the underlying mechanism responsible for the vasomotor response to hyperosmolarity has not been elucidated.

It is well established that the vascular endothelium, in response to various stimuli, participates in the regulation of vascular tone by releasing vasoactive substances (3, 8) or by transducing electronic potentials to the underlying vascular smooth muscle (5). A study of isolated coronary arterial rings indicates that hyperosmolarity-induced relaxation is mainly due to hyperpolarization of vascular smooth muscle (17). Because interstitial osmolarity has been implicated as a factor that regulates coronary vascular tone and hyperpolarization via activation of potassium channels is a major vasodilatory mechanism (28), the purposes of this study were *1*) to characterize the microvascular response to hyperosmolarity and *2*) to examine whether the endothelium and specific potassium channels are involved in the dilation of coronary arterioles to hyperosmotic stimulation. To accomplish these goals, the response of coronary arterioles (60–100 μm in diameter) to extravascular hyperosmolarity was examined in the presence and absence of endothelium, and the role of potassium channels in hyperosmotic vasodilation was determined by using various potassium channel inhibitors.

MATERIALS AND METHODS

General preparation. Pigs (10–15 kg; either sex) were sedated with Telazol (tiletamine and zolapepam, 1:1, 4.4 mg/kg im) and xylazine (2.2 mg/kg im) and then anesthetized and heparinized with pentobarbital sodium (20 mg/kg iv) and heparin (1,000 U/kg iv), respectively, via the marginal ear

vein. Pigs were intubated and ventilated with room air. After a left thoracotomy was performed, the heart was electrically fibrillated, excised, and immediately placed in cold (5°C) saline solution.

Isolation and cannulation of microvessels. The techniques for isolation of porcine coronary arterioles have been described previously (20). In brief, a mixture of india ink and gelatin in physiological saline solution (PSS) containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgCl_2$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 3-(*N*-morpholino)propanesulfonic acid was perfused into the left anterior descending artery and the circumflex artery to enable visualization of the coronary microvessels. The subepicardial coronary arterioles (internal diameter 60–100 μm, length without branches 0.6–0.8 mm) were dissected from surrounding cardiac tissue under cold (5°C) PSS containing bovine albumin (1%; US Biochemical) at pH 7.4. Each isolated microvessel was then transferred for cannulation to a Lucite vessel chamber containing albumin-PSS (pH 7.4) equilibrated with room air at ambient temperature. One end of the microvessel was cannulated with a glass micropipette filled with filtered PSS-albumin, and the outside of the microvessel was securely tied to the pipette with 11–0 ophthalmic suture (Alcon). The ink-gelatin solution inside the vessel was flushed out at a low perfusion pressure (<20 cmH_2O). The other end of the vessel was cannulated with a second micropipette and secured with suture. We have previously shown that the ink-gelatin solution has no detectable detrimental effect on either endothelial or vascular smooth muscle function (19,20).

Instrumentation. After a vessel was cannulated, the preparation was transferred to the stage of an inverted microscope (Diaphot 300, Nikon) coupled to a CCD camera (TM-34KC, Pulnix) and video micrometer (Microcirculation Research Institute, Texas A&M University Health Science Center). Internal diameters of the vessels were measured throughout the experiment by using videomicroscopic techniques incorporated with the MacLab data-acquisition system (ADInstruments) (19). The micropipettes were connected to independent pressure-reservoir systems, and intraluminal pressures were measured through side arms of the two reservoir lines by low-volume displacement strain-gauge transducers (Statham P23 Db, Gould). The isolated vessels were pressurized without flow by setting both reservoirs at the same hydrostatic level. Leaks were detected by closing off the system to the reservoirs and examining for a decline in intraluminal pressure. Preparations with leaks were excluded from further study.

Experimental protocols. Each cannulated vessel was bathed in albumin-PSS and equilibrated with room air. The pH and P_{O_2} values in the vessel bath were 7.4 and 149 ± 1 mmHg, respectively. The bath temperature was maintained at 36–37°C by an external heat exchanger. The vessel was set to its in situ length and allowed to develop spontaneous tone at 60 cmH_2O intraluminal pressure without flow. This pressure is comparable with that in coronary arterioles of the same size in vivo (7). After a stabilization period of 60 min, the effect of hyperosmolarity on vascular diameter was studied by adding D-glucose or D-sucrose to the vessel bath. The concentration of glucose or sucrose in the vessel bath was incrementally increased from control (5 mM) to 10, 20, and 40 mM, which corresponded to the osmolarity (measured by a vapor pressure osmometer, model 5500, Wescor) of 300, 310, 325, and 345 mosM, respectively. The change in vessel diameter was then measured after each step of osmolarity alterations. After the diameter-osmolarity relationship was examined, the arteriole was washed with albumin-PSS (300 mosM) and equilibrated for 30 min. In some control experiments, the glucose- and sucrose-induced dilation was studied repeatedly to confirm its reproducibility.

To assess the role of endothelium in the vascular response to hyperosmolarity, the diameter-osmolarity relation was studied before and after endothelial denudation. To examine whether endothelial hyperpolarization contributes to the hyperosmolarity-induced coronary arteriolar dilation, the intraluminal fluid of the vessel was replaced with a solution containing a high concentration of potassium (80 mM) to inhibit endothelial hyperpolarization. To accomplish this protocol, an isotonic high-potassium albumin-PSS solution was prepared by substituting 75 mM NaCl with an equimolar amount of KCl. After 10 min of incubation, the vasodilation to hyperosmotic glucose was examined. To evaluate the role of different potassium channels in hyperosmotic vasodilation to glucose, the vasomotor response was examined before and after intraluminal incubation of the intact vessels with various potassium-channel inhibitors. Specifically, the involvement of ATP-sensitive potassium (K_{ATP}) channels was evaluated by the selective inhibitor glibenclamide (1 μM) (28, 36). The role of calcium-activated potassium (K_{Ca}) channels and the inward rectifier potassium (K_{ir}) channels was examined by the specific inhibitor iberiotoxin (100 nM) and $BaCl_2$ (10 μM) (28), respectively. The effect of inhibition of K_{ATP} channels by a high concentration of $BaCl_2$ (100 μM) (28) was also examined. In addition, the effect of glibenclamide (1 μM) on vasodilation to sucrose-induced hyperosmolarity was evaluated. In each protocol, the intraluminal solution (PSS-albumin) was replaced by PSS-albumin containing an inhibitor. The technique for exchange of intraluminal solutions has been established previously (18). In the process of replacing solutions, vessels dilated transiently because of the initiation of intraluminal flow [flow-induced vasodilation (19)]. After the completion of the solution exchange, vessels gradually regained tone within 10 min and were incubated with the inhibitor for 20 min. The vasodilation to hyperosmolarity was examined before and after administration of inhibitor.

To study the involvement of nitric oxide and prostaglandins in hyperosmolarity-induced vasodilation, the isolated intact vessels were treated with the specific inhibitors N^G-nitro-L-arginine methyl ester (L-NAME; 10 μM) and indomethacin (10 μM), respectively. To examine the involvement of endothelium-derived hyperpolarizing factor (EDHF), which has recently been demonstrated as a cytochrome *P*-450-derived arachidonic acid metabolite in the coronary microcirculation (4), vessels were treated with the cytochrome *P*-450 inhibitor clotrimazole (30 μM). In these protocols, the inhibitors were administrated intraluminally and incubated with the vessels for 30 min. The arteriolar response to glucose-induced hyperosmolarity was examined before and after exposure to each inhibitor.

An as-yet unidentified EDHF has been shown to elicit vasodilation by opening vascular smooth muscle K_{ATP} channels (6, 36) as well as large- (12) and small-conductance (27) K_{Ca} channels. The possible involvement of these smooth muscle potassium channels in the vasodilation to hyperosmolarity was examined by extraluminal administration (20 min) of their specific inhibitors glibenclamide (5 μM), iberiotoxin (100 nM), and apamin (1 μM), respectively.

Finally, to evaluate whether glibenclamide had a nonspecific effect on vasodilatory function, the dose-dependent dilation of isolated vessels to sodium nitroprusside (10^{-8}–10^{-5} M) was examined in the absence and presence of intraluminal glibenclamide (1 μM for 20 min) or KCl (80 mM for 10 min). At the end of each experiment, vessels were relaxed completely with nitroprusside sodium (100 μM) to obtain the maximum diameter at 60 cmH_2O intraluminal pressure.

Endothelium denudation. A nonionic detergent, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, 0.4%), was intraluminally perfused into the vessel for 1–2 min to remove endothelial cells (15). After disruption of the endothelium, as verified by the absence of vasodilation in response to the endothelium-dependent vasodilator bradykinin (100 nM), the vessel was perfused with PSS-albumin for 5 min to remove CHAPS. To ensure that the vascular smooth muscle function was not compromised by CHAPS treatment, dose-dependent dilation of the vessel in response to sodium nitroprusside (10^{-8}–10^{-5} M) was examined before and after denudation. Only vessels that exhibited normal spontaneous tone, showed no vasodilation to bradykinin, and showed unaltered vasodilation to nitroprusside after endothelial removal were accepted for data analysis.

Chemicals. Drugs were obtained from Sigma Chemical, except as specifically stated. Iberiotoxin, $BaCl_2$, L-NAME, bradykinin, and sodium nitroprusside were dissolved in PSS. Glibenclamide and indomethacin were dissolved in dimethyl sulfoxide (DMSO) and then diluted in PSS to obtain the desired final concentration. Clotrimazole was dissolved in ethanol and diluted in PSS. The final concentrations of DMSO and ethanol were 0.025 and 0.01%, respectively. A vehicle-control study indicated that this concentration of DMSO and ethanol had no effect on the arteriolar responses to hyperosmolarity.

Data analysis. All diameter changes were normalized to the maximum dilation in the presence of sodium nitroprusside (100 μM) and expressed as a percentage of maximum dilation. All data are presented as means ± SE. The changes in osmolarity in bath solution by glucose and sucrose and the vasodilation to various levels of osmolarity were analyzed with one-way analysis of variance (ANOVA) and tested with Fisher's protected least significant difference multiple-range test. Statistical comparisons of hyperosmolarity-induced vasodilation under different treatments were performed with two-way ANOVA. Differences in baseline diameter before and after pharmacological interventions and the vascular responses to bradykinin before and after intervention were compared using Student's paired *t*-test. Significance was accepted at $P < 0.05$.

RESULTS

Glucose- and sucrose-induced hyperosmolarity and coronary arteriolar dilation. All isolated arterioles (internal diameter 81 ± 2 μm) developed a similar level of spontaneous tone (69 ± 1% of their maximum diameter) within 40 min at 36–37°C bath temperature and 60 cmH_2O intraluminal pressure without flow. Both glucose (20 mM) and sucrose (20 mM) produced a marked dilation of an isolated arteriole from the baseline diameter of 90–114 μm (Fig. 1*A*). This dilation was sustained for more than 30 min, and the diameter gradually returned to the baseline level after we replaced the vessel bath with control solution (Fig. 1*A*). To examine the corresponding changes of osmolarity with glucose or sucrose, the concentration of glucose or sucrose in the vessel bath was incrementally increased

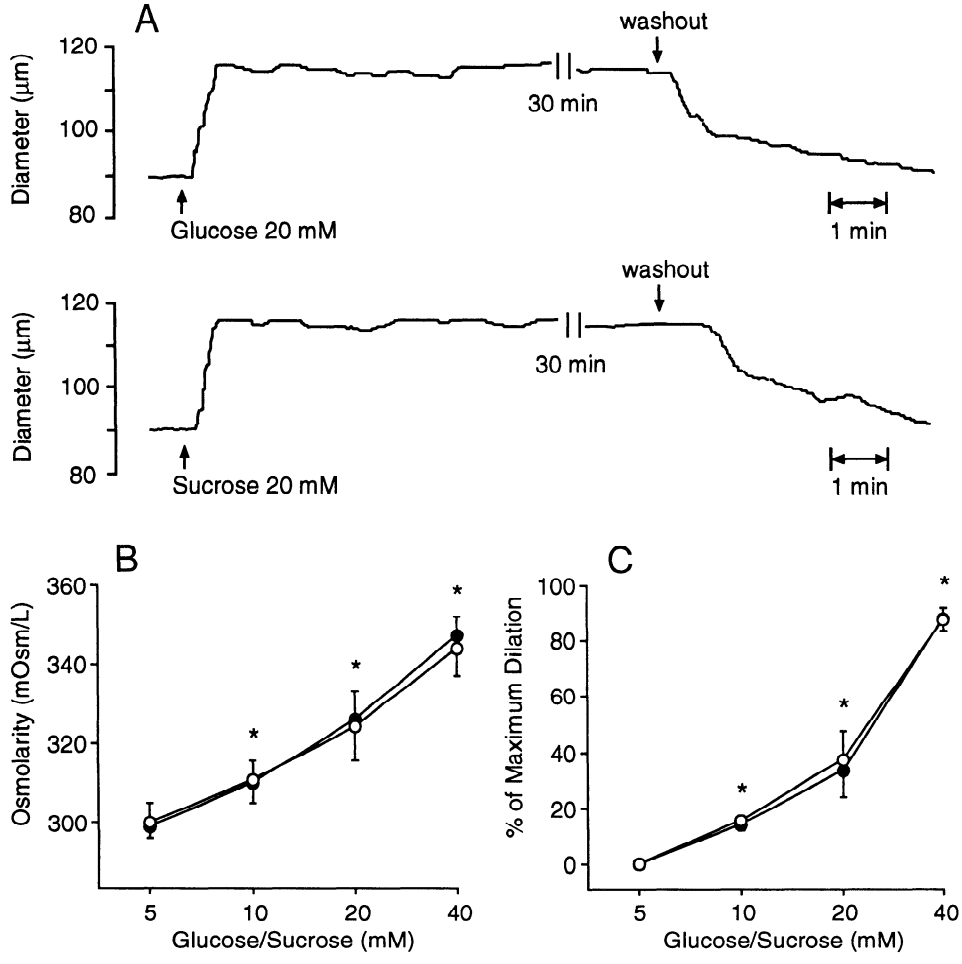

Fig. 1. Effects of glucose and sucrose on osmolarity of extravascular solution and on coronary arteriolar diameter. *A*: 20 mM glucose and sucrose produced a sustained arteriolar dilation. Diameters returned to baseline level after washout. *B*: glucose (○) and sucrose (●) produced an identical concentration-osmolarity relationship ($n = 6$; $^*P < 0.05$ vs. 5 mM). *C*: glucose (○) and sucrose (●) increased coronary arteriolar diameter in a concentration-dependent manner (resting diameter 77 ± 8 μm, maximum diameter 116 ± 12 μm, $n = 6$; $^*P < 0.05$ vs. 5 mM). Extent of glucose-induced vasodilation was identical to that of sucrose-induced vasodilation.

and the changes in osmolarity were measured. As shown in Fig. 1*B*, the increase in glucose concentration from control (5 mM) to 10, 20, and 40 mM produced a corresponding increase in osmolarity from 300 ± 5 to 311 ± 5, 324 ± 8, and 344 ± 7 mosM, respectively. The same increment of sucrose concentrations produced an identical increase in the osmolarity of the solution (Fig. 1*B*). Isolated coronary arterioles dilated equally in response to glucose- and sucrose-induced hyperosmolarity in a concentration-dependent manner (Fig. 1*C*). When the osmolarity of the solution was increased from 300 (control) to 310 mosM (e.g., with glucose or sucrose 10 mM), a small but significant increase in diameter (~10–15% of maximum dilation) was consistently observed. In general, arteriolar diameter increased within 10 s and reached a new steady state within 1 min after each stepwise increase in osmolarity. The arterioles were almost maximally dilated (>90%) at 40 mM glucose or sucrose, which corresponded to an osmolarity of 345 mosM (Fig. 1, *B* and *C*). After the vessel bath was replaced with isotonic PSS-albumin (300 mosM), the diameters returned to the control level within 5 min. The vasodilation to glucose and sucrose was reproducible after reequilibration of the vessels for 30 min (n = 5).

Role of endothelium. Figure 2 summarizes the effect of endothelial denudation on vascular dilation in response to hyperosmolarity. Before endothelial denudation, bradykinin (100 nM) produced coronary arteriolar dilation from a baseline of 77 ± 9 μm to 100 ± 12 μm ($P < 0.05$). Disruption of the endothelium by CHAPS did not significantly alter vascular tone (77 ± 9 μm before denudation vs. 71 ± 10 μm after denudation), but abolished the vasodilation to bradykinin (71 ± 10 μm of baseline vs. 72 ± 13 μm after bradykinin, $P > 0.05$). In addition, the dose-dependent vasodilation to sodium nitroprusside (10^{-8}–10^{-5} M) was not altered after endothelial denudation. As shown in Fig. 2, the vasodilatory response of coronary arterioles to glucose-induced hyperosmolarity was abolished after endothelial removal.

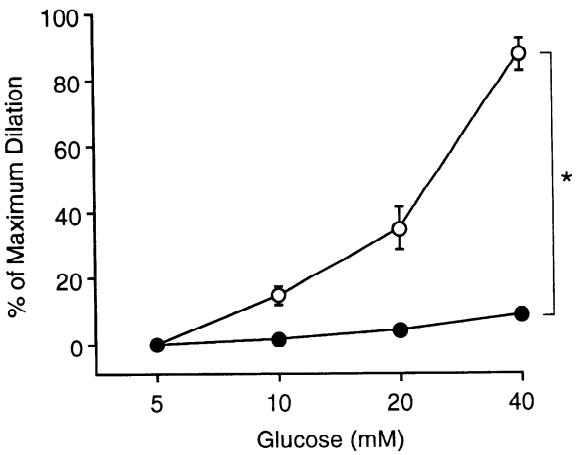

Fig. 2. Effect of endothelial removal on coronary arteriolar dilation in response to hyperosmolarity. ○, Control; ●, denudation. Coronary arteriolar dilation in response to hyperosmotic glucose solution was abolished after endothelial denudation. Resting diameters before and after denudation were 77 ± 9 μm and 71 ± 10 μm, respectively, and maximum diameter was 117 ± 14 μm (n = 4). *$P < 0.05$ between two curves.

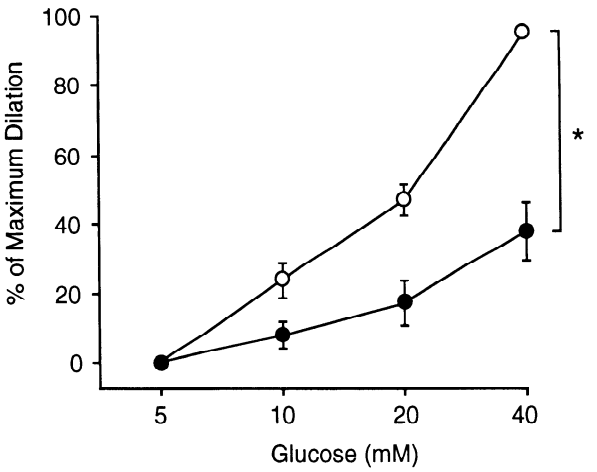

Fig. 3. Effect of intraluminal administration of KCl on glucose-induced dilation. Intraluminal KCl (80 mM) significantly attenuated glucose-induced vasodilation. Resting diameters before (○) and after intraluminal administration of KCl (●) were 86 ± 11 μm and 85 ± 14 μm, respectively, and maximum diameter was 126 ± 20 μm (n = 4). *$P < 0.05$ between two curves.

Contribution of endothelial hyperpolarization. To examine whether hyperosmolarity-induced vasodilation is mediated by the hyperpolarization of endothelium, a high concentration of KCl (80 mM) was administered intraluminally to inhibit the endothelial hyperpolarization. The high intraluminal concentration of KCl did not significantly alter resting vascular tone but significantly attenuated vasodilation to hyperosmotic glucose, as shown in Fig. 3.

Effects of potassium-channel inhibitors. Intraluminal incubation of the isolated vessels with glibenclamide, $BaCl_2$, or iberiotoxin did not alter resting vascular diameter. As shown in Fig. 4*A*, glibenclamide (1 μM) attenuated vasodilation in response to glucose-induced hyperosmolarity. In the presence of glibenclamide, the vasodilation to 10 or 20 mM glucose was completely abolished and the dilation to the highest concentration (40 mM) of glucose was significantly reduced from 93 ± 2% (control) to 31 ± 8%. The hyperosmolarity-induced dilation was also significantly attenuated by a higher concentration of $BaCl_2$ (100 μM, Fig. 4*B*) in a similar manner to the attenuation produced by glibenclamide (Fig. 4*A*). In contrast, neither a low concentration of $BaCl_2$ (10 μM, Fig. 5A) nor iberiotoxin (100 nM, Fig. 5*B*) influenced the hyperosmolarity-induced vasodilation.

Effect of glibenclamide on sucrose-induced dilation. To assess whether the inhibitory effect of intraluminal glibenclamide on the glucose-induced vasodilation was specific to the vasodilation to hyperosmolarity, another set of experiments was performed in isolated vessels using a hyperosmotic sucrose solution. The vasodilation in response to a graded increase in sucrose concentration was significantly attenuated after exposure of the vessels to glibenclamide (1 μM, intraluminally) (Fig. 6). The inhibitory effects of glibenclamide on vasodilations in response to hyperosmotic sucrose or glucose solutions were essentially identical (Figs. 4*A* and 6).

Contributions of endothelium-derived vasodilators. Intraluminal incubation of coronary arterioles with L-NAME (10 μM) or indomethacin (10 μM) did not significantly alter vascular diameter. Likewise, vasodilation in response to glucose-induced hyperosmolarity was not altered either by L-NAME or by indomethacin (Table 1). Intraluminal administration of clotrimazole (30 μM) slightly increased vascular diameter from 71 ± 2 μm to 80 ± 5 μm (n = 4). Clotrimazole significantly attenuated the vascular response to bradykinin (1 nM) by reducing vasodilation from 69 ± 10% to 9 ± 4%, but it did not alter the arteriolar dilation to hyperosmotic glucose solution (Table 1). Because the endothelium may release an unidentified EDHF to produce vasodilation via opening of smooth muscle K_{ATP} or K_{Ca} (large and small conductance) channels, these pathways were examined by extraluminal incubation of the vessels with the specific potassium-channel inhibitors glibenclamide, iberiotoxin, or apamin. None of these inhibitors affected the vasodilation to hyperosmolarity (Table 2).

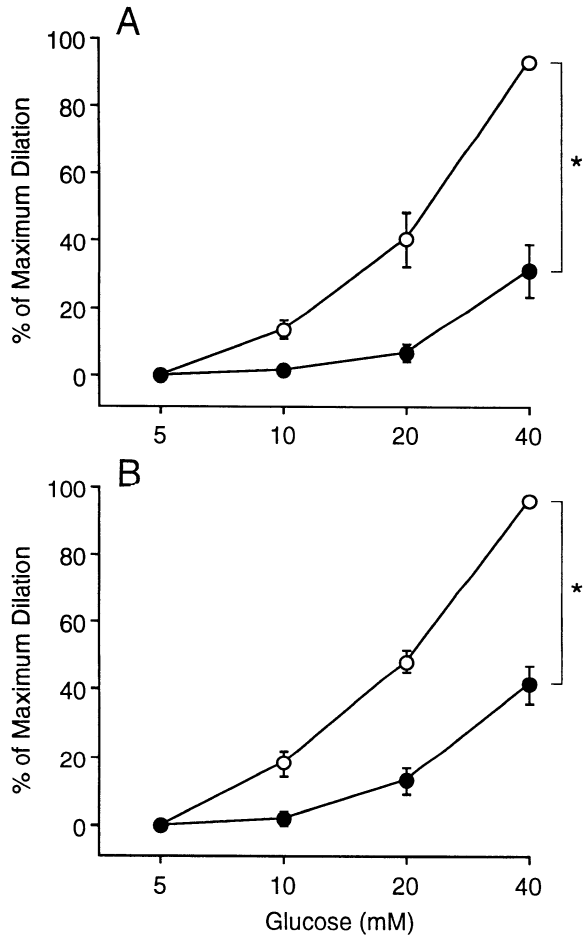

Fig. 4. Effects of the ATP-sensitive potassium-channel inhibitors glibenclamide (A) and $BaCl_2$ (B) on the coronary arteriolar dilation in response to hyperosmolarity. Intraluminal glibenclamide (1 μM) or $BaCl_2$ (100 μM) attenuated the vasodilatory response of coronary arterioles to hyperosmotic glucose solution. A: resting diameters before (○) and after glibenclamide (●) were 70 ± 4 μm and 72 ± 4 μm, respectively, and maximum diameter was 96 ± 6 μm (n = 6). B: resting diameters before (○) and after $BaCl_2$ (●) were 97 ± 1 μm and 108 ± 2 μm, respectively, and maximum diameter was 138 ± 3 μm (n = 3). *$P < 0.05$ between two curves.

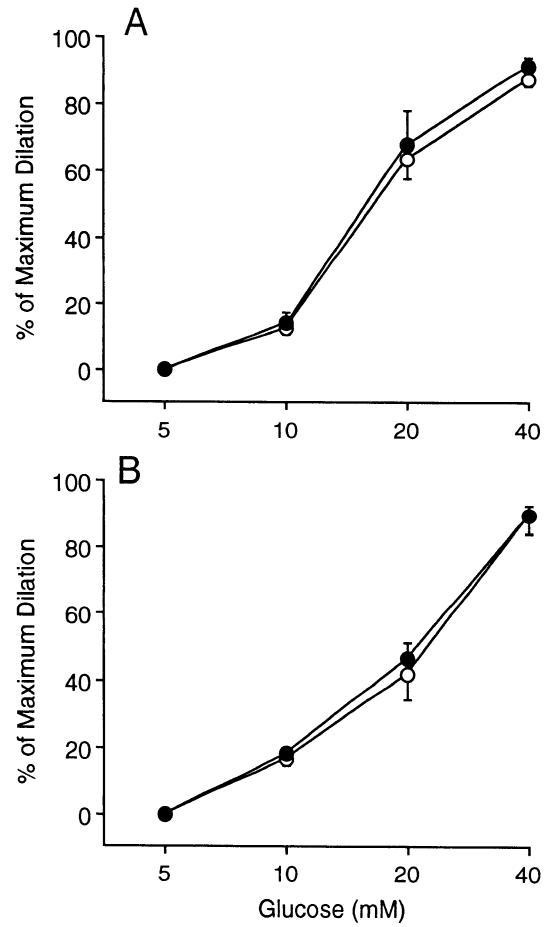

Fig. 5. Effects of a low concentration of $BaCl_2$ (A) and iberiotoxin (B) on coronary arteriolar dilation in response to hyperosmolarity. Intraluminal administration of $BaCl_2$ (10 μM) or iberiotoxin (100 nM) did not affect vasodilation in response to hyperosmotic glucose solution. A: resting diameters before (○) and after $BaCl_2$ (●) were 74 ± 6 μm and 77 ± 4 μm, respectively, and maximum diameter was 120 ± 5 μm (n = 4). B: resting diameters before (○) and after iberiotoxin (●) were 88 ± 11 μm and 88 ± 9 μm, respectively, and the maximum diameter was 121 ± 16 μm (n = 4).

Effect of glibenclamide on vasodilatory function. Sodium nitroprusside (10^{-8}–10^{-5} M) produced dose-dependent dilation of isolated coronary arterioles (Fig. 7). This dilation was not altered in the presence of intraluminal glibenclamide (1 μM), indicating that the vasodilatory capacity of these vessels was not affected by glibenclamide.

DISCUSSION

The major findings of the present study are as follows: *1*) The increase in extravascular osmolarity by glucose or by sucrose produces an identical concentration-dependent dilation of coronary arterioles. *2*) Disruption of endothelium abolishes the coronary arteriolar dilation in response to hyperosmolarity. *3*) Preventing endothelial hyperpolarization attenuates hyperosmotic vasodilation. *4*) Vasodilation in response to hyperosmolarity is also attenuated by intraluminal administration of glibenclamide or a high concentration of $BaCl_2$, but not by L-NAME, indomethacin, clotrimazole, iberiotoxin, or a low concentration of $BaCl_2$. These findings

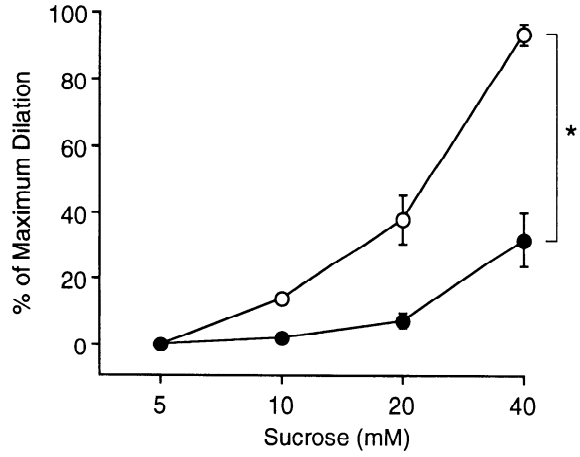

Fig. 6. Effect of intraluminal administration of glibenclamide on sucrose-induced dilation. Vasodilatory response of coronary arterioles to hyperosmotic sucrose solution was significantly attenuated by intraluminal glibenclamide (1 μM). Resting diameters before (○) and after glibenclamide treatment (●) were 86 ± 5 μm and 88 ± 5 μm, respectively, and maximum diameter was 136 ± 10 μm (n − 4). *$P <$ 0.05 between two curves.

indicate that the hyperosmolarity-induced coronary arteriolar dilation is endothelium dependent but is independent of nitric oxide, prostaglandins, or EDHF released from the endothelium. In addition, K_{Ca} and K_{ir} channels are not involved in this dilation. Because hyperosmotic vasodilation is attenuated by intraluminal administration of either glibenclamide or a high concentration of $BaCl_2$ or KCl in a similar manner, it is suggested that the endothelial hyperpolarization, via opening of endothelial K_{ATP} channels, is responsible for this vasomotor response.

Methodological considerations. In the present study, without any influences from alterations in flow, pressure, or other regulatory mechanisms, hyperosmotic glucose and sucrose produced a sustained (>30 min) dilation of isolated coronary arterioles (Fig. 1*A*). The extent of vasodilation was dependent on the concentration of glucose and sucrose (Fig. 1*C*). It is worth noting that no pharmacological preconstrictor was used for tone generation in our preparation. Therefore, the confounding influences from pharmacologically induced tone were eliminated. To ensure that the glucose-induced dilation specifically resulted from an increase in osmolarity, the response of isolated arterioles to various concentrations of sucrose was also tested. As shown in Fig. 1, *B* and *C*, the degree of glucose-induced vasodilation was identical to that produced by a sucrose solution of equal osmolarity, suggesting that hyperosmolarity per se produces dilation of these vessels.

Table 1. *Effect of* L-*NAME, indomethacin, or clotrimazole on coronary arteriolar dilation in response to hyperosmotic glucose solution*

Interventions	n	Resting Diameter, μm	Glucose Concentration, mM 5	10	20	40
			% of Maximum Dilation			
Group 1	5					
Control		83 ± 15	0.0	18 ± 4	46 ± 6	88 ± 5
L-NAME		81 ± 15	0.0	18 ± 4	45 ± 9	92 ± 6
Group 2	5					
Control		76 ± 8	0.0	13 ± 3	48 ± 3	96 ± 1
Indomethacin		75 ± 8	0.0	15 ± 4	47 ± 5	95 ± 1
Group 3	4					
Control		71 ± 2	0.0	21 ± 5	47 ± 8	93 ± 1
Clotrimazole		80 ± 5	0.0	15 ± 3	46 ± 7	85 ± 3

Values are means ± SE; n, number of vessels. L-NAME, N^G-nitro-L-arginine methyl ester; MD, maximum diameter. *Group 1*: MD = 124 ± 8 μm; *group 2*: MD = 107 ± 10 μm; *group 3*: MD = 106 ± 7 μm. Based on paired t-test, neither intraluminal L-NAME, indomethacin, nor clotrimazole affected the vasodilatory response of coronary arterioles to hyperosmotic glucose solution.

Table 2. *Effects of extraluminal administration of potassium-channel inhibitors on coronary arteriolar dilation in response to hyperosmotic glucose solution*

Interventions	n	Resting Diameter, μm	Glucose Concentration, mM 5	10	20	40
			% of Maximum Dilation			
Group 1	6					
Control		85 ± 5	0.0	14 ± 3	44 ± 3	93 ± 3
Glibenclamide		85 ± 4	0.0	15 ± 4	44 ± 7	93 ± 3
Group 2	5					
Control		75 ± 6	0.0	17 ± 2	40 ± 5	91 ± 5
Iberiotoxin		74 ± 6	0.0	17 ± 3	41 ± 6	91 ± 3
Group 3	4					
Control		74 ± 2	0.0	15 ± 4	44 ± 7	93 ± 1
Apamin		76 ± 2	0.0	12 ± 4	40 ± 4	90 ± 4

Values are means ± SE; n, number of vessels. *Group 1*: MD = 131 ± 6 μm; *group 2*: MD = 103 ± 10 μm; *group 3*: MD = 116 ± 8 μm. Based on paired t-test, arteriolar dilation in response to hyperosmotic glucose solution was not altered by extraluminal glibenclamide, iberiotoxin, or apamin.

In the present study, a potential deficiency of the in vitro model should be considered. For example, our isolated microvessels were studied in the absence of flow, a condition that would not occur in the heart in vivo. Exposure of the microvessels to hyperosmolarity in the nonperfused vessels may cause an increase in luminal ionic concentration as a result of flux of water from the lumen to the bath. Therefore, the observed vasodilation may be a result of changes in luminal ionic concentration rather than a direct hyperosmotic stimulation to the microvessels. In addition, a constant flux of isosmotic blood in vivo would possibly buffer the osmotic effects from the interstitium. Therefore, the observed hyperosmotic vasodilation in the present study might not be applicable to the perfused vessels as seen in vivo. To address this issue, the vascular response to hyperosmolarity (i.e., sucrose) was examined in three microvessels perfused with flow (6.6 μl/min, V = 2.3 mm/min). Initially, the vasomotor response of the blood vessel to flow needs to be eliminated, because we have previously shown that coronary arterioles dilate in response to an increased flow (19). Because this flow-induced vasodilation is mediated by the endothelial release of nitric oxide (19), the vascular response to hyperosmolarity was studied in the L-NAME-pretreated microvessels. It was found that L-NAME blocked the flow-induced dilation, which is consistent with results reported in our previous studies (19). In addi-

tion, these perfused vessels dilated in response to the hyperosmolar solution in the same fashion as that observed in the nonperfused vessels. Based on the length of blood vessels subjected to flow (~0.5–0.6 mm), the calculated transit time was ~0.20–0.25 min. As shown in Fig. 1, the hyperosmolarity produced a sustained vasodilation in 1 min, and this period of time would allow intraluminal fluid to be exchanged four to five times. Because fresh solution was continuously supplied to the vessels during flow, it is believed that the contribution of increased luminal ionic concentration to hyperosmotic vasodilation is insignificant in this preparation. In addition, vasodilation in the presence of L-NAME was also observed when the vessels were perfused with a hyperosmolar sucrose solution (40 mosM). Collectively, it appears that hyperosmotic vasodilation can be elicited via both the luminal and the interstitial side, and the phenomenon is independent of flow or changes in luminal ionic concentrations. This contention is also supported by in vivo studies showing that intra-arterial infusion of hyperosmolar solution produces vascular dilation (13, 24, 38), and microapplication of mannitol or NaCl to the pial arterioles produces vasodilation in an osmolarity-dependent (315–360 mosM) manner (39). A similar finding was also reported in the intact skeletal muscle microcirculation (14).

Role of endothelium and potassium channels. In a study of rabbit coronary arterial rings, it was shown that vascular relaxation in response to hyperosmolarity is mainly a result of hyperpolarization (17). Our denudation data (Fig. 2) suggest that the hyperpolarizing signal may have originated from the endothelial cells. This contention is supported by the result of inhibition of endothelial hyperpolarization with a high intraluminal concentration of KCl (80 mM) in which the hyperosmotic vasodilation was significantly attenuated (Fig. 3). We have previously shown that intraluminal administration of KCl does not alter resting vascular tone and vascular smooth muscle function (15, 18) but does inhibit endothelial hyperpolarization in response to various stimuli in isolated vessel preparations (18, 32). The inhibition of hyperosmotic vasodilation by intraluminal KCl indicates that endothelial hyperpolarization is an essential factor responsible for vasodilation to hyperosmolarity.

Because activation of potassium channels and subsequent hyperpolarization has been shown to be a major vasodilatory mechanism (28), we examined the role of potassium channels in hyperosmolarity-induced vasodilation. In the present study, intraluminal administration of glibenclamide (1 μM) significantly attenuated coronary arteriolar dilation in response to hyperosmotic glucose (Fig. 4*A*) or sucrose (Fig. 6), suggesting that the opening of endothelial K_{ATP} channels is essential for hyperosmotic vasodilation. This contention relies on the selective action of intraluminal glibenclamide on the endothelium. In our previous study, we demonstrated that K_{ATP} channel-mediated endothelium-dependent vasodilation to adenosine is completely abolished by intraluminal glibenclamide (1 μM) (18), indicating that endothelial K_{ATP} channels are selectively

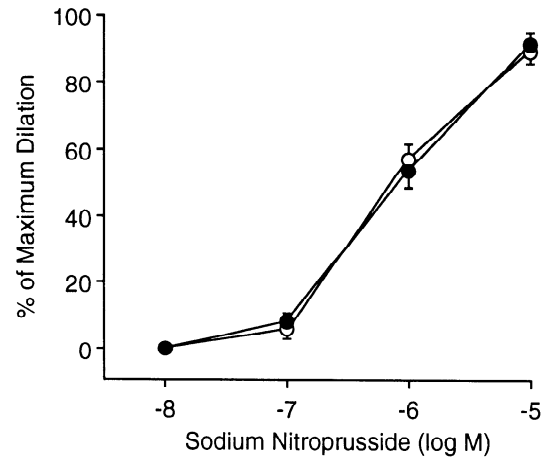

Fig. 7. Effect of glibenclamide on sodium nitroprusside-induced coronary arteriolar dilation. Dose-dependent dilation of coronary arterioles in response to sodium nitroprusside was not altered after intraluminal administration of glibenclamide (1 μM). Resting diameters before (○) and after glibenclamide treatment (●) were 90 ± 7 μm and 89 ± 8 μm, respectively, and maximum diameter was 131 ± 11 μm (n = 4).

inactivated by intraluminal administration of glibenclamide. This notion was further supported by endothelial denudation experiments (18). In the present study, the resting vascular diameter and dose-dependent vasodilation in response to sodium nitroprusside (10^{-8}–10^{-5} M) were not altered by intraluminal glibenclamide (Fig. 7), indicating that the inhibitory action of glibenclamide on hyperosmotic vasodilation is selective for endothelial K_{ATP} channels as opposed to a nonspecific loss of vasodilatory capacity or damage to vascular smooth muscle.

The involvement of endothelial K_{ATP} channels in hyperosmolarity-induced dilation is further supported by the inhibitory effect of $BaCl_2$ (100 μM intraluminally). Barium is less potent in comparison with glibenclamide in blocking K_{ATP} channels, and a relatively high concentration (100 μM) is usually required for significant blocking (28). This is consistent with our finding that a 100-fold higher concentration of barium than glibenclamide was required to inhibit vasodilation to hyperosmolarity (Fig. 4*B*). Because barium is a more effective inhibitor of K_{ir} channels (dissociation constant 2 μM) (31), it is possible that the hyperosmotic vasodilation is also mediated by the opening of K_{ir} channels. To address this issue, the effect of a low concentration of barium was examined. Previously, we reported that a low concentration of KCl (10–20 mM) increases coronary arteriolar diameter by 10–30% (41). This dilation is mediated by the opening of K_{ir} channels, and a low concentration of barium (10 μM) selectively inhibits the activity of these channels (41). We found that the dilation of microvessels to hyperosmotic glucose was not altered when 10 μM of barium was administered intraluminally (Fig. 5*A*). Therefore, these results do not support the idea that K_{ir} channels contribute to the hyperosmotic vasodilation in isolated coronary arterioles.

In the present study, the hyperosmolarity-induced dilation was also not altered by iberiotoxin, the specific inhibitor of the K_{Ca} channel (Fig. 5*B*). The potency of iberiotoxin in inhibiting K_{Ca} channels in arterial smooth

muscle was reported to be <10 nM (for 50% inhibition) (28), and a very low dose of iberiotoxin (1 nM) is sufficient to block K_{Ca} channel-mediated, shear-induced dilation of isolated vessels (9). Because vasodilation in response to hyperosmolarity was not altered by the relatively high concentration of iberiotoxin (100 nM) (Fig. 5*B*), it is unlikely that the opening of K_{Ca} channels in the endothelium is involved in coronary vasodilation in response to hyperosmolarity.

Role of nitric oxide, prostacyclin, and EDHF in hyperosmolarity-induced dilation. It is well documented that vascular endothelium plays a crucial role in modulating vascular tone by release of vasodilators (i.e., nitric oxide, prostacyclin, and EDHF) (3, 8) and by conduction of a hyperpolarization signal to the underlying vascular smooth muscle (5). Because nitric oxide (26) and prostacyclin (16, 27) can initiate vasodilation via opening of vascular K_{ATP} channels in certain tissues, it is possible that the observed vasodilatory response was mediated by the release of these two endogenous vasodilators. However, coronary arteriolar dilation in response to hyperosmolarity was not altered by L-NAME or indomethacin (Table 1), specific inhibitors for nitric oxide and prostaglandin synthesis, respectively. The concentration of inhibitors used in the present study (10 μM) has been shown to be effective in our previous isolated vessel preparations (19). It appears that hyperosmolarity does not induce release of nitric oxide or prostacyclin from blood vessels, and thus the opening of K_{ATP} channels elicited by hyperosmolarity is independent of these two endogenous vasodilators.

Recently, it was demonstrated that vascular dilation in response to bradykinin, especially in the coronary microcirculation, is mediated by the release of a cytochrome *P*-450-derived arachidonic acid metabolite that exhibits the characteristic features of EDHF (4). In the present study, inhibition of cytochrome *P*-450 by clotrimazole significantly attenuated vasodilation to bradykinin, confirming the involvement of cytochrome *P*-450 metabolites in the bradykinin-induced vasodilation of coronary microvessels. However, clotrimazole did not affect vasodilation in response to hyperosmolarity (Table 1), indicating that cytochrome *P*-450-derived EDHF is not involved in the hyperosmotic vasodilation. In some preparations, vasodilation can also be elicited by the release of an as-yet unidentified EDHF (10, 40) via opening of smooth muscle K_{ATP} channels (6, 36), large-conductance K_{Ca} channels (12), or small-conductance K_{Ca} channels (27). However, blocking these channels by extraluminal administration of their specific inhibitors had no effect on hyperosmotic vasodilation (Table 2). We (15), Kuo and Chancellor (18), and other laboratories (9, 27, 28) have shown that the concentration of inhibitors used in the present study is effective in blocking smooth muscle potassium channels. In general, these results indicate that hyperosmotic vasodilation is not mediated by the activation of smooth muscle K_{ATP} and K_{Ca} (large or small conductance) channels and also indirectly suggest that the release of cytochrome *P*-450-independent EDHF during hyperosmotic stimulation is unlikely.

Mechanistic speculations. Recent studies in various tissues indicate that K_{ATP} channels are inactivated by physiological concentrations of ATP (5–10 mM) but, in the presence of Mg^{2+}, a low concentration of ADP (100 μM) is able to reverse the inhibitory effect of ATP (11, 29). It is possible that the shrinkage of endothelium during exposure to hyperosmotic solutions may increase intracellular ADP concentration and/or activate other regulatory mechanisms such as integrin-mediated mechanotransduction pathways (33) and thus lead to the opening of K_{ATP} channels. Indeed, it was found that endothelial cells respond to a twofold increase in osmolarity with ~30% shrinkage of total cell volume within 1 min (23). However, a comparable increase in osmolarity reduces vascular smooth muscle cell volume by only 14%, and this process requires ~20 min to reach a steady state (1). On the basis of these findings, endothelial cells appear to be more sensitive and responsive to osmotic alterations in terms of the rate and extent of volume changes.

Although the exact signal for the opening of endothelial K_{ATP} channels is still unclear, it is possible that the endothelial hyperpolarization produced by opening K_{ATP} channels during hyperosmotic stimulation contributes to smooth muscle relaxation by means of electrical propagation. This electrical communication mechanism has been demonstrated in the porcine coronary artery for kinin-induced vasodilation (5). Alternatively, endothelial hyperpolarization may increase cellular second messengers and subsequently cause vasodilation by transferring these factors to the underlying smooth muscle cells via myoendothelial junctions, as recently suggested by microcirculatory studies (21). Irrespective of these speculations, the results of the present study provide us with initial information for future investigation of the regulation of vasomotor function by interstitial osmolarity.

Physiological-pathophysiological significance. It has been shown that an increased metabolism in skeletal muscle (i.e., exercise) leads to an increased rate of release of osmotically active products and subsequently causes local hypertonicity (35). The vasodilation associated with a 20–40 mosM increase in venous plasma osmolarity has been demonstrated in the working skeletal muscle of human (22) and other animal species (24, 25), suggesting that the interstitial hyperosmolarity may be a factor contributing to the development of functional hyperemia (22, 24, 25). Although information regarding the changes in interstitial osmolarity subsequent to an increase in myocardial metabolism is lacking, the present study demonstrates that coronary microvessels are capable of sensing and responding to the physiological and/or pathophysiological increases, if any, in interstitial osmolarity. We have also demonstrated that the endothelium, in addition to modulating vascular permeability and interstitial osmolarity, plays an important role in the regulation of vascular tone in response to osmotic changes. Because the hyperosmolarity-induced vasomotor response is mediated by the K_{ATP} channels, it is conceivable that dysfunction of these channels would impair the vasodilatory function during exposure to a hyperosmotic environment and could possibly aggravate the inadequacy of flow supply and

oxygen transport to the tissue during metabolic stress, i.e., hypoxia/ischemia.

We thank Drs. Michael J. Davis and Travis W. Hein for suggestions and for review of the manuscript.

This study was supported by National Heart, Lung, and Blood Institute Grant HL-48179.

Address reprint requests to L. Kuo.

Received 31 October 1996; accepted in final form 28 February 1997.

REFERENCES

1. **Arvill, A., B. Johansson, and O. Jonsson.** Effects of hyperosmolarity on the volume of vascular smooth muscle cells and the relation between cell volume and muscle activity. *Acta Physiol. Scand.* 75: 484–495, 1969.
2. **Avolio, A. P., J. A. E. Spaan, and J. D. Laird.** Plasma protein concentration and control of coronary vascular resistance in isolated rat heart. *Am. J. Physiol.* 238 (*Heart Circ. Physiol.* 7): H471–H480, 1980.
3. **Bassenge, E., and R. Busse.** Endothelial modulation of coronary tone. *Prog. Cardiovasc. Dis.* 30: 349–380, 1988.
4. **Bauersachs, J., M. Hecker, and R. Busse.** Display of the characteristics of endothelium-derived hyperpolarizing factor by a cytochrome P450-derived arachidonic acid metabolite in the coronary microcirculation. *Br. J. Pharmacol.* 113: 1548–1553, 1994.
5. **Beny, J. L., and C. Pacicca.** Bidirectional electrical communication between smooth muscle and endothelial cells in the pig coronary artery. *Am. J. Physiol.* 266 (*Heart Circ. Physiol.* 35): H1465–H1472, 1994.
6. **Brayden, J. E.** Membrane hyperpolarization is a mechanism of endothelium-dependent cerebral vasodilation. *Am. J. Physiol.* 259 (*Heart Circ. Physiol.* 28): H668–H673, 1990.
7. **Chilian, W. M., C. L. Eastham, and M. L. Marcus.** Microvascular distribution of coronary vascular resistance in beating left ventricle. *Am. J. Physiol.* 251 (*Heart Circ. Physiol.* 20): H779–H788, 1986.
8. **Cohen, R. A., and P. M. Vanhoutte.** Endothelium-dependent hyperpolarization: beyond nitric oxide and cyclic GMP. *Circulation* 92: 3337–3349, 1995.
9. **Cooke, J. P., Jr., E. Rossitch, N. A. Andon, J. Loscalzo, and V. J. Dzau.** Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. *J. Clin. Invest.* 88: 1663–1671, 1991.
10. **Corriu, C., M. Feletou, E. Canet, and P. M. Vanhoutte.** Inhibition of the cytochrome P450-mono-oxygenase and endothelium-dependent hyperpolarizations in the guinea-pig isolated carotid artery. *Br. J. Pharmacol.* 117: 607–610, 1996.
11. **Davies, N. W., N. B. Standen, and P. R. Stanfield.** ATP-dependent potassium channels of muscle cells: their properties, regulation, and possible functions. *J. Bioenerg. Biomembr.* 23: 509–535, 1991.
12. **Eckman, D. M., J. D. Frankovich, and K. D. Keef.** Comparison of the actions of acetylcholine and BRL 38227 in the guinea-pig coronary artery. *Br. J. Pharmacol.* 106: 9–16, 1992.
13. **Gazitua, S., J. B. Scott, B. Swindall, and F. J. Haddy.** Resistance responses to local changes in plasma osmolality in three vascular beds. *Am. J. Physiol.* 220: 384–391, 1971.
14. **Gray, S. D.** Effect of hypertonicity on vascular dimensions in skeletal muscle. *Microvasc. Res.* 3: 117–124, 1971.
15. **Ishizaka, H., and L. Kuo.** Acidosis-induced coronary arteriolar dilation is mediated by ATP-sensitive potassium channels in vascular smooth muscle. *Circ. Res.* 78: 50–57, 1996.
16. **Jackson, W. F., A. König, T. Dambacher, and R. Busse.** Prostacyclin-induced vasodilation in rabbit heart is mediated by ATP-sensitive potassium channels. *Am. J. Physiol.* 264 (*Heart Circ. Physiol.* 33): H238–H243, 1993.
17. **Keef, K. D., and G. Ross.** Relaxation induced by KCl, NaCl and sucrose in rabbit coronary arteries. *Pflügers Arch.* 409: 308–313, 1987.
18. **Kuo, L., and J. D. Chancellor.** Adenosine potentiates flow-induced dilation of coronary arterioles by activating K_{ATP} channels in endothelium. *Am. J. Physiol.* 269 (*Heart Circ. Physiol.* 38): H541–H549, 1995.
19. **Kuo, L., W. M. Chilian, and M. J. Davis.** Interaction of pressure- and flow-induced responses in porcine coronary resistance vessels. *Am. J. Physiol.* 261 (*Heart Circ. Physiol.* 30): H1706–H1715, 1991.
20. **Kuo, L., M. J. Davis, and W. M. Chilian.** Myogenic activity in isolated subepicardial and subendocardial coronary arterioles. *Am. J. Physiol.* 255 (*Heart Circ. Physiol.* 24): H1558–H1562, 1988.
21. **Little, T. L., J. Xia, and B. R. Duling.** Dye tracers define differential endothelial and smooth muscle coupling patterns within the arteriolar wall. *Circ. Res.* 76: 498–504, 1995.
22. **Lundavall, J., S. Mellander, and T. White.** Hyperosmolality and vasodilatation in human skeletal muscle. *Acta Physiol. Scand.* 77: 224–233, 1969.
23. **Mazzoni, M. C., E. Lundgren, K.-E. Arfors, and M. Intaglietta.** Volume changes of an endothelial cell monolayer on exposure to an isotonic media. *J. Cell. Physiol.* 140: 272–280, 1989.
24. **Mellander, S., B. Johansson, S. Gray, O. Jonsson, J. Lundvall, and B. Ljung.** The effects of hyperosmolarity on intact and isolated vascular smooth muscle. Possible role in exercise hyperemia. *Angiologica* 4: 310–322, 1967.
25. **Mellander, S., and J. Lundvall.** Role of tissue hyperosmolality in exercise hyperemia. *Circ. Res.* 28, *Suppl.* 1: I-39–I-45, 1971.
26. **Murphy, M. E., and J. E. Brayden.** Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. *J. Physiol. (Lond).* 486: 47–58, 1995.
27. **Murphy, M. E., and J. E. Brayden.** Apamin-sensitive K^+ channels mediate an endothelium-dependent hyperpolarization in rabbit mesenteric arteries. *J. Physiol. (Lond).* 489: 723–734, 1995.
28. **Nelson, M. T., and J. M. Quayle.** Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C799–C822, 1995.
29. **Nichols, C. G., and W. J. Lederer.** Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. *Am. J. Physiol.* 261 (*Heart Circ. Physiol.* 30): H1675–H1686, 1991.
30. **Olsson, R. A., and R. Bünger.** Metabolic control of coronary blood flow. *Prog. Cardiovasc. Dis.* 29: 369–387, 1987.
31. **Quayle, J. M., J. G. McCarron, J. E. Brayden, and M. T. Nelson.** Inward rectifier K^+ currents in smooth muscle cells from rat resistance-sized cerebral arteries. *Am. J. Physiol.* 265 (*Cell Physiol.* 34): C1363–C1370, 1993.
32. **Rubanyi, G. M., A. D. Freay, A. Johns, and C. Van Breemen.** Elevated transmural pressure inhibits the release of EDRF by mechanism(s) similar to high K^+ and barium. In: *Resistance Arteries, Structure and Function,* edited by M. J. Mulvany, C. Aalkjær, A. M. Heagerty, N. C. B. Nyborg, and S. Strandgaard. Amsterdam: Elsevier Science, 1991, p. 226–232.
33. **Schwartz, M. A., and D. E. Ingber.** Integrating with integrins. *Mol. Biol. Cell* 5: 389–393, 1994.
34. **Scott, J. B., and D. Rodawski.** Role of hyperosmolarity in the genesis of active and reactive hyperemia. *Circ. Res.* 28, *Suppl.* 1: I-26–I-32, 1971.
35. **Sparks, H. V., Jr.** Effect of local metabolic factors on vascular smooth muscle. In: *Handbook of Physiology. The Cardiovascular System. Vascular Smooth Muscle.* Bethesda, MD: Am. Physiol. Soc., 1980, sect. 2, vol. II, chapt. 17, p. 475–513.
36. **Standen, N. B., J. M. Quayle, N. W. Davies, J. E. Brayden, Y. Huang, and M. T. Nelson.** Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* 245: 177–180, 1989.
37. **Tranum-Jensen, J., M. J. Janse, J. W. T. Fiolet, W. J. G. Krieger, C. N. D'Alnoncourt, and D. Durrer.** Tissue osmolality, cell swelling, and reperfusion in acute regional myocardial ischemia in the isolated porcine heart. *Circ. Res.* 49: 364–381, 1981.
38. **Vlahakes, G. J., S. R. Giamber, K. O. Rothaus, and W. J. Powell.** Hyperosmotic mannitol and collateral blood flow to ischemic myocardium. *J. Surg. Res.* 47: 438–446, 1989.
39. **Wahl, M., W. Kuschinsky, O. Bosse, and T. Thurau.** Dependency of pial arterial and arteriolar diameter on perivascular osmolarity in the cat: a microapplication study. *Circ. Res.* 32: 162–169, 1972.
40. **Weintraub, N. L., S. N. Joshi, C. A. Branch, A. H. Stephenson, R. S. Sprague, and A. J. Lonigro.** Relaxation of porcine coronary artery to bradykinin: role of arachidonic acid. *Hypertension* 23: 976–981, 1994.
41. **Wu, X., M. J. Davis, H. Ishizaka, and L. Kuo.** Coronary arteriolar dilation to KCl is mediated by an inwardly rectifying K^+ channel in smooth muscle (Abstract). *Microcirculation* 3: 110, 1996.