Gender-specific K⁺-channel contribution to adenosine-induced relaxation in coronary arterioles

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Heaps, Cristine L, and Douglas K. Bowles. Gender-specific K⁺-channel contribution to adenosine-induced relaxation in coronary arterioles. J Appl Physiol 92: 550–558, 2002. First published October 5, 2001; 10.1152/japplphysiol.00566.2001.—We examined the contribution of K⁺-channel activity on basal tone and adenosine-mediated relaxation of coronary arterioles isolated from sexually mature male and female miniature swine. Arterioles (≈100–200 μm ID) isolated from the apical region of the heart were cannulated and studied using videodimensional analysis under constant intraluminal pressure. Coronary arterioles from male and female pigs demonstrated similar levels of basal tone and reductions in basal diameter in response to the K⁺-channel blockers 4-aminopyridine (4-AP; 1 mM), tetraethylammonium (1 mM), and glibenclamide (Glib; 10 μM), with 4-AP producing significantly greater constriction than tetraethylammonium or Glib. After endothelin-induced preconstriction, relaxation responses to adenosine were not significantly different between coronary arterioles of male and female pigs. Inhibition of 4-AP-sensitive channels significantly impaired adenosine-mediated relaxation in arterioles from male but not female pigs. However, inhibition of K⁺ channels with iberiotoxin (100 nM) or Glib had no effect on adenosine-induced relaxation in either sex. Results obtained in the presence of nitric oxide synthase inhibition suggest a potential interaction of 4-AP-sensitive channels and nitric oxide at low adenosine concentrations. In conclusion, our data indicate that 4-AP-sensitive channels 1) contribute significantly to basal tone in coronary arterioles of both male and female pigs, 2) contribute to adenosine-mediated relaxation in male but not female pigs, and 3) can contribute to adenosine-induced relaxation independent of nitric oxide production in male pigs. These data are consistent with a significant role for voltage-dependent K⁺ channels in adenosine-mediated relaxation of coronary arterioles from males.
to the vasodilatory effects of adenosine is nitric oxide dependent, and 3) examine whether gender influences adenosine-induced K⁺-channel activation.

METHODS

Animals. Male and female Yucatan miniature swine were obtained from the breeder (Charles River, Wilmington, MA) and housed in animal facilities at the University of Missouri College of Veterinary Medicine for 17–20 wk until sexually mature (~1 yr of age). All animal protocols were in accordance with the US Government “Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training” and approved by the University of Missouri Animal Care and Use Committee.

Isolation of coronary arterioles. Animals were anesthetized using ketamine (35 mg/kg im), rompun (2.25 mg/kg im), and pentothal sodium (10 mg/kg iv), followed by administration of heparin (1,000 U/kg iv). Animals were euthanized by removal of hearts, which were immediately placed in cold (4°C) Krebs bicarbonate buffer. The apical region of the heart was isolated and placed in a chilled (4°C) dissection chamber containing physiological saline solution (PSS; in mM): 145 NaCl, 4.7 KCl, 2 CaCl₂, 1.17 MgSO₄, 1.2 NaH₂PO₄, 5 glucose, 2 pyruvate, 0.02 EDTA, and 3 MOPS, pH 7.4, and 1 g/100 mL bovine serum albumin. Similarly sized single coronary arterioles from male and female pigs (~100- to 200-μm luminal diameter) were dissected free of surrounding myocardium with the aid of a dissection microscope and transferred to a Lucite vessel chamber containing PSS for cannulation. The length of arteriolar segments isolated for cannulation was typically ~1 mm. Arterioles were cannulated on one end with a glass micropipette filled with PSS and tied securely to the pipette using 11-0 suture. The arteriole was gently flushed, and the other end was cannulated with a second micropipette and tied.

Microvessel videodimensional instrumentation. The cannulated arteriole was transferred to the stage of an inverted microscope (Olympus IX50) equipped with a ×10 objective (numerical aperture, 0.25) and coupled with a video camera (Olympus N10), video monitor (Sony), and video micrometer (Microcirculation Research Institute, Texas A&M University, College Station, TX). Data acquisition and analysis were accomplished by using Axoscan 8.0 software (Axon Instruments). Both micropipettes were connected to a single-reservoir system that adjusted the intraluminal pressure of the arteriole at 40 mmHg without allowing flow through the vessel lumen. Leaks were detected by pressurizing the arteriole to 40 mmHg and then verifying that intraluminal diameter remained constant when the valve to the reservoir system was closed. Only arterioles that were free of leaks were studied. The vessel chamber bath was gradually warmed and maintained at 37°C for the duration of the experiment. Luminal diameter was monitored continuously throughout the experiment.

Experimental protocol. After a 1-h equilibration period at 40 mmHg, during which time the vessels established a stable basal tone, the effects of the K⁺-channel blockers 4-aminopyridine (4-AP; 1 mM), tetraethylammonium (TEA; 1 mM), and glibenclamide (Glib; 10 μM) on basal diameter were evaluated. Vessels were incubated with each K⁺-channel blocker until a steady-state diameter was attained (~20 min).

After incubation with K⁺-channel blockers, arterioles were further treated with endothelin until a preconstriction level of ~40–50% maximal diameter was attained. For control experiments (no K⁺-channel blocker present), vessels were preconstricted to the same level (~40–50%) using only endothelin. Adenosine concentration–response relationships were determined by cumulative additions of concentrated stock solutions directly to the tissue bath. Adenosine concentration–response relationships were repeated in the presence of the nitric oxide synthase inhibitor N⁵,N⁶-dimethylarginine (L-NAME; 300 μM) and the cyclooxygenase inhibitor indomethacin (Indo; 5 μM). At completion of the experimental protocol, maximal (passive) intraluminal diameters (Dₚ) of coronary arterioles were measured at 40-mmHg intraluminal pressure in Ca²⁺-free PSS containing 1 mM EGTA and the Ca²⁺-channel blocker nifedipine (2 μM). All drug applications were made to the tissue bath.

Smooth muscle cell dissociation. Coronary microvessels (~150-μm luminal diameter) were placed in low-Ca²⁺ (0.1 mM) physiological buffer containing 294 U/ml collagenase, 5 U/ml elastase, 2 mg/ml bovine serum albumin, 1 mg/ml soybean trypsin inhibitor, and 0.4 mg/ml DNase I. Cells were enzymatically dissociated by incubation in a 37°C water bath for 1 h. The enzyme solution was replaced with enzyme-free, low-Ca²⁺ solution, and single smooth muscle cells were isolated with gentle trituration by micropipette. Isolated smooth muscle cells were maintained in low-Ca²⁺ solution at 4°C until use (0–2 h).

Voltage clamp. Whole cell currents were determined by using a standard whole cell voltage-clamp technique as used routinely (4). Cells were initially superfused with low-Ca²⁺ PSS containing (in mM) 138 NaCl, 5 KCl, 0.1 CaCl₂, 1 MgCl₂, 10 glucose, and 20 HEPE, pH 7.4, during gigaseal formation. Heat-polished glass pipettes (2–5 MΩ) were filled with a solution containing (in mM) 120 KCl, 10 NaCl, 1 MgCl₂, 10 EGTA, and 10 HEPE, pH 7.1, with KOH. For single-channel experiments, outside-out patches were obtained using (in mM) 10 NaCl, 135 KCl, 1 MgCl₂, 10 glucose, and 20 HEPE, pH 7.4, for both the bath and pipette solution. Ionic currents were amplified by an Axopatch 200B patch-clamp amplifier (Axon Instruments). Currents were low-pass filtered with a cutoff frequency of 1,000 Hz, digitized at 2.5 kHz, and stored on computer. Leak subtraction was not performed. Data acquisition and analysis were accomplished using pClamp 8.0 software (Axon Instruments). Cells were continuously perfused under gravity flow. All experiments were conducted at room temperature (22–25°C).

Drugs and solutions. Stock solutions for 4-AP, TEA, ibetrixin (IBTX), L-NAME, endothelin, and adenosine were prepared in distilled water. Nifedipine and Indo stock solutions were prepared in ethanol. Glib stock solution was prepared in DMSO. Vehicle concentrations did not exceed 0.1% of vessel bath volume.

Data analysis. Spontaneous tone was calculated as [1 – (Dₚ/Dₛₛₛ)] × 100, where Dₚ is active (presence of tone) diameter. Student’s paired t-tests were used to test for differences between pre- and postvessel diameters, where one treatment was evaluated. For endothelin preconstriction, data are presented as percent possible constriction, [(Dₛₛₛ – Dₛₛₛ/Dₚ]) × 100, where Dₛₛₛ is the steady-state diameter in the presence of endothelin. Student’s unpaired t-tests were used to evaluate differences between group means where one treatment was evaluated. Relaxation responses to adenosine are presented as the percent increase in diameter relative to the maximal possible relaxation, [(Dₛₛₛ – Dₛₛₛ/Dₚ)] × 100, to normalize for differences in initial and passive diameters between vessels. Adenosine concentration–response curves of
arterioles from male and female pigs were analyzed using two-way repeated-measures analysis of variance and the Greenhouse-Geisser adjustment to control for type I error due to unequal group sizes (22). Mean differences were ascertained using Fisher's least significant difference when either the main interaction or drug effect was significant. For all analyses, a P value < 0.05 was considered significant. Data are presented as means ± SE, and n values in parentheses reflect the number of animals.

RESULTS

Vessel characteristics. Maximal $D_P$ of coronary arterioles measured at 40-mmHg intraluminal pressure in Ca$^{2+}$-free PSS plus nifedipine were not significantly different between male and female pigs (163 ± 15 and 162 ± 16 μm, respectively). Coronary arterioles from both male and female pigs developed a similar mean level of spontaneous tone (8.6 ± 2.4 and 7.7 ± 1.1%, respectively) during the equilibration period at 37°C and 40-mmHg intraluminal pressure. These levels of spontaneous tone are similar to those observed previously (27).

Effect of K$^+$-channel blockers on basal diameter. Application of 4-AP, TEA, and Glib produced similar degrees of constriction in coronary arterioles of male and female pigs, with 4-AP producing a significantly greater reduction in intraluminal diameter compared with TEA and Glib (Fig. 1). Neither TEA nor Glib produced significant decreases in basal diameter, as demonstrated by paired t-tests comparing arteriolar diameter before and after drug application. $K_{ATP}$-channel blockade by Glib produced no change in basal diameter in arterioles from male pigs (Fig. 1). Although TEA is relatively nonselective and inhibits more than just $K_{Ca}$ channels, the insignificant reduction in basal diameter observed using TEA indicated that the use of IBTX, a selective $K_{Ca}$-channel blocker, on basal diameter was unnecessary.

Adenosine-mediated concentration-response curves. The level of endothelin-induced preconstriction was similar between coronary arterioles from male and female pigs (50.1 ± 3.4 and 48.3 ± 5.2% of maximal intraluminal diameter, respectively). The concentration of endothelin required to attain this level of preconstriction was not significantly different in arterioles from male and female pigs (1.1 ± 0.5 and 1.3 ± 0.5 nM, respectively) and was similar to that observed previously in female pigs (21). Concentration-response curves for adenosine are presented in Fig. 2 and were not significantly different between arterioles from male and female pigs. Adenosine concentrations causing 50% relaxation were not calculated because maximal responses were not consistently observed.

Role of K$^+$ channels in adenosine-mediated relaxation. Figure 3 demonstrates that both TEA and 4-AP significantly attenuated adenosine-mediated relaxation in arterioles from male but not female pigs. Based on the reported relative selectivity of 1 mM TEA and 4-AP for inhibition of $K_{Ca}$ and $K_V$ channels, respectively (27), these data suggest that adenosine activates both $K_{Ca}$ and $K_V$ channels as a mechanism for vasodilatation in arterioles from males. However, further examination of $K_{Ca}$-channel contribution to adenosine-mediated relaxation using IBTX, a more selective inhibitor of $K_{Ca}$ channels, indicated that adenosine does not activate $K_{Ca}$ channels in arterioles from male pigs (Fig. 4). Impaired adenosine-mediated relaxation in the presence of the TEA, but not IBTX, is consistent with nonselective inhibition by TEA of another K$^+$-channel subfamily in addition to $K_{Ca}$ channels. Because TEA demonstrated no significant effect on adenosine-mediated relaxation in female pigs, we did not examine the effect of IBTX in females. Inhibition of $K_{ATP}$ channels with Glib was without effect on adenosine-mediated relaxation in both male and female animals.

Figure 5 compares the group mean responses of arterioles isolated from male pigs over the most physiologically relevant range of adenosine concentrations.
The vasodilatory responses at these low adenosine concentrations appear primarily mediated by 4-AP-sensitive channel activity. Furthermore, KV-channel activity remains considerable at higher adenosine concentrations, as demonstrated by significant attenuation of adenosine-mediated relaxation in the presence of 4-AP (Fig. 3A). Application of the nitric oxide synthase inhibitor L-NAME produced a reduction in basal vessel diameter of coronary arterioles isolated from male pigs (n = 6). Pretreatment of arterioles from male pigs with L-NAME significantly altered the vasodilatory response to adenosine only at lower concentrations (10⁻⁹ and 10⁻⁸ M adenosine; Fig. 6A). Application of the prostacyclin inhibitor Indo did not alter adenosine-mediated relaxation in arterioles from male pigs (Fig. 6B). In the presence of L-NAME, the inhibitory effect of KV-channel blockade with 4-AP on adenosine-mediated relaxation was maintained (Fig. 7).

Role of nitric oxide and prostacyclin in adenosine-mediated relaxation. Previous studies have indicated that adenosine-mediated relaxation in porcine coronary arterioles is attenuated to the same extent by either inhibition of nitric oxide synthase or endothelial removal (13), indicating that nitric oxide is the sole endothelium-derived vasodilator involved in adenosine-induced relaxation in these vessels. Additional studies have reported a direct activation of vascular smooth muscle K⁺ channels by nitric oxide (3, 28) and adenosine-stimulated endothelial K⁺-channel contribution to nitric oxide production (13). Based on these findings, we specifically examined the contribution of 4-AP-sensitive channels to adenosine-mediated relaxation during nitric oxide synthase inhibition in arterioles from male pigs. Application of the nitric oxide synthase inhibitor L-NAME produced a reduction in basal vessel diameter of coronary arterioles isolated from male pigs (n = 6). Pretreatment of arterioles from male pigs with L-NAME significantly altered the vasodilatory response to adenosine only at lower concentrations (10⁻⁹ and 10⁻⁸ M adenosine; Fig. 6A). Application of the prostacyclin inhibitor Indo did not alter adenosine-mediated relaxation in arterioles from male pigs (Fig. 6B). In the presence of L-NAME, the inhibitory effect of KV-channel blockade with 4-AP on adenosine-mediated relaxation was maintained (Fig. 7).

Effect of TEA and IBTX on coronary arteriolar smooth muscle K⁺ currents. Because of the contradictory effects of TEA and IBTX on arteriolar dilation, we...
examined whether TEA produced nonselective inhibition of Kv-channel currents in smooth-muscle cells isolated from arterioles of male pigs. In whole cell patch clamp, pipette solution containing 10 mM EGTA was used to chelate intracellular Ca^{2+} and, thereby, reduce activation of KCa-channel currents. These conditions allowed us to evaluate K^{+}-channel blocker effects on macroscopic Kv-channel currents (37). Figure 8A shows representative currents obtained during a step depolarization to +30 mV from a holding potential of either −80 or 0 mV. Sustained depolarization effectively eliminated outward current at +30 mV, indicating that ~100% of this current demonstrates voltage-dependent inactivation, consistent with Kv and not KCa current as the dominant current measured under these experimental conditions. Further evidence for this is provided in Fig. 8, B and C, where 4-AP (1 and 5 mM) reduced K^{+} currents, but IBTX (100 nM) had no effect on outward current. Therefore, under these conditions, the measured K^{+} current demonstrates voltage-dependent inactivation and is IBTX insensitive, consistent with Kv current. The addition of TEA (1 mM) in the presence of IBTX produces a significant reduction in K^{+} current, providing evidence of inhibition of Kv current by 1 mM TEA in these cells (Fig. 8D).

Outside-out patch experiments were performed to verify that IBTX at 100 nM does, in fact, inhibit KCa channels in these arterioles. Single-channel recordings are shown in Fig. 8E, indicating a channel with a conductance of ~250 pS, consistent with large-conductance KCa channels. Application of 100 nM IBTX completely abolished large-conductance KCa-channel activity (Fig. 8F). These results indicate that TEA is not entirely selective for KCa channels in our preparation but rather appears to partially inhibit the Kv subfamily of currents.

**DISCUSSION**

These studies provide the first evidence for significant 4-AP-sensitive channel contribution to adenosine-mediated relaxation in pressurized arterioles. These data are consistent with a significant role for Kv channels in adenosine-mediated relaxation in our model. We also document the novel finding that the contribution of Kv channels to adenosine-mediated relaxation in porcine coronary arterioles is sex specific, as 4-AP significantly attenuated adenosine-induced vasodilation in coronary arterioles isolated from male, but not female, pigs. Furthermore, KCa- or KATP-channel blockade did not significantly alter the relaxation response to adenosine in arterioles from pigs of either sex. We also demonstrate that 4-AP inhibited adenosine-mediated relaxation similarly in the absence or presence of nitric oxide synthase inhibition, suggesting that, in our model, adenosine-mediated activation of Kv channels can occur independent of nitric oxide. However, nitric oxide synthase inhibition did attenuate adenosine-induced vasodilatation, indicating that adenosine is partly dependent on nitric oxide for its relaxation effects. Finally, our study demonstrates a significant role for Kv channels in the maintenance of basal diameter in coronary arterioles from both male and female pigs and a relatively minor role for KCa and KATP channels.

In agreement with our findings, previous studies have reported that inhibition of KCa channels does not alter the vasodilatory response to adenosine in pres-
surized porcine coronary arterioles (13). However, other studies have implicated a significant role for KCa-channel activation in adenosine-mediated relaxation in pressurized canine coronary arterioles (7, 31). KCa-channel blockade was accomplished with IBTX in each of these studies; therefore, the discrepant findings suggest species differences in adenosine activation of KCa channels. Contrary to our findings, previous studies have also reported that inhibition of KATP channels significantly attenuated adenosine-mediated relaxation in pressurized porcine coronary arterioles (13, 19). The discrepancy between these reports (19) and our data may result from developmental differences between animal models used in these studies (neonatal vs. adult pigs) or the use of endothelin preconstriction in our study. Indeed, endothelin-induced vasoconstriction is mediated in part via Kv, KCa, and KATP-channel inhibition (25, 26, 35), as is likely the case with most vasoconstrictors (29). Our data suggest that adenosine may overcome the endothelin-mediated inhibition of Kv but not KATP channels. Interestingly, previous studies have provided evidence against KATP-channel activation by adenosine in porcine coronary arterial rings preconstricted pharmacologically (23).

Evaluation of adenosine-mediated relaxation in the presence of endothelin preconstriction should provide important insight into in vivo regulation of coronary blood flow under both physiological and pathophysiological conditions. Endothelin levels are elevated in patients with congestive heart failure, hypertension, and postmyocardial infarction and contribute to myocardial ischemic-reperfusion injury (12, 32, 38). Understanding the mechanism by which vasodilators counter endothelin-induced vasoconstriction may aid in developing pharmacological tools to counter the pathophysiological effects of endothelin. Furthermore, the finding that 4-AP-sensitive vasodilatation to adenosine is sex specific suggests that sex-specific therapies may be necessary.

The influence of gender on adenosine-mediated relaxation of coronary arterioles has not been assessed previously. Furthermore, comparison of Kv-channel activity under basal conditions or as a mechanism of adenosine-mediated relaxation between sexes has not been reported previously. The underlying mechanisms for the sex-related differences observed in our study remain unknown; however, it is probable that sex hormones are directly or indirectly involved. Although sex hormones are not present in our in vitro preparation, hormone-stimulated alterations in protein expression may contribute to sex-related differences. Both estrogen and testosterone activate Kv channels to produce
vascular smooth muscle relaxation (8, 39). However, whether estrogen and/or testosterone contribute to the observed sex-related differences by altering the expression of K⁺ channels or other mediators of adenosine-induced relaxation, including adenosine-receptor subtype expression and/or components of the cAMP pathway, has not been reported. Furthermore, our finding that adenosine-mediated relaxation was not significantly different between arterioles of male and female pigs indicates that arterioles of female pigs utilize an additional vasodilatory pathway to compensate for the lack of K⁺-channel contribution.

Aiello et al. (1, 2) have reported that both PKA and isoproterenol stimulate Kᵥ channels in isolated vascular smooth-muscle cells. Similar to isoproterenol, adenosine is proposed to activate K⁺ channels via cAMP/PKA-dependent phosphorylation or direct G-protein activation of the channel (9, 33). However, the contribution of Kᵥ channels to adenosine or other cAMP-mediated relaxation in isolated, intact arteries has not been reported previously. Our findings provide the first evidence that Kᵥ-channel activation contributes considerably to adenosine-induced relaxation, independent of nitric oxide synthase.

Endothelium-dependent K⁺-channel activation has also been implicated in dilation of coronary arterioles to adenosine (13). These studies have demonstrated that endothelium-dependent K⁺-channel contribution to adenosine-induced coronary arteriolar dilation was attenuated to the same extent by nitric oxide synthase inhibition and endothelial denudation, suggesting that endothelium-dependent K⁺-channel activation is entirely nitric oxide dependent (13). Our data also demonstrate that inhibition of nitric oxide synthase, but not prostacyclin, attenuates adenosine-mediated relaxation. Based on these findings, we specifically examined the dependence of Kᵥ-channel activation during adenosine-induced dilation on nitric oxide in arterioles from male pigs. Kᵥ-channel blockade abolished adenosine-mediated relaxation at low adenosine concentrations in the presence or absence of L-NAME. This was surprising because our data also indicate that nitric oxide contributes significantly to adenosine-mediated relaxation in our model. A model of endothelium-dependent Kᵥ-channel activation implicated in dilation of coronary arterioles to adenosine proposed previously (13) fits our data incorporating endothelial Kᵥ channels. We propose that inhibition of adenosine-mediated endothelial Kᵥ-channel activation (4-AP) reduces nitric oxide production, eliminating the nitric oxide component of adenosine-mediated relaxation. Therefore, in addition to inhibition of direct activation of smooth muscle Kᵥ channels by adenosine, 4-AP also eliminates smooth muscle relaxation via inhibition of nitric oxide production. Thus Kᵥ-channel blockade produces complete inhibition of adenosine-mediated relaxation at low adenosine concentrations. However, our data cannot discount a direct activation of smooth-muscle Kᵥ channels by nitric oxide (18) as a contributor to adenosine-mediated relaxation. Together, these data suggest that 1) nitric oxide contributes to vasodilation through activation of smooth-muscle Kᵥ channels, 2) endothelial Kᵥ-channel activation stimulates nitric oxide production, or 3) a combination of both.

We also determined the contribution of Kᵥ, Kᵥ Ca, and Kᵥ ATP channels to basal arteriolar diameter. K⁺-channel activity primarily determines smooth muscle resting membrane potential (10) and, thereby, the basal diameter of arteries (6, 17). Furthermore, increased endothelial K⁺-channel activity hyperpolarizes endothelial cells, thereby stimulating nitric oxide production to reduce smooth-muscle contraction. Coronary arterioles from both male and female pigs demonstrated similar reductions in basal diameter in response to 4-AP, TEA, and Glib. 4-AP profoundly reduced basal diameter of porcine coronary arterioles, suggesting that Kᵥ channels play a major role in the maintenance of basal tone, and, therefore, vascular resistance, in arterioles of both male and female pigs. Previous studies have also indicated a significant role for Kᵥ channels in the regulation of basal tone in large porcine coronary arteries (30, 34) and in pressurized cerebral arterioles (17). Furthermore, the effect of TEA and Glib on basal tone observed in our study was similar to that observed previously in both coronary arterioles (7) and arterial rings (5), consistent with (19, 30, 34) little to no contribution of Kᵥ Ca, and Kᵥ ATP channels to the regulation of basal diameter in porcine coronary arterioles in vitro.

For these studies, adenosine-mediated relaxation responses were examined after a steady-state level of preconstriction (~40–50% maximal diameter) was attained. For control experiments (no K⁺-channel blocker present), vessels were preconstricted using only endothelin, whereas, in the presence of K⁺-channel blockers or L-NAME, typically less endothelin was required to attain similar levels of preconstriction. This raises the possibility that differences between adenosine-induced relaxation in the absence and presence of K⁺-channel blockers and/or L-NAME may be attributable to variable concentrations of endothelin. However, our data suggest that differences in endothelin concentration did not influence adenosine-mediated relaxation because arterioles preconstricted with lower concentrations of endothelin (K⁺-channel blocker and/or L-NAME present) demonstrated less relaxation than vessels preconstricted with higher endothelin concentrations (control experiments). We also found no sex difference in endothelin concentration required to attain similar levels of preconstriction. This finding is in contrast to those of others using larger porcine coronary arteries (14, 24) and supports heterogeneity in physiological and pharmacological responses in vessels of different sizes (4, 15, 20).

The selectivity of K⁺-channel blockers in vascular smooth muscle has been reviewed previously (29). TEA preferentially blocks Kᵥ Ca currents at concentrations of 1 mM (one-half block at 0.2 mM) (29). However, using whole cell patch clamp, we demonstrate inhibition of Kᵥ current with 1 mM TEA in smooth muscle cells isolated from coronary arterioles of male pigs. These data compelled us to examine the effect of a more
selective inhibitor of K$_{Ca}$ channels, IBTX, on adenosine-mediated relaxation. IBTX is highly selective for K$_{Ca}$ channels (one-half block, 1–10 nM) and has no effect on K$_V$, K$_{ATP}$, or inward-rectifying K$^+$ (K$_{IR}$) channels (29). Glib is a highly selective blocker of K$_{ATP}$ channels (one-half block at 20–100 nM) with no effect on K$_V$, K$_{Ca}$, or K$_{IR}$ channels at a concentration of 10 μM, as used in our study (29). 4-AP is a selective inhibitor of Kv channels (one-half block at 0.2–1.1 mM) and does not inhibit K$_{Ca}$ or K$_{IR}$ channels at these concentrations but may inhibit K$_{ATP}$ currents (29). Because K$_{ATP}$-channel blockade (Glib) did not alter significantly to the maintenance of basal diameter in coronary arterioles isolated from male, but not female, pigs. Our data also indicate that adenosine-mediated Kv-channel activation can occur independent of nitric oxide. Furthermore, Kv channels contributed significantly to the maintenance of basal diameter in coronary arterioles from both male and female pigs. We conclude that Kv channels can be vital to the mechanism of action of endogenous vasodilators and, therefore, may be an important pharmacological target for the treatment of vascular pathologies such as hypertension and ischemia.

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