Hypercholesterolemia abolishes voltage-dependent $K^+$ channel contribution to adenosine-mediated relaxation in porcine coronary arterioles


Hypercholesterolemia abolishes voltage-dependent $K^+$ channel contribution to adenosine-mediated relaxation in porcine coronary arterioles. Am J Physiol Heart Circ Physiol 288: H568–H576, 2005. First published September 30, 2004; doi:10.1152/ajpheart.00157.2004.—Hypercholesterolemic patients display reduced coronary flow reserve in response to adenosine infusion. We previously reported that voltage-dependent $K^+$ ($K_v$) channels contribute to adenosine-mediated relaxation of coronary arterioles isolated from male miniature swine. For this study, we hypothesized that hypercholesterolemia attenuates $K_v$ channel contribution to adenosine-induced vasodilatation. Pigs were randomly assigned to a control or high fat/high cholesterol diet for 20–24 wk, and then killed. After completion of the experimental treatment, arterioles (~150 μm luminal diameter) were isolated from the left-ventricular free wall near the apical region of the heart, cannulated, and pressurized at 40 mmHg. Adenosine-mediated relaxation was significantly attenuated in both endothelium-intact and -denuded arterioles from hypercholesterolemic compared with control animals. The classic $K_v$ channel blocker, 4-aminopyridine (1 mM), significantly attenuated adenosine-mediated relaxation in arterioles isolated from control but not hypercholesterolemic animals. Furthermore, the nonselective $K^+$ channel blocker, tetraethylammonium (TEA; 1 mM) significantly attenuated adenosine-mediated relaxation in arterioles from control but not hypercholesterolemic animals. In additional experiments, coronary arteriolar smooth muscle cells were isolated, and whole cell $K_v$ currents were measured. $K_v$ currents were significantly reduced (~15%) in smooth muscle cells from hypercholesterolemic compared with control animals. Furthermore, $K_v$ current sensitive to low concentrations of TEA was reduced (~45%) in smooth muscle cells from hypercholesterolemic compared with control animals. Our data indicate that hypercholesterolemia abolishes $K_v$ channel contribution to adenosine-mediated relaxation in coronary arterioles, which may be attributable to a reduced contribution of TEA-sensitive $K_v$ channels in smooth muscle of hypercholesterolemic animals.

MATERIALS AND METHODS

Animals. Male Yucatan miniature swine were obtained from the breeder (Sinclair Farms; Columbia, MO) and housed in animal facilities at the University of Missouri College of Veterinary Medicine. Animals were matched for body mass and assigned to either a control or high fat/high cholesterol diet for 20–24 wk. Pigs on the control diet were fed Laboratory Mini-Pig Breeder Chow (PMI Feeds) with calories provided by 23% protein, 8% fat and 69% carbohydrate. The high cholesterol diet consisted of Mini-Pig Chow supplemented (by weight) with 2.0% cholesterol, 17.1% coconut oil, 2.4% corn oil, and 0.7% sodium cholate with calories provided by 13% protein, 46% fat and 41% carbohydrate. Pigs were fed to maintain a matched body mass throughout the study. Animals were fed an average of 15–20 g/kg once daily, and water was provided ad libitum. All animal protocols were in accordance with the U.S. Government’s “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 by using ketamine (35 mg/kg im), rompun (2.25 mg/kg im), and thio-pentobarbital (10 mg/kg iv), followed by administration of heparin (1,000 U/kg iv). Animals were killed by removal of hearts, which were immediately placed in cold (4°C) Krebs bicarbonate buffer. The left ventricular free wall, near the apical region of the heart, was isolated and placed in a chilled (4°C) dissection chamber containing PSS (in mM): 138 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 glucose, and 20 HEPES, pH 7.4. Coronary arterioles from pigs (~150 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

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isolated was typically ~1–1.5 mm. Arterioles were cannulated on one end with a glass micropipette filled with PSS-albumin and tied securely to the pipette using 11–0 ophthalmic suture. The arteriole was gently flushed, and the other end was cannulated with a second micropipette and tied. PSS-albumin contained (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, plus 1 g/100 ml bovine serum albumin. For arterioles in which the endothelium was denuded, arterioles were cannulated on one end with a glass micropipette and 0.4% CHAPS (prepared in PSS) was passed through the vessel lumen for ~1.5–2 min at 40 mmHg. The arteriole lumen was then flushed with PSS-albumin for ~3 min, 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 110), video monitor (Sony), and video micrometer (Microcirculation Research Institute; Texas A&M University, College Station, TX). Data acquisition and analysis were accomplished by using Axoscope 8.0 software (Axon Instruments). Both micropipettes were connected to a single reservoir system adjusted to set 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 (PSS-albumin) was gradually warmed and maintained at 37°C for the duration of the experiment. Luminal diameter was monitored continuously throughout the experiment.

Experimental protocol. Arterioles underwent a 1-h equilibration period at 40 mmHg during which time the vessels established a stable basal tone. For experiments in which a K⁺ channel blocker was present, arterioles were further preconstricted with endothelin-1 until a preconstriction level of ~40–60% maximal diameter was attained. For control experiments (no K⁺ channel blocker present), vessels were preconstricted to the same level (~40–60%) using only endothelin-1. Adenosine concentration-response relationships were determined by cumulative additions of concentrated stock solutions directly to the tissue bath. Adenosine concentration was increased when the response to the previous concentration had stabilized. The order of the adenosine curves (in the absence and presence of K⁺ channel blockade) was randomized to control for potential changes in vessel responsiveness over time. 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 drugs applications were made to the tissue bath.

Smooth muscle cell dissociation. Coronary arterioles (~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 then replaced with enzyme-free low-Ca²⁺ solution, and the entire vessel was dispersed with gentle trituration by micropipette for isolation of single smooth muscle cells. Smooth muscle cells were morphologically distinguishable from other cells types in the dispersion, such as endothelial cells and fibroblasts. Isolated cells were maintained in low-Ca²⁺ solution at 4°C until used (0–6 h).

Whole cell voltage clamp. Whole cell K⁺ currents were obtained from single cells using standard whole cell voltage-clamp techniques as used routinely (5, 13). Experiments were conducted under physiological K⁺ concentrations. Because membrane depolarization activates both Kₑ and large-conductance Ca²⁺-dependent K⁺ (BKCa) channels, we utilized low extracellular Ca²⁺ (0.1 mM) and 10 mM EGTA in the pipette to chelate intracellular Ca²⁺ and thereby minimize the contribution of BKCa current to outward K⁺ current (30). We also limited the depolarizing command pulses to +20 mV to minimize activation of BKCa channels (22). The contribution of Kᵥ,TPP channels to whole cell K⁺ current was minimized by inclusion of 2 mM ATP in the pipette solution. These conditions allowed us to isolate Kₑ currents (13, 22, 30). Cells were initially superfused with PSS containing (in mM): 138 NaCl, 5 KCl, 0.1 CaCl₂, 1 MgCl₂, 10 glucose, 20 HEPES, pH 7.4. Heat-polished glass pipettes (2–5 MΩ) were filled with a solution containing (in mM): 120 KCl, 10 NaCl, 1 MgCl₂, 10 EGTA, 10 HEPES, 2 Na₂ATP, 0.5 Tris-GTP, pH 7.1 with KOH. Stock solutions of selected pharmacological K⁺ channel blockers were added to the superfusate. Ionic currents were amplified with an Axopatch 200B patch-clamp amplifier (Axon Instruments). Currents were elicited by 500-ms step depolarizations to potentials ranging from ~60 to +20 (in 10-mV increments) from a holding potential of ~80 mV. Steady-state inactivation of outward current was measured by using 5-s conditioning pulses (~70 to +40 mV) in 10-mV increments from a holding potential of ~80 mV, followed by a 600-ms test potential of +30 mV. Currents were low-pass filtered with a cutoff frequency of 1,000 Hz, digitized at 2.5 kHz, and stored on computer. Data acquisition and analysis were accomplished by using pClamp 8.0 software (Axon Instruments). Leak subtraction was not performed. Cells were continuously perfused under gravity flow at room temperature (22–25°C).

Drugs and solutions. Stock solution of 4-aminopyridine (4-AP) was prepared with distilled H₂O and 1 M HCl to a final pH of 7.4. Tetraethylammonium (TEA), iberiotoxin and adenosine stocks were prepared in distilled H₂O. Endothelin stock was prepared in PSS. Nifedipine stock solution was prepared in ethanol. Vehicle concentrations did not exceed 0.1%. Drugs were obtained from Sigma (St. Louis, MO) unless otherwise noted. Smooth muscle cell dispersion chemicals were obtained from Worthington Chemicals (Freehold, NJ) and endothelin-1 from Peninsula Laboratories (San Carlos, CA).

Data analysis. For endothelin preconstriction, data are presented as percent possible constriction, [(Dₚ – Dₘ)/(Dₚ)] × 100, where Dₚ is the passive internal diameter and Dₘ is the steady-state internal 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 internal diameter relative to the maximal possible relaxation [(Dₘ – Dₚ)/(Dₚ)] × 100, where Dₚ is the endothelin-preconstricted baseline diameter to normalize for differences in initial and passive diameters between vessels. Adenosine concentration-response curves of arterioles were analyzed by using two-way repeated measures ANOVA and the Greenhouse-Geisser adjustment to control for type I error due to unequal group sizes (19). Mean differences were ascertained by using Bonferroni multiple comparison tests when either the main interaction or drug effect was significant.

Steady-state activation curves (g/gmax) were constructed as conductance (g = FVn – Eₖ) at each test potential relative to conductance at +20 mV (gmax), where I is the current amplitude at each potential (Vₘ) and Eₖ is the calculated reversal potential for the outward K⁺ current. Steady-state inactivation curves were calculated as current (I) relative to maximal current (Imax) attained during the step depolarization to +30 mV after each conditioning pulse. Data for both activation and inactivation curves were obtained at the end of each command pulse for each step depolarization. Activation and inactivation data were fit to a conventional Boltzmann distribution equation, I = Iₘₐₓ/[1 + exp((Vₘₐₓ – Vₙ₅)/K)], where I is the outward current at a given test potential (Vₘₚ), Iₘₐₓ is the maximal current, Vₙ₅ is the membrane potential producing half-maximal activation/inactivation and K is the slope. K, Vₙ₅, and IC₅₀ values were compared between groups using unpaired t-tests.

Concentration-dependent inhibition of whole cell Kₑ currents by 4-AP and TEA were best-fit using either a single- or two-component equation. Data were curve fit using the averaged data from the cells.
RESULTS

Effectiveness of the high fat/high cholesterol diet. Animal body weight did not differ between control and hypercholesterolemic animals at the time of death (41.0 ± 1.5 vs. 43.5 ± 1.3 kg, respectively). Total serum cholesterol levels and low-density lipoprotein (LDL)-cholesterol were both significantly elevated by 4 wk after initiation of the high cholesterol diet compared with control, and remained at greater levels throughout the study (Fig. 1, A and B, respectively). These cholesterol levels are consistent with other studies (4, 28) using this animal model.

Characteristics of arterioles. Maximal $D_P$ of cannulated coronary arterioles measured at 40 mmHg intraluminal pressure in Ca$^{2+}$-free PSS plus nifedipine was not significantly different between pigs fed the control compared with high cholesterol diet under both endothelium-intact and -denuded conditions (Table 1). The level of preconstriction (% maximal intraluminal diameter) was similar between coronary arterioles of pigs fed the control compared with high cholesterol diets under both endothelium-intact and denuded conditions (Table 1). The concentration of endothelin-1 required to attain this level of preconstriction was not significantly different between groups but tended to be reduced in endothelium-denuded arterioles compared with endothelium-intact arterioles (Table 1). Endothelium denudation was verified by complete block of relaxation to the endothelium-dependent vasodilator bradykinin (10 nM).

Adenosine-mediated concentration-response curves. Concentration-response curves for adenosine in arterioles from control and hypercholesterolemic animals are compared in Fig. 2. Figure 2A represents adenosine curves in arterioles with an intact endothelium, whereas in Fig. 2B, arterioles have been denuded of endothelium. These data demonstrate that adenosine-mediated vasodilatation was significantly attenuated in arterioles from hypercholesterolemic compared with control animals under both endothelium-intact and denuded conditions.

Role of $K_v$ channels in adenosine-mediated relaxation. As illustrated in Fig. 3, the classic $K_v$ channel blocker, 4-AP, significantly attenuated adenosine-induced relaxation in arterioles from control (Fig. 3A) but not hypercholesterolemic (Fig. 3B) animals. These data confirm our previous report that $K_v$ channels contribute significantly to adenosine-induced relaxation in coronary arterioles from male pigs (13). In contrast, hypercholesterolemia abolished the contribution of $K_v$ channels to adenosine-mediated relaxation. These studies were confirmed in endothelium-denuded arterioles of control and

Table 1. Arteriolar characteristics in the absence (control) and presence of 4-AP

<table>
<thead>
<tr>
<th>Diet</th>
<th>Endothelium</th>
<th>$n$</th>
<th>$D_P$ (mm)</th>
<th>%preconstr</th>
<th>ET (nM)</th>
<th>%preconstr</th>
<th>ET (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4-AP</td>
<td></td>
</tr>
<tr>
<td>NF</td>
<td>Intact</td>
<td>9</td>
<td>145.3±21.0</td>
<td>57.3±5.5</td>
<td>0.7±0.2</td>
<td>61.0±4.7</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>NF</td>
<td>Denuded</td>
<td>8</td>
<td>152.5±16.0</td>
<td>48.3±5.1</td>
<td>0.5±0.1</td>
<td>53.4±4.3</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>HF</td>
<td>Intact</td>
<td>11</td>
<td>154.4±11.0</td>
<td>55.8±2.7</td>
<td>0.8±0.2</td>
<td>64.1±3.1</td>
<td>0.3±0.1</td>
</tr>
<tr>
<td>HF</td>
<td>Denuded</td>
<td>7</td>
<td>129.6±13.0</td>
<td>51.9±3.4</td>
<td>0.4±0.1</td>
<td>51.8±5.3</td>
<td>0.4±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. NF, normal fed diet; HF, high-fat fed diet; $D_P$, passive (maximal) arteriole diameter; %preconstr, % of maximal diameter to which arterioles were preconstricted; ET, concentration of endothelin required to obtain % preconstriction. $n$, number of arterioles studied. No significant differences exist. 4-AP, 4-aminopyridine.
cell $K_v$ current at test potentials ($-40 \text{ mV}$) near resting membrane potential are especially noteworthy, because this falls within the physiological range of membrane potential for smooth muscle in arterioles of this size (14). Whether changes in the intracellular milieu under whole cell recording conditions influence these properties is unknown. Figure 6, C and D, compares the steady-state activation and inactivation properties of $K_v$ currents from control and hypercholesterolemic animals. As illustrated in Fig. 6C, the test potential at which outward current was half-activated was significantly shifted toward more positive membrane potentials in hypercholesterolemic compared with control animals ($-5.0 \pm 0.8 \text{ vs. } -7.9 \pm 0.4 \text{ mV}$, respectively). The slope value, which provides an indication of the sensitivity of the current to voltage, was not significantly altered by diet ($9.9 \pm 0.7 \text{ vs. } 9.4 \pm 0.4 \text{ mV}$, respectively). Comparison of steady-state inactivation for currents from control and hypercholesterolemic animals demonstrates that neither $V_{0.5}$ ($-16.6 \pm 0.3 \text{ and } -15.3 \pm 0.9 \text{ mV}$, respectively) nor the slope for inactivation ($-11.0 \pm 0.3 \text{ and } 11.4 \pm 0.9 \text{ mV}$, respectively) was altered by diet (Fig. 6D).

**Pharmacological characterization of $K_v$ current.** To further characterize outward $K_v$ current in arteriolar smooth muscle cells, we examined the response of whole cell $K_v$ current to the $K^+$ channel blockers 4-AP and TEA. Figure 7, A and B, shows representative current traces obtained under control conditions and in response to increasing concentrations of 4-AP and TEA, respectively, in both control and hypercholesterolemic animals. Current was measured at +10 mV (end of pulse) from a hypercholesterolemic animals (Figs. 4, A and B, respectively). Further studies demonstrated that the nonselective $K^+$ channel blocker, TEA (1 mM), significantly attenuated adenosine-mediated relaxation in arterioles from control but not hypercholesterolemic animals (Fig. 5).

**Voltage-dependence of $K^+$ current.** We compared the basic biophysical properties of whole cell $K_v$ currents from coronary arteriolar smooth muscle of both control and hypercholesterolemic animals. Currents were elicited by 500-ms step-depolarizations to potentials ranging from $-60$ to $+20 \text{ mV}$ from a holding potential of $-80 \text{ mV}$ (Fig. 6A). We effectively eliminated the BK$_{Ca}$ current contribution to total outward $K^+$ current at test potentials equal or negative to $+20 \text{ mV}$ as evidenced by the lack of inhibition of whole cell $K^+$ current by the selective BK$_{Ca}$ channel blocker iberiotoxin (100 nM; Fig. 6B, inset) as documented previously (13). Current is plotted as the mean value of the outward current for the last 50 ms of each test potential and is normalized to cell membrane capacitance (pA/pF). Cell capacitance was significantly greater in smooth muscle cells isolated from hypercholesterolemic compared with control animals ($13.4 \pm 0.5 \text{ vs. } 11.5 \pm 0.5 \text{ pF}$). Comparison of the current-voltage relationships ($I-V$) indicated that smooth muscle cells from hypercholesterolemic pigs displayed significantly reduced whole cell $K_v$ currents when compared with control animals (Fig. 6B). Significant differences in whole
holding potential of $-80 \text{ mV}$ and plotted as the percentage of control current remaining as a function of the drug concentration after steady-state inhibition was attained (Fig. 7, C and D). Concentration-dependent inhibition of whole cell $K_v$ current was quantified by using a best-fit approach. Although multiple $K_v$ channel isoforms with varying drug sensitivities likely contribute to $K_v$ current in coronary arteriolar smooth muscle, the number of components established by curve fitting is the minimum number that represents the experimental data. In both control and hypercholesterolemic animals, blockade of whole cell current with 4-AP was best fit with a two-component equation, indicating that $K_v$ current from both animal treatment groups displayed both high- and low-sensitive components to 4-AP inhibition (Fig. 7, C and D). Similarly, inhibition of $K_v$ currents by TEA in cells from control animals was best fit with a two-component equation (Fig. 7, D and F). In contrast, sensitivity of $K_v$ currents to TEA in cells from hypercholesterolemic animals was best fit with a single-component fit, displaying only a low-sensitive component and an absence of a corresponding high-sensitive component found in control cells (Fig. 7, D and F). The relative amplitude ($F_1$) of the two-component fits for 4-AP-sensitive data was 79 and 90% for cells from control and hypercholesterolemic animals, respectively, and 57% for TEA-sensitive data from control cells.

To further confirm the loss of a high $K_v$-sensitive component with hypercholesterolemia, we compared $K_v$ current sensitive to 500 $\mu\text{M}$ TEA in cells from control and hypercholesterolemic animals. TEA-sensitive currents were obtained by subtraction of currents in the presence of TEA from control currents (Fig. 8A). Comparison of the $I-V$ relationships indicated that smooth muscle cells from hypercholesterolemic pigs displayed significantly reduced TEA-sensitive $K_v$ currents when compared with control animals (Fig. 8B). These data indicate that hypercholesterolemia selectively abolishes the function and/or expression of a highly TEA-sensitive $K_v$ channel. Interestingly, the mathematical difference in TEA-sensitive $K_v$ current (Fig. 8B) between cells from control and hypercholesterolemic pigs is similar to the difference observed in total $K_v$ current (Fig. 6B), suggesting that the loss of the TEA-sensitive $K_v$ current accounts entirely for the difference in the total $K_v$ current.

**DISCUSSION**

This study documents the novel finding that adenosine-mediated relaxation is significantly impaired in both endothelium-intact and -denuded coronary arterioles of hypercholesterolemic animals. Furthermore, hypercholesterolemia abolishes the $K_v$ channel contribution to adenosine-mediated relaxation in both endothelium-intact and -denuded coronary arterioles. These data are consistent with the conclusion that the impaired adenosine-mediated relaxation observed in arterioles from hypercholesterolemic animals is attributable to an impaired adenosine-activation of $K_v$ channels. Because nearly all $K_v$ channel isoforms display $IC_{50}$ values below or very near 1 mM 4-AP (for review, see Refs. 6 and 8), we postulate that...
This concentration should provide partial-to-full block of most Kv channel isoforms. Thus we propose that the lack of effect of 1 mM 4-AP on adenosine-mediated relaxation in arterioles from hypercholesterolemic animals is indicative of elimination of Kv channel contribution to adenosine-mediated relaxation. We also establish for the first time that Kv channels contribute significantly to adenosine-mediated relaxation in endothelium-denuded coronary arterioles, extending our previous finding in endothelium-intact arterioles (13) and providing the first evidence that Kv channels contributing to adenosine-mediated vasodilatation in coronary arterioles reside in smooth muscle.

It is well established that coronary flow reserve is reduced in hypercholesterolemic patients, providing evidence of abnormal vasodilatory response of the coronary microcirculation to intravenous adenosine or dipyridamole (11, 12, 24, 32). However, the present data are the first in vitro studies to document that hypercholesterolemia attenuates adenosine-mediated vasodilatation in coronary arterioles.

We also establish for the first time that Kv channels contribute to adenosine-mediated relaxation responses to adenosine are not altered in coronary arterioles (27) and femoral and iliac arteries (18) of atherosclerotic monkeys. In contrast, others have documented that relaxation responses to adenosine are not altered in coronary arterioles (27) and femoral and iliac arteries (18) of atherosclerotic monkeys. The disparate findings between the present study and previous findings in coronary arterioles are currently unresolved but may be attributed to differences in the level at which vessels were pressurized in these studies (i.e., 20 vs. 40 mmHg) or different pharmacological preconstrictors (U-46619 vs. endothelin) before adenosine administration. An intraluminal pressure of 40 mmHg was used in our experiments, because this is near the intraluminal pressure measured under physiological conditions in coronary arterioles of this size (7). Finally, it is of interest to note that a trend for impaired adenosine-mediated relaxation was observed by Sellke et al. (27); however, statistical differences may have been masked by the relatively high degree of variability.

Fig. 6. Effect of hypercholesterolemia on whole cell Kv current and current kinetics in coronary arteriole smooth muscle cells. A: representative current traces for whole cell Kv current of cells from both control and HC animals. Currents were elicited by 500-ms step depolarizations (tp) to potentials ranging from −60 to +20 (in 10 mV increments) from a holding potential (hp) of −80 mV. Cell capacitances for representative traces were 10.3 and 13.1 pF for control and HC, respectively. B: comparison of I-V relationships obtained by plotting mean current at the end of the steps as a function of the indicated step potential. Whole cell Kv current was significantly diminished in myocytes from HC compared with control animals. Left inset: differences in Kv current at negative membrane potentials are emphasized by plotting on smaller y-axis scale. Right inset: outward K+ currents (IKv) were not affected by inclusion of iberiotoxin (IbTx; 100 nM) in superfusate at step depolarizations equal or negative to +20 mV (hp = −80 mV), indicating no contamination of large-conductance Ca2+-dependent K+ (BKCa) current in whole cell measures. C: steady-state activation curves were constructed as conductance (g = IVm − EK) at each test potential relative (end of pulse) to conductance at +20 mV (gmmax), where I was the steady-state K+ current amplitude at each potential (Vm) and EK was the calculated reversal potential for the outward K+ current. Relative conductance (g/gmmax) was fit to a Boltzmann distribution equation as described in MATERIALS AND METHODS. The test potential at which outward current was half-activated was significantly shifted toward more positive membrane potentials in HC compared with control animals (inset), whereas slope was not altered by diet. D: steady-state inactivation of outward current was measured by using 5-s conditioning pulses (−70 to +40 mV) in 10-mV increments from a holding potential of −80 mV, followed by a 600-ms test potential of +30 mV. Data were fit to a Boltzmann distribution equation. Comparison of inactivation kinetics indicated no effect of diet on half-maximal inactivation (inset) or slope. Data are average of smooth muscle cells from 6 animals. Numbers in parentheses indicate number of cells. Error bars in B, C, and D, some of which are smaller than symbol, represent SE. *P ≤ 0.05 HC vs. control.
Adenosine is proposed to mediate its endothelium-independent vasodilatory effect via activation of cAMP-dependent PKA. An increasing body of evidence generated from our laboratory (13) and others (1, 2, 9, 15, 17, 26) indicates that PKA and substances known to enhance cellular PKA levels, such as adenosine, isoproterenol, forskolin, and dibutyryl cAMP, stimulate Kv channels in coronary and other smooth muscle cell types. Furthermore, a previous study (17) indicated that pathophysiological conditions associated with oxidative stress impair PKA-mediated vasodilatation of coronary arterioles, attributed, in part, to reduced activity of Kv channels. The deleterious effects of hypercholesterolemia on vascular reactivity of coronary arterioles have also been associated with increased oxidative stress (25), suggesting increased oxidative stress associated with hypercholesterolemia as a potential mechanism for the impaired coupling of adenosine with Kv channels observed in the present study.

We also report the novel finding that whole cell Kv currents are significantly reduced in coronary arteriolar smooth muscle cells from hypercholesterolemic compared with control animals, supporting a previous report of decreased whole cell K⁺ currents in portal vein smooth muscle of hypercholesterolemic rabbits (10). These data suggest that the impaired adenosine activation of Kv channels in arterioles of hypercholesterolemic animals may be attributed to reduced Kv channel activity or expression rather than an impaired coupling of adenosine with Kv channels. We have previously reported that the selective BKCa channel blocker, iberiotoxin (100 nM), did not alter adenosine-mediated relaxation in coronary arterioles, whereas TEA (1 mM) significantly attenuated adenosine-induced relaxation (13). Additional studies documented inhibition of whole cell Kv currents by TEA (1 mM) in the presence of iberiotoxin (13). These previous findings indicate that in addition to its effects on BKCa channels, TEA also blocks Kv channels, even at low concentrations. Based on these data, we have demonstrated that TEA-sensitive Kv channels contribute to adenosine-mediated relaxation in arterioles from control, but not hypercholesterolemic animals. Consistent with this finding, arteriolar smooth muscle from hypercholesterolemic animals demonstrates a dramatic (~45%) reduction in TEA-sensitive
Kv current. Furthermore, pharmacological characterization of smooth muscle whole cell Kv current indicates that current from hypercholesterolemic animals does not display the high TEA-sensitive component observed in cells from control animals. These findings suggest that a subset of Kv channels, sensitive to relatively low concentrations of TEA, may be absent or nonfunctional in coronary arteriolar smooth muscle of hypercholesterolemic animals. Furthermore, this subset of Kv channels (or a member of this subset) may be the isoforms activated by adenosine in arteriolar smooth muscle of control animals. These findings suggest that a subset of Kv channels, which appears to be attributable to a reduced availability of TEA-sensitive Kv channels in hypercholesterolemic animals. We also demonstrate that whole cell Kv currents are reduced in smooth muscle cells from hypercholesterolemic compared with control animals, which appears to be attributable to a reduced availability of TEA-sensitive Kv channels in hypercholesterolemic animals. We propose that the impaired adenosine-induced vasodilation observed in coronary arterioles from hypercholesterolemic animals is attributable to decreased activity of TEA-sensitive Kv channel isoforms. However, we cannot discount the possibility that transduction mechanisms (e.g., PKA pathway) that couple adenosine with Kv channel isoforms may be impaired under hypercholesterolemic conditions. Studies examining Kv channel isoform expression and the coupling of adenosine with Kv channels will be required to validate this proposed mechanism.
REFERENCES


