Endothelin-1 Inhibits Inward Rectifier K^+ Channels in Rabbit Coronary Arterial Smooth Muscle Cells Through Protein Kinase C

Won Sun Park, Jin Han,* Nari Kim,* Jae Boum Youm,* Hyun Joo,* Hyung Kyu Kim,* Jae-Hong Ko,† and Yung E. Earm†*

Abstract: We studied inward rectifier K^+ (Kir) channels in smooth muscle cells isolated from rabbit coronary arteries. In cells from small- (<100 μm, SCASMC) and medium-diameter (100 ~ 200 μm, MCASMC) coronary arteries, Kir currents were clearly identified (11.2 ± 0.6 and 4.2 ± 0.6 pA pF^{-1} at –140 mV in SCASMC and MCASMC, respectively) that were inhibited by Ba^{2+} (50 μM). By contrast, a very low Kir current density (1.6 ± 0.4 pA pF^{-1}) was detected in cells from large-diameter coronary arteries (>200 μm, LCASMC). The presence of Kir2.1 protein was confirmed in SCASMC in a Western blot assay. Endothelin-1 (ET-1) inhibited Kir currents in a dose-dependent manner. The inhibition of Kir currents by ET-1 was abolished by pretreatment with the protein kinase C (PKC) inhibitor staurosporine (100 nM) or GF 109203X (1 μM). The PKC activators phorbol 12,13-dibutyrate (PDBu) and 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) reduced Kir currents. The ET_A-receptor inhibitor BQ-123 prevented the ET-1–induced inhibition of Kir currents. The amplitudes of the ATP-dependent K^+ (K_{ATP}), Ca^{2+}-activated K^+ (BK_{Ca}), and voltage-dependent K^+ (K_V) currents, and effects of ET-1 on these channels did not differ between SCASMC and LCASMC. From these results, we conclude that Kir channels are expressed at a higher density in SCASMC than in larger arteries and that the Kir channel activity is negatively regulated by the stimulation of ET_A-receptors via the PKC pathway.

Key Words: coronary artery, endothelin-1, inward rectifier K^+ channel, Kir2.1, protein kinase C

(*J Cardiovasc Pharmacol*™ 2005;46:681–689)

Received for publication July 7, 2005; accepted August 6, 2005.

From the *Mitochondrial Signaling Laboratory, Department of Physiology and Biophysics, College of Medicine, Biohealth Products Research Center, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea; †Department of Physiology and National Research Laboratory for Cellular Signaling, Seoul National University College of Medicine, Seoul, Korea.

This work was supported by a National Research Laboratory Grant and Grants R05-2002-000-00905-0, R05-2003-000-00413-0 and R05-2004-000-00905-0 by the Korea Science and Engineering Foundation from the Ministry of Science and Technology, BK21 Human Life Sciences of the Ministry of Education, and the Research Project on the Production of Bio-organs, Ministry of Agriculture and Forestry, Republic of Korea.

Reprints: Yung E. Earm, Department of Physiology, Seoul National University College of Medicine, 28 Yonkeun-Dong, Chongno-Gu, Seoul 110-799, Korea (e-mail: earmye@snu.ac.kr).

In general, small arteries play a major role in the control of systemic blood pressure and local blood flow. Small arteries differ in several ways from larger ones. For example, small coronary arteries respond actively to changes in luminal flow, intravascular pressure, and the concentration of local metabolites, whereas large coronary arteries are relatively insensitive to these factors.

The local metabolites are considered to act directly on arterial smooth muscle and cause vasodilation. These include a fall in pH, the release of products of purine metabolism, endothelium-dependent hyperpolarizing factors (EDHF), and an elevation of local extracellular K^+ concentration.[1] K^+ is one of several factors that link increases in blood flow, membrane potential, and vascular tone.[2] Extracellular K^+ concentrations increase to greater than 10 mM during coronary and cerebral ischemia.[3,4] Most systemic arteries constrict in response to an increase in extracellular K^+ concentrations; however, moderate increases in extracellular K^+ concentrations (~15 mM) in small coronary and cerebral arteries lead to vasodilation and increased blood flow.[5,6]

A number of mechanisms have been suggested to explain K^+-induced vasodilations including activation of Kir channels and Na^+/K^+ ATPases.[2,5] The vasodilation of small arteries induced by extracellular K^+ is blocked by low concentrations of extracellular Ba^{2+} (<50 μM), but not by blockers of ATP-dependent K^+ (K_{ATP}), Ca^{2+}-activated K^+ (BK_{Ca}), or voltage-dependent K^+ (K_V) channels; inhibitors of Na^+-K^+ ATPase; or the removal of the endothelium. These observations suggested that K^+ dilated small arteries through activation of Kir channels.

Although several reports have identified and characterized the responses of Kir channels in rat and pig small-diameter coronary arteries and arterioles in relation to small increases in extracellular K^+ concentrations, little is known about the modulation of Kir channels in rabbit coronary arteries.

Endothelin-1 (ET-1), a 21-amino-acid peptide secreted by vascular tissue, is the most potent vasoconstrictor described to date.[7] Previous studies of vessels of the systemic circulation revealed that ET-1 modulated various types of ion channels, including K_{ATP}, BK_{Ca}, Kv, Ca^{2+}, Cl^-, and nonselective cation (NSC) channels.[8–13] However, it is not clear whether the effects of ET-1 on ion channels are owing to direct coupling between the channels and ET receptors (ET_A or ET_B) or whether the effects are secondary to other cellular events, such as increases in intracellular Ca^{2+} concentration, depolarization, or the

activation of protein kinase C (PKC). Moreover, the effect of ET-1 on Kir channels in vascular smooth muscle cells has not been studied.

To address these questions in this study, we examined the distribution of Kir channels in rabbit coronary arteries with different diameters using the whole-cell patch clamp technique and Western blot analysis. In addition, the regulation of Kir channels by ET-1 and its mechanism were investigated.

METHODS

Cell Preparation

New Zealand White rabbits (1.5 ~ 2.0 kg) were anesthetized with sodium pentobarbitone (50 mg kg^{-1}) and simultaneously injected with heparin (100 U kg^{-1}). The hearts were removed and immersed in normal Tyrode solution. The left anterior descending (LAD) coronary arteries and all branches connected to LAD were dissected. Before enzymatic treatment of the coronary arteries (see below), the diameter of each dissected artery was measured using a video edge detector (Crescent Electronics, Sandy, UT). Each artery was cut open along the longitudinal axis. The arteries were transferred to Ca^{2+}-free normal Tyrode solution that contained papain (1.0 mg/ml), bovine serum albumin (BSA, 1.5 mg/ml), and dithiothreitol (DTT, 1.0 mg/ml). After a 25-minute incubation, the arteries were transferred to Ca^{2+}-free normal Tyrode solution containing collagenase (2.8 mg/ml), BSA, and DTT, and then incubated for ~20 minutes. Following the enzyme treatment, each artery was rinsed with Kraft-Brühe (KB) solution. Individual cells were dispersed in this solution by gentle agitation of the tissue with a fire-polished Pasture pipette.

Solutions

Normal Tyrode solution contained (in mM): NaCl, 140; KCl, 5.4; NaH_2PO_4, 0.3; $CaCl_2$, 1.8; $MgCl_2$, 0.5; HEPES, 5; glucose, 16.6; adjusted to pH 7.4 with NaOH. The 140 K^+ external solution was made by substituting NaCl for KCl in the normal Tyrode solution. KB solution contained (in mM): potassium hydroxide (KOH), 70; L-glutamate, 50; KH_2PO_4, 20; KCl, 55; taurine, 20; $MgCl_2$, 3; glucose, 20; HEPES, 10; EGTA, 0.5; adjusted to pH 7.3 with KOH. The pipette-filling solution contained (in mM): K-aspartate, 112; KCl, 25; NaCl, 5; $MgCl_2$, 3; Mg-ATP, 4; EGTA, 10; $CaCl_2$, 1; HEPES, 10; adjusted to pH 7.2 with KOH. To record the K_{ATP} currents, the concentration of Mg-ATP was lowered to 0.1 mM. For the cell-attached (BK_{Ca} channel) experiment, we used the following bath and pipette solution (in mM): KCl, 140; $MgCl_2$, 2; EGTA, 3; HEPES, 10; $CaCl_2$, 2.3; adjusted to pH 7.4. For the cell-attached (Kir channel) experiment, we used a low Ca^{2+} bath and pipette solution containing (in mM): KCl, 140; NaH_2PO_4, 0.33; $CaCl_2$, 0.1; $MgCl_2$, 2; HEPES, 5; glucose, 16.6; adjusted to pH 7.4 with KOH.

Drugs

ET-1, phorbol 12,13-dibutyrate (PDBu), 1-oleoyl-2-acetyl-sn-glycerol (OAG), pinacidil, iberiotoxin, BQ-123, BQ-788, and glibenclamide were purchased from Sigma (St. Louis, MO). Staurosporine and GF 109203X were purchased from Tocris Cookson (Ellisville, MO).

Electrophysiology

Membrane currents were recorded in the whole-cell and cell-attached configurations. The amplifier (Axopatch-1C or Axopatch 200A, Axon Instruments, Union, CA) was interfaced with a personal computer through a Digidata 1200 interface (Axon Instruments). The voltage and current signals were filtered at 0.5 ~ 1.0 kHz and were sampled at a rate of 1 ~ 3 kHz. Patch pipettes (3 MΩ) were pulled from borosilicate capillaries (Clark Electromedical Instruments, Pangbourne, UK) using a Narishige PP-83 puller (Narishige, Tokyo, Japan).

Western Blot

Strips of endothelium-denuded coronary arteries were homogenized in a hand-held Micro-tissue Grinder (PYREX) in a 1:2 volume of ice-cold storage buffer (in mM: 100 KPO_4, 1 EDTA, 1 dithiothreitol, 0.1 phenylmethylsulfonyl fluoride, and 30% glycerol, pH 7.25). The homogenate was centrifuged at 3500 g for 15 minutes at 4°C. The supernatant was collected and stored at −80°C until used for Western blot analysis.

Proteins (10 μg from 3 rabbits) from the endothelium-denuded coronary arteries were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gels were transferred to Immobilon-P membranes (Millipore, Billerica, MA), which were blocked overnight in Tris-buffered saline (20 mM Tris and 150 mM NaCl, pH 8.0) containing 5% nonfat dry milk and then probed with β-tubulin antiserum (Sigma) at a dilution of 1:1000 and antiserum for Kir2.1, 2.2, and 2.3 (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:500 for 1h at room temperature. The membranes were incubated with secondary antibodies, a goat anti-mouse IgG for β-tubulin (Santa Cruz), a mouse anti-goat IgG for Kir2.1, 2.2, and 2.3 (Santa Cruz). The secondary antibodies were conjugated to horseradish peroxidase at a dilution of 1:5000 for 1h at room temperature. Immunoreactivity was visualized using enhanced chemiluminescence (Amersham ECL Western blotting detection kit, Amersham Biosciences, Piscataway, NJ).

Statistics

Data are presented as the means ± SEM. Statistical analyses were performed using Student's *t* test. The differences between two groups were considered statistically significant at $P < 0.05$.

RESULTS

Ba^{2+}-Sensitive Kir Currents in Rabbit Coronary Arterial Smooth Muscle Cells

Smooth muscle cells were isolated from rabbit coronary arteries with small (<100 μm, SCASMC), medium (100 ~ 200 μm, MCASMC), or large (>200 μm, LCASMC) diameters. Figure 1 illustrates the Kir currents recorded from LCASMC (A and B), MCASMC (C and D), and SCASMC (E and F). To increase the magnitude of the Kir currents, both the extracellular and intracellular K^+ concentrations were maintained at 140 mM. Kir currents were recorded in response to a hyperpolarizing voltage pulse from a holding potential of –60 to –140 mV and were compared with the current response

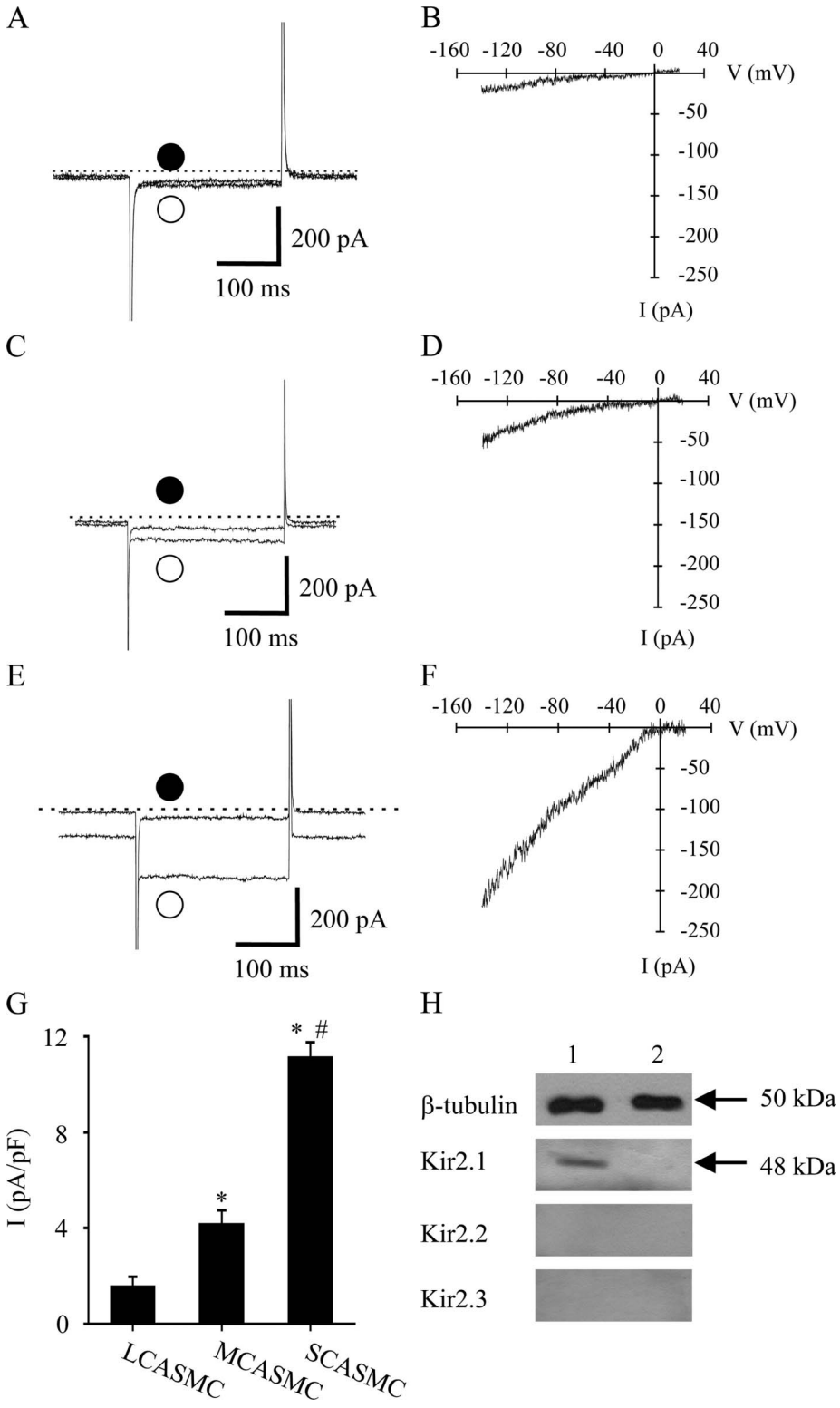

FIGURE 1. Inward rectifier K^+ (Kir) currents in rabbit coronary arterial smooth muscle cells. (A, C, and E) Whole-cell K^+ currents recorded in response to a voltage step from a holding potential of –60 to –140 mV for 200 milliseconds. A, Cells from large-diameter coronary arteries (LCASMC). C, Cells from medium-diameter coronary arteries (MCASMC). E, Cells from small-diameter coronary arteries (SCASMC). The dotted line is the zero current level (○: control, ●: 50 μM Ba^{2+}). (B, D, and F) Current-voltage relationships of 50 μM Ba^{2+}-sensitive currents in symmetrical K^+ of 140 mM in LCASMC (B), MCASMC (D), and SCASMC (F). The whole-cell current was recorded in response to a voltage step from –60 to –140 mV for 50 milliseconds, followed by a depolarizing voltage ramp from –140 to +20 mV at 0.5 mV ms^{-1}. The fraction of the current that was Ba^{2+}-sensitive was determined by subtracting the current in the presence of 50 μM Ba^{2+} from that in the control condition. G, Density of Kir currents in LCASMC (n = 5), MCASMC (n = 6), and SCASMC (n = 6). The current was normalized to the cell capacitance and is the 50 μM Ba^{2+}-sensitive current measured at –140 mV. *$P < 0.01$ versus LCASMC; #$P < 0.01$ versus MCASMC. H, Identification of Kir channel subtypes in rabbit coronary arterial smooth muscle. Western blot analysis of the expression of β-tubulin, Kir2.1, Kir2.2, and Kir2.3 proteins in SCASMC (1) and LCASMC (2) (all n = 4).

in the presence of 50 μM Ba^{2+} (Fig. 1A, C, and E). In addition, a ramp-depolarizing pulse (from –140 to 20 mV, 0.5 V s^{-1}) was applied to obtain a brief current-voltage relationship (I/V curve). Figure 1B, D, and F demonstrate the inwardly rectifying property of the Ba^{2+}-sensitive currents in coronary artery myocytes. Ba^{2+}-sensitive inward K^+ currents were detected in MCASMC (Fig. 1C and D) and SCASMC (Fig. 1E and F) but little in LCASMC (Fig. 1A and B). The reversal

potential was –1.8 ± 0.3 (n = 8). The densities of the Ba^{2+}-sensitive currents recorded at –140 mV are summarized in Figure 1G (LCASMC, 1.6 ± 0.4 pA pF^{-1}; MCASMC, 4.2 ± 0.6 pA pF^{-1}; SCASMC, 11.2 ± 0.6 pA pF^{-1}).

To further identify the subtypes of Kir channels expressed in coronary arteries, Western blot analysis was performed with specific antibodies for Kir2.1, Kir2.2, and Kir2.3 in SCASMC (1) and LCASMC (2). Kir2.2 and 2.3 were not detected in either type of cell, whereas Kir2.1 was detected only in SCASMC (the band at ~48 kDa in Fig. 1H). Similar results were obtained from four different experiments. We confirmed that these antibodies are working properly by detecting Kir2.1, Kir2.2, and Kir2.3 in rabbit ventricle (for Kir2.1 and Kir2.2) or hippocampus (for Kir2.1, Kir2.2 and Kir2.3) expressing these channels (data not shown). Therefore, we concluded that Kir2.1 is expressed in native smaller coronary artery smooth muscle cells.

Effects of ET-1 on Kir Currents

We investigated the effect of ET-1 on Kir currents in SCASMC and MCASMC. ET-1 caused a significant reduction in the magnitude of the Kir currents recorded at –140 mV. The mean degree of inhibition of the Kir current caused by ET-1 (30 nM) was 57.6 ± 3.8% (n = 4) and 36.6 ± 4.3% (n = 4) in SCASMC and MCASMC, respectively (Fig. 2A and C); the remaining current was further inhibited by the addition of Ba^{2+} in combination with ET-1. To confirm these data, the current-voltage relationship was measured from the voltage ramps, as depicted in Figure 1. The Ba^{2+}-sensitive current declined when 30 nM ET-1 was added (Fig. 2B and D). The dose-response curve indicated that SCASMC were slightly more sensitive to the ET-1-induced inhibition of Kir currents, as compared with MCASMC (K_d values: 17.4 ± 5.9 and 73.6 ± 5.3 nM in SCASMC and MCASMC, respectively, Fig. 2E).

To further characterize the effect of ET-1 on Kir channels, we made cell-attached patches for SCASMC bathed in symmetrical (140 mM) K^+. The pipette potential was clamped to +140 mV. Therefore, the holding potential corresponded to –140 mV. Figure 2F shows single-channel recordings from a cell-attached patch at a pipette potential of +140 mV. Under this condition, spontaneous channel openings were observed. However, ET-1 (30 nM) significantly reduced the Kir channel activity (*NP*o = 0.990 ± 0.003 in control versus 0.670 ± 0.124 for ET-1, n = 4). We confirmed the presence of Kir channel with Ba^{2+}-containing pipette (50 μM) in different cells. These results are summarized in Figure 2G, and the current-voltage relationship for unitary currents is shown in Figure 2H. The mean amplitudes averaged from five patches are plotted as a function of the membrane potential. The current-to-voltage relationship was almost linear, and the single-channel slope conductance was 21 pS.

Effect of ET_A and ET_B Antagonists on ET-1–Mediated Inhibition of Kir Currents

We tested the relative contributions of the ET_A and ET_B receptors to ET-1–mediated inhibition of Kir currents in SCASMC using the ET_A receptor-specific inhibitor BQ-123 (3 μM) and the ET_B receptor-specific inhibitor BQ-788 (3 μM). The ET_A receptor blockade significantly reduced the effect on ET-1–mediated Kir current inhibition (Fig. 3A, n = 5), whereas the ET_B receptor blockade had no effect on ET-1–mediated inhibition of Kir currents (Fig. 3B, n = 5). These results suggest that ET_A receptor stimulation is responsible for Kir channel inhibition by ET-1.

Protein Kinase C Mediates Inhibitory Effects of ET-1 on Kir Channels

To test whether the inhibition of Kir currents by ET-1 was mediated by the PKC pathway, we determined the effect of two specific PKC inhibitors, staurosporine and GF 109203X (GFX), on the inhibition of Kir currents induced by ET-1 (30 nM). Staurosporine (100 nM) alone had no significant effect on the magnitude of the Kir current. By contrast, in the presence of staurosporine, the ET-1–induced inhibition of Kir currents was sharply reduced in both SCASMC (Fig. 4A) and MCASMC (Fig. 4B) at a potential of –140 mV. ET-1 inhibited Kir currents by only 8.2 ± 1.2% in SCASMC and by 6.1 ± 1.9% in MCASMC in the presence of staurosporine, as compared with the control effects of ET-1 in the absence of staurosporine (57.6 ± 3.8 and 36.6 ± 4.3% inhibition in SCASMC and MCASMC, respectively). The mean results from four trials under these conditions are shown in Figure 4C. Pretreatment with another PKC inhibitor, GFX (1 μM), also strongly reduced the inhibitory effect of ET-1 (9.2 ± 1.7 and 4.4 ± 1.1% inhibition in SCASMC and MCASMC, respectively, Fig. 4C, n = 4).

To further examine the role of PKC in the inhibition of Kir currents, the effects of a specific activator of PKC, PDBu (1 μM), and a membrane-permeable analogue of diacylglycerol, OAG (1 μM), were tested. The addition of either PDBu or OAG caused a significant decrease in the Kir current (Fig. 4D, E, and F). The magnitude of the reduction was similar to that seen with ET-1–induced inhibition. The application of 1 μM PDBu decreased Kir currents by a mean of 64.6 ± 5.2% in SCASMC (n = 5, Fig. 4D) and 39.0 ± 3.5% in MCASMC (n = 4, Fig. 4E), respectively. We obtained similar results when OAG was added (60.5 ± 4.4 and 31.8 ± 2.5% inhibition in SCASMC and MCASMC, respectively, Fig. 4F, n = 4). These results suggested that the activation of PKC is the major mechanism by which ET-1 inhibited Kir channels in our preparation.

Activity of K_{ATP}, BK_{Ca}, and K_V Channels in LCASMC and SCASMC

To elucidate whether the different levels of expression in coronary arteries with different sizes are specific to Kir channels, the activities of other types of K^+ channels (K_{ATP}, BK_{Ca}, and K_V channels) were compared between SCASMC and LCASMC.

To activate the K_{ATP} channels in SCASMC and LCASMC maximally, the intracellular concentration of ATP was lowered to 0.1 mM, and 10 μM pinacidil was added to the bath perfusate. In addition, the whole-cell current was recorded at a holding potential of –60 mV to minimize the activity of the K_V channel, and the extracellular concentration of K^+ was increased to 140 mM. An increase in the extracellular K^+ concentration from 5.4 to 140 mM increased the

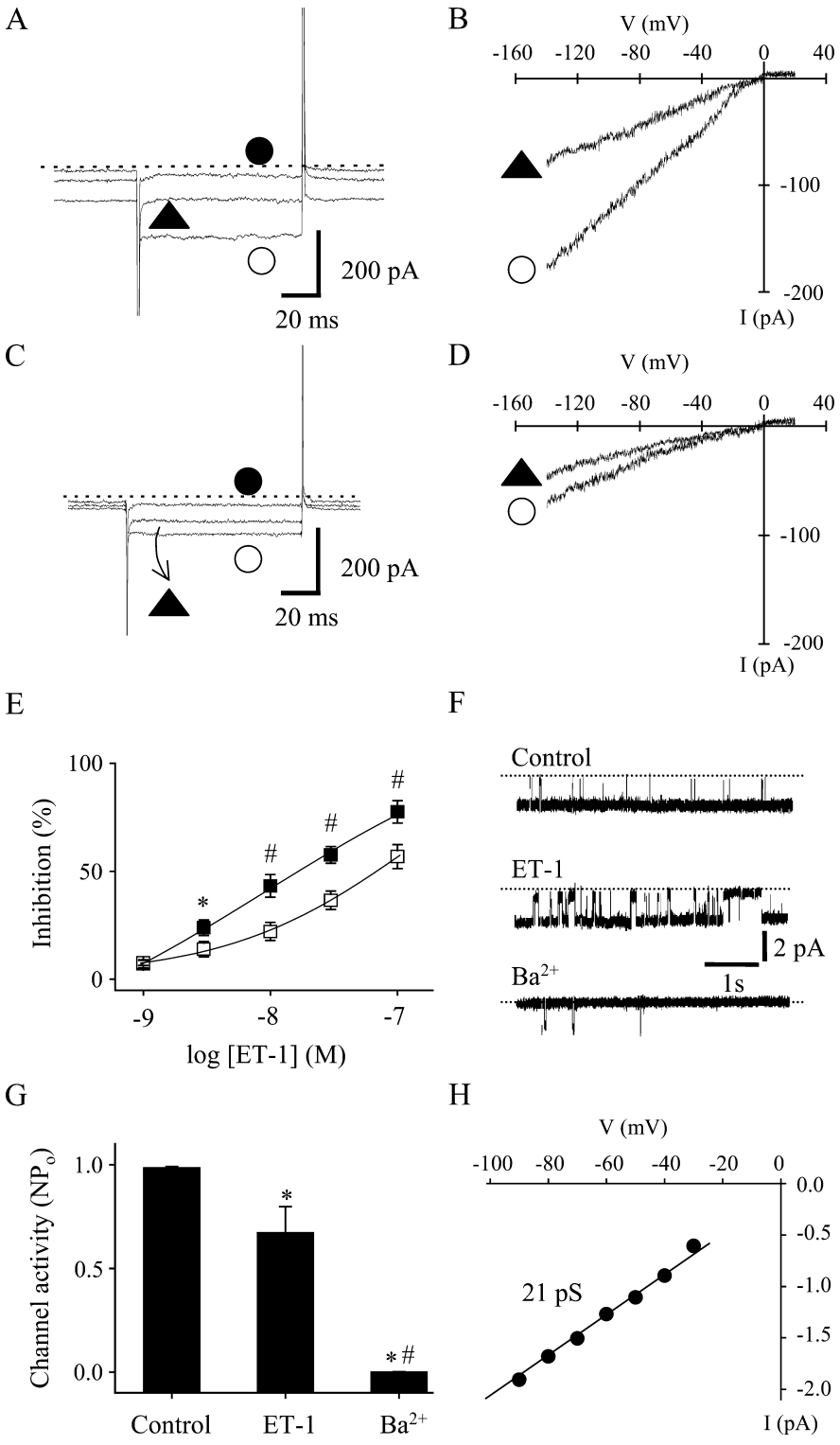

FIGURE 2. Effect of endothelin (ET)-1 on whole-cell Kir currents. A and C, 30 nM ET-1 inhibited the Kir currents in SCASMC (A) and MCASMC (C). 50 μM Ba^{2+} inhibited the remaining current (○: control, ●: Ba^{2+}, ▲: ET-1). B and D, Current-voltage relationship curves (Ba^{2+}-sensitive current) before (○) and after (▲) the application of ET-1 in SCASMC (B) and MCASMC (D). E, Average percent inhibition of Kir currents induced by different concentrations of ET-1 in SCASMC (■) and MCASMC (□) at –140 mV (all n = 4). *$P <$ 0.01 versus 10^{-9} M ET-1; #$P <$ 0.001 versus 10^{-9} M ET-1. F, Single-channel currents measured at –140 mV in control, ET-1, and Ba^{2+}. The dotted line represents the closed-channel level. G, The average open probability (*NP*o) obtained at –140 mV in four patches. *$P <$ 0.01 versus control; #$P <$ 0.001 versus ET-1. H, Mean single-channel current-voltage relationship. Each point represents the average of single-channel amplitudes obtained from five cells. The points have been fitted by a straight line, and the slope gives an estimated mean conductance of 21 pS.

inward current, whereas pinacidil activated a much greater inward current in SCASMC (Fig. 5A) and LCASMC (Fig. 5B). The inward currents were rapidly reversed to the control levels by the glibenclamide (10 μM), which suggests that the pinacidil-induced inward current is the K_{ATP} current. As shown in Figure 5C, however, the amplitudes of K_{ATP} currents were similar in SCASMC and LCASMC (11.1 ± 0.5 and 11.3 ± 0.6 pA $pF,^1$ n = 5, respectively).

We also examined the effect of ET-1 on K_{ATP} currents in SCASMC and LCASMC. As shown in Figure 5D, ET-1 caused a comparable dose-dependent inhibition of K_{ATP} currents in SCASMC and LCASMC, and the magnitude of the

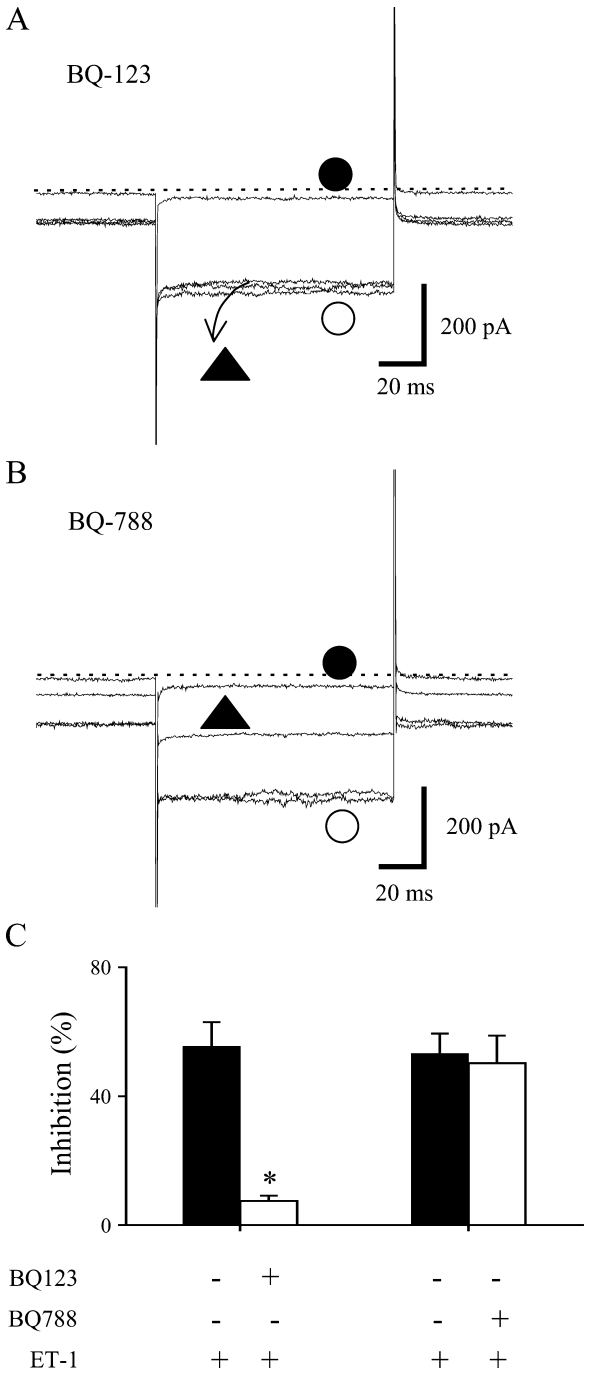

FIGURE 3. Effect of BQ-123 and BQ-788 on ET-1–mediated inhibition of Kir currents. A and B, Effect of 3 μM BQ-123 (A) and 3 μM BQ-788 (B) on ET-1 (30 nM)–induced inhibition of Kir currents in SCASMC (○: control, ●: Ba^{2+}, ▲: ET-1). C, Average percent inhibition of Kir current at −140 mV. All n = 5; *$P < 0.001$ versus BQ123 (-).

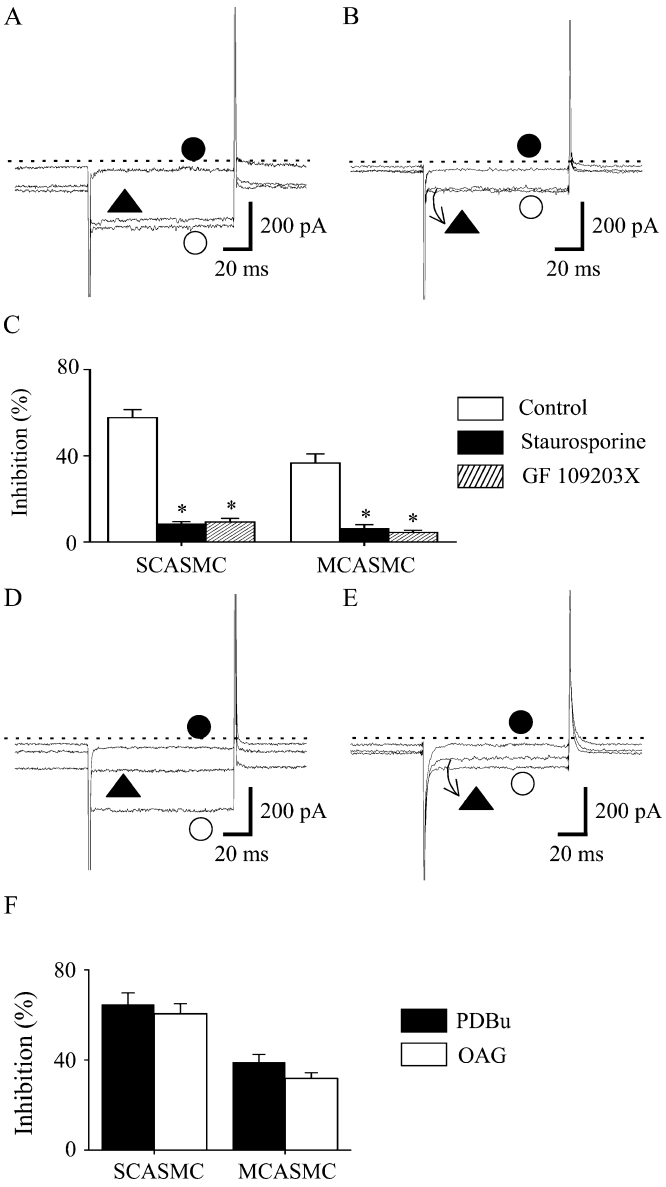

FIGURE 4. The effect of PKC inhibitors and activators on the ET-1–induced inhibition of Kir currents. A and B, Effect of staurosporine on the ET-1–induced inhibition of Kir currents in SCASMC (A) and MCASMC (B) (○: control, ●: Ba^{2+}, ▲: ET-1). C, The inhibition of Kir by 30 nM ET-1 under control conditions (n = 4) and in cells pretreated with the PKC inhibitor staurosporine (100 nM, n = 4) or GFX 109203X (1 μM, n = 4) at –140 mV in SCASMC and MCASMC. *$P < 0.001$ versus control. D and E, Effect of PKC activation with PDBu on Kir currents in SCASMC (D) and MCASMC (E) (○: control, ●: Ba^{2+}, ▲: PDBu). F, Percent inhibition of Kir current by PDBu (1 μM) and OAG (1 μM) at –140 mV in SCASMC and MCASMC.

inhibitory effect of ET-1 was similar in both SCASMC and LCASMC (all n = 4).

We also compared BK_{Ca} and K_V currents in SCASMC and LCASMC. To record the BK_{Ca} current, the intracellular and extracellular free Ca^{2+} concentration was maintained at 300 nM, as calculated using Fabiato's[14] computer program (see Methods). The BK_{Ca} current was markedly inhibited in SCASMC (Fig. 6A) and LCASMC (Fig. 6B) by iberiotoxin (IbTX), a potent inhibitor of BK_{Ca}. The percentage of inhibition by IbTX was not significantly different between SCASMC

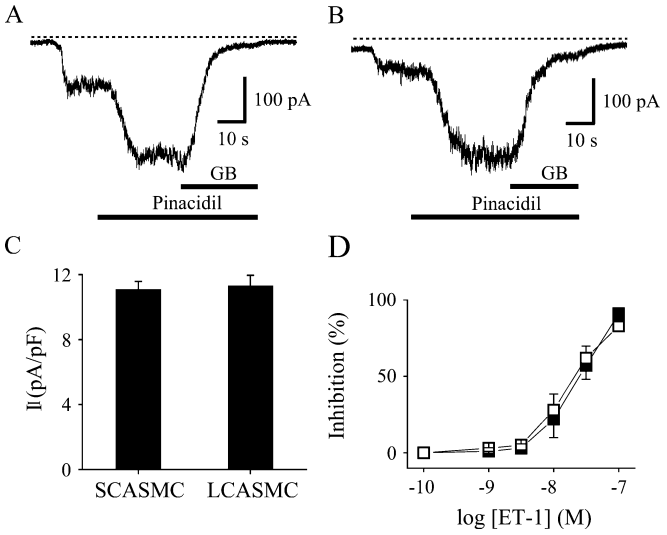

FIGURE 5. Distribution of K_{ATP} currents in SCASMC and LCASMC. A and B, Recording of whole-cell current from a cell held at –60 mV showing pinacidil activation of glibenclamide (GB, 10 μM)-sensitive K^+ currents in SCASMC (A) and LCASMC (B). In each recording shown, the dashed line indicates the zero current level. C, Density of K_{ATP} currents in SCASMC and LCASMC. The current was normalized to cell capacitance. D, ET-1 blocked the K_{ATP} current in a dose-dependent manner (■: SCASMC and □: LCASMC).

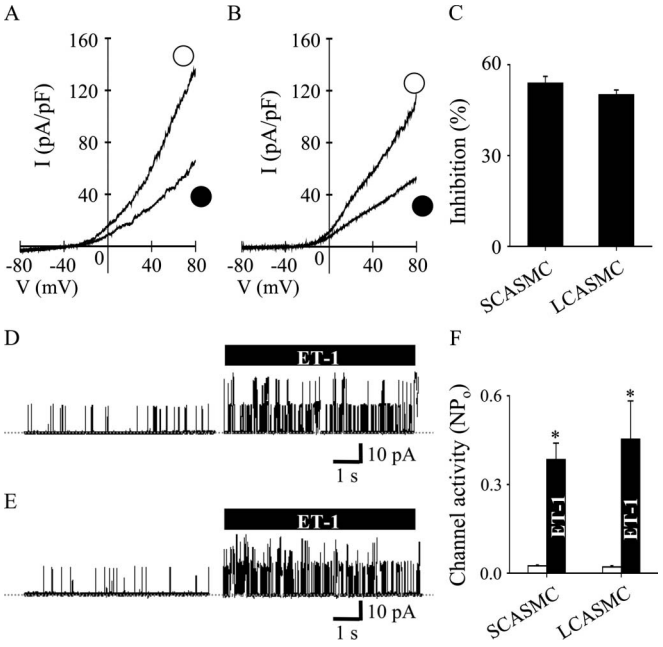

FIGURE 6. Ca^{2+}-activated K^+ currents in SCASMC and LCASMC. A and B, Averaged currents recorded in response to a voltage ramp (from −80 to +80 mV for 320 milliseconds) for SCASMC (A) and LCASMC (B) superfused with control solution (○) and IbTX-containing solution (●). C, The bars show mean (±SEM) inhibition of BK_{Ca} by IbTX at +70 mV. D and E, Effect of 5 nM ET-1 on the activity of the BK_{Ca} channel at +70 mV in SCASMC (D) and LCASMC (E). Upward deflections are outward channel openings. F, Graph shows the effects of ET-1 on the *NP*o in SCASMC and LCASMC. Channel openings were increased by the application of 5 nM ET-1; *$P < 0.001$ versus ET-1 (-).

(54.0 ± 2.12%, n = 4) and LCASMC (50.2 ± 1.44%, n = 4, Fig. 6C). We also tested the effects of ET-1 on the activity of the BK_{Ca} channel. At 5 nM, ET-1 significantly increased the channel mean open-time and the frequency of channel opening (*NP*o = 0.025 ± 0.003 for control versus 0.384 ± 0.056 for ET-1 in SCASMC, Fig. 6D; *NP*o = 0.021 ± 0.004 for control versus 0.453 ± 0.129 in LCASMC, Fig. 6E, all n = 5). This effect was not different between SCASMC and LCASMC (Fig. 6F).

K_V currents were recorded in response to voltage steps from a holding potential of −60 mV to test pulse potentials between –120 and +60 mV, in 20-mV increments, in a bath solution that contained IbTX to reduce BK_{Ca} currents. The mean current density appeared to be slightly greater in LCASMC than in SCASMC, but this difference was not statistically significant (Fig. 7C, all n = 4). The magnitude of the inhibition induced by 30 nM ET-1 in both cell types developed for 5 minutes and was not significantly different between cell types (SCASMC: 23.2 ± 2.9%, LCASMC: 21.2 ± 3.2% inhibition, Fig. 7A, B, and D, n = 5).

DISCUSSION

The major findings of this study were: (1) the Kir current density was greater in cells isolated from small-diameter coronary arteries than in cells from larger arteries; (2) ET-1 caused a concentration-dependent inhibition of Kir in SCASMC and MCASMC; (3) the ET-1–induced inhibition of the Kir channel was mediated by activation of the ET_A receptor; (4) the inhibitory effect of ET-1 on the Kir current was attenuated by the inhibition of PKC and was mimicked by PKC activation; (5) K_{ATP}, BK_{Ca}, and K_V currents and the effects of ET-1 on these channels did not differ between SCASMC and LCASMC.

The Kir channels identified in coronary and cerebral artery smooth muscle cells have the characteristics of the Kir2 subfamily: strong inward rectification, which is partly due to inhibitory effects of outward current by internal Mg^{2+}, conductance dependent on the extracellular K^+ concentration, a voltage- and time-dependent gating process, and consensus sites for phosphorylation by protein kinases A and C.[15–17] Recently, several reports have identified transcripts for Kir2.1 in smooth muscle cells of the coronary artery, but not transcripts for the other members of the Kir2 subfamily (Kir2.2 and 2.3).[18] The targeted disruption of the Kir2.1 gene produces arteries that fail to dilate in response to the modest elevations in extracellular K^+ that are typically associated with periods of hypoxia and ischemia, which indicated that Kir2.1 plays an essential role in the regulation of vascular tone under conditions of metabolic stress.[19] In this study, we first detected Kir2.1 protein in freshly isolated SCASMC, but not in LCASMC, by Western blotting. Moreover, we found only one form of the Kir channel, namely Kir2.1, in native SCASMC.

Although Zhang et al[20] first demonstrated that ET-1 inhibited Kir channels in cultured bovine pulmonary arterial endothelial cells, ET-1 modulation of Kir channels in rabbit

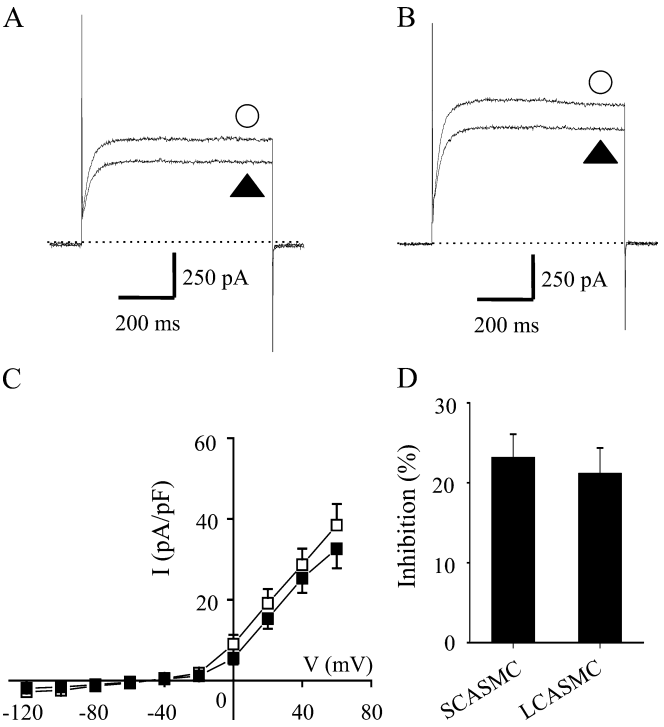

FIGURE 7. Voltage-dependent K^+ currents in SCASMC and LCASMC. C, Mean current-voltage relationship for K_v currents in SCASMC (■) and LCASMC (□). A and B, K_v currents were elicited by applying 600-millisecond depolarizing pulses from a holding potential of −60 to +40 mV in SCASMC (A) and LCASMC (B), respectively. The traces of the currents obtained in the absence and presence of 30 nM EI-1 (○: control, ▲: ET-1, n = 5). The average inhibition of K_v current by 30 nM ET-1 is shown in D.

coronary arterial smooth muscle cells has not been reported previously; however, Miyoshi et al[9] showed that the K_{ATP} channels in porcine coronary artery, which have a molecular structure similar to that of Kir channels, are inhibited by ET-1. In this report, the addition of endothelin to the pipette solution specifically blocked K_{ATP} channels in a concentration-dependent manner in the cell-attached configuration. Our recent paper also showed that ET-1 blocked K_{ATP} channels by activation of PKC.[21] In this report, the inhibitory effect of ET-1 on K_{ATP} channels was abolished when smooth muscle cells were pretreated with PKC inhibitors (GF 109203X and staurosporine). Also, vasoconstrictors other than ET-1 (eg, angiotensin II, phenylephrine, serotonin, histamine, and neuropeptide Y) have been shown to reduce K_{ATP} currents via the activation of PKC.[22,23] Therefore, the best candidate for the mechanism of the inhibition of Kir channels by ET-1 might be related to PKC-mediated pathways. In fact, in our study, the inhibitory effect of ET-1 on Kir currents is mediated by a PKC signal transduction pathway via activation of the ET_A receptor (Figs. 2, 3, and 4).

Although we have presented evidence that Kir currents are abundant only in small arteries, many previous reports have focused on the roles of K_{ATP}, BK_{Ca}, and K_V channels. K_{ATP} and BK_{Ca} channels are important for regulating basal tone in coronary,[24] mesenteric,[25] cerebral,[26] and saphenous[27] arteries. In pulmonary arteries, K_{ATP}[28] and K_V[29] channels are thought to regulate the resting membrane potential. Previous reports have suggested that large-diameter coronary arteries also express several types of channels, including K_{ATP}, BK_{Ca}, and K_V channels.[30–32] However, the distribution of K_{ATP}, BK_{Ca}, and K_V channels relative to vascular size has not been investigated previously. In the present study, we did not find any differences in the distributions of K_{ATP}, BK_{Ca}, and K_V channels between SCASMC and LCASMC. These results suggest that the variations in responses that are related to differences in the arterial diameter might be a result of the differential expression of Kir channels.

Electrophysiological studies in systemic arteries demonstrate that ET-1 either increases or decreases activity of BK_{Ca} channels depending on concentration.[33] In pulmonary arterial smooth muscle cell, less than 5 nM ET-1 activates the BK_{Ca} current by increasing intracellular Ca^{2+} concentration, whereas, at a higher concentration (>10 nM), ET-1–induced BK_{Ca} current inhibition predominates. Although the mechanism of BK_{Ca} channel inhibition by ET-1 is not known clearly, ET-1 has a inhibitory effect on BK_{Ca} channels directly and/or by activation of PKC.[11,34,35] Our recent paper[21] demonstrated that ET-1 inhibited the K_{ATP} channels by activation of Ca^{2+}-independent PKC subtype (ε subtype) in native coronary and pulmonary arterial smooth muscle cells. For K_V channels, Shimoda et al[13] suggested that ET-1 inhibited the K_V channel. These effects of ET-1 were attenuated by inhibition of PLC and PKC. However, this report suggested that both Ca^{2+}-dependent and Ca^{2+}-independent subtype of PKC might be involved. In this article, we also suggested that ET-1 inhibited Kir channels by activation of PKC. Thus, these inhibitions of K^+ (BK_{Ca}, K_{ATP}, K_V, and Kir) channels by ET-1 may cause membrane depolarization, which causes vasoconstriction and increases the vascular resistance in coronary arterial smooth muscle.

Johnson et al[36] suggested that Kir channels contribute to resting tone of rat middle cerebral artery since Ba^{2+} constricted middle cerebral arteries at resting tone. The open Kir channels in these arteries maintained the resting diameter 8% to 12% more dilated than if these channels were closed or not present. Also, the membrane potential of resting coronary and cerebral artery smooth muscle cells was between –40 and –50 mV, which is positive relative to the reversal potential of K^+ channels at physiological K^+ gradient; therefore, the physiological role for Kir channels requires outward currents through the channels. Outward currents through the Kir channels have a physiological role in regulating the membrane potential and thus vascular tone. Therefore, Kir channels that are open during the resting condition maintain coronary vessels in a relatively hyperpolarized state and, consequently, maintain the arteries in a partially dilated state. In this way, the Kir channels contribute to the membrane potential and resting tone through the coronary arteries. In addition, if the Kir channels are responsible, at least in part, for determining the membrane potential in these cells, the closure of the channels, such as by vasoconstrictors (ET-1), would be an effective mechanism causing depolarization, subsequent voltage-dependent Ca^{2+} entry, and consequent vasoconstriction.

REFERENCES

1. Edwards FR, Hirst GDS, Silverberg GD. Inward rectification in rat cerebral arterioles; involvement of potassium ions in autoregulation. *J Physiol*. 1988;404:455–466.
2. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol*. 1995;268:C799–C822.
3. Weiss JN, Lamp ST,Shine KI. Cellular K^+ loss and anion efflux during myocardial ischemia and metabolic inhibition. *Am J Physiol*. 1989;256: H1165–H1175.
4. Sieber FE, Wilson DA, Hanley DF, et al. Extracellular potassium activity and cerebral blood flow during moderate hypoglycemia in anesthetized dogs. *Am J Physiol*. 1993;264:H1774–H1780.
5. Knot HJ, Zimmermann PA,Nelson MT. Extracellular K^+-induced hyperpolarizations and dilatations of rat coronary and cerebral arteries involve inward rectifier K^+ channels. *J Physiol*. 1996;492:419–430.
6. Chrissobolis S, Ziogas J, Chu Y, et al. Role of inwardly rectifying K^+ channels in K^+-induced cerebral vasodilatation in vivo. *Am J Physiol*. 2000;279:H2704–H2712.
7. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*. 1988; 332:411–415.
8. Inoue Y, Oike M, Nakao K, et al. Endothelin augments unitary calcium channel currents on the smooth muscle cell membrane of guinea-pig portal vein. *J Physiol*. 1990;423:171–191.
9. Miyoshi Y, Nakaya Y, Wakatsuki T, et al. Endothelin blocks ATP-sensitive K^+ channels and depolarizes smooth muscle cells of porcine coronary artery. *Circ Res*. 1992;70:612–616.
10. Van Renterghem C, Lazdunski M. Endothelin and vasopressin activate low conductance chloride channels in aortic smooth muscle cells. *Pflugers Arch*. 1993;425:156–163.
11. Minami K, Hirata Y, Tokumura A, et al. Protein kinase C-independent inhibition of the Ca^{2+}-activated K^+ channel by angiotensin II and endothelin-1. *Biochem Pharmacol*. 1995;49:1051–1056.
12. Nakajima T, Hazama H, Hamada E, et al. Endothelin-1 and vasopressin activate Ca^{2+}-permeable non-selective cation channels in aortic smooth muscle cells: mechanism of receptor-mediated Ca^{2+} influx. *J Mol Cell Cardiol*. 1996;28:707–722.
13. Shimoda LA, Sylvester JT, Sham JS. Inhibition of voltage-gated K^+ current in rat intrapulmonary arterial myocytes by endothelin-1. *Am J Physiol*. 1998;274:L842–L853.
14. Fabiato A. Computer programs for calculating total from specific free or free from specified total ionic concentrations in aqueous solutions containing multiple metals and ligands. *Methods Enzymol*. 1988;157: 378–417.
15. Quayle JM, Dart C, Standen NB. The properties and distribution of inward rectifier potassium currents in pig coronary arterial smooth muscle. *J Physiol*. 1996;494:715–726.
16. Henry P, Pearson WL, Nichols CG. Protein kinase C inhibition of cloned inward rectifier (HRK1/K_{IR}2.3) K^+ channels expressed in Xenopus oocytes. *J Physiol*. 1996;495:681–688.
17. Xu X, Rials SJ, Wu Y, et al. The properties of the inward rectifier potassium currents in rabbit coronary arterial smooth muscle cells. *Pflugers Arch*. 1999;438:187–194.
18. Bradley KK, Jaggar JH, Bonev AD, et al. Kir2.1 encodes the inward rectifier potassium channel in rat arterial smooth muscle cells. *J Physiol*. 1999;515:639–651.
19. Zaritsky JJ, Eckman DM, Wellman GC, et al. Targeted disruption of Kir2.1 and Kir2.2 genes reveals the essential role of the inwardly rectifying K^+ current in K^+-mediated vasodilation. *Circ Res*. 2000;87:160–166.
20. Zhang H, Inazu H, Weir B, et al. Endothelin-1 inhibits inward rectifier potassium channels and activates nonspecific cation channels in cultured endothelial cells. *Pharmacology.* 1994;49:11–22.
21. Park WS, Ko EA, Han J, et al. Endothelin-1 acts via protein kinase C to block K_{ATP} channels in rabbit coronary and pulmonary arterial smooth muscle cells. *J Cardiovasc Pharmacol*. 2005;45:99–108.
22. Bonev AD, Nelson MT. Vasoconstrictors inhibit ATP-sensitive K^+ channels in arterial smooth muscle through protein kinase C. *J Gen Physiol*. 1996; 108:315–323.
23. Kubo M, Quayle JM, Standen NB. Angiotensin II inhibition of ATP-sensitive K^+ currents in rat arterial smooth muscle cells through protein kinase C. *J Physiol*. 1997;503:489–496.
24. Samaha FF, Heineman FW, Ince C, et al. ATP-sensitive potassium channel is essential to maintain basal coronary vascular tone in vivo. *Am J Physiol*. 1992;262:C1220–C1227.
25. Nelson MT, Patlak JB, Worley JF, et al. Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol*. 1990;259:C3–18.
26. Brayden JE, Nelson MT. Regulation of arterial tone by activation of calcium-dependent potassium channels. *Science*. 1992;256:532–535.
27. Berczi V, Stekiel WJ, Contney SJ, et al. Pressure-induced activation of membrane K^+ current in rat saphenous artery. *Hypertension*. 1992;19: 725–729.
28. Clapp LH, Gurney AM. ATP-sensitive K^+ channels regulate resting potential of pulmonary arterial smooth muscle cells. *Am J Physiol*. 1992; 262:H916–H920.
29. Yuan XJ. Voltage-gated K^+ currents regulate resting membrane potential and $[Ca^{2+}]i$ in pulmonary arterial myocytes. *Circ Res*. 1995;77:370–378.
30. Yamamura H, Nagano N, Hirano M, et al. Activation of Ca^{2+}-dependent K^+ current by nordihydroguaiaretic acid in porcine coronary arterial smooth muscle cells. *J Pharmacol Exp Ther.* 1999;291:140–146.
31. Volk KA, Matsuda JJ, Shibata EF. A voltage-dependent potassium current in rabbit coronary artery smooth muscle cells. *J Physiol*. 1991;439:751–768.
32. Wellman GC, Quayle JM, Standen NB. ATP-sensitive K^+ channel activation by calcitonin gene-related peptide and protein kinase A in pig coronary arterial smooth muscle. *J Physiol*. 1998;507:117–129.
33. Hu SL, Kim HS, Jeng AY. Dual action of endothelin-1 on the Ca^{2+}-activated K^+ channel in smooth muscle cells of porcine coronary artery. *Eur J Pharmacol*. 1991;194:31–36.
34. Dixon BS, Sharma RV, Dickerson T, et al. Bradykinin and angiotensin II: activation of protein kinase C in arterial smooth muscle. *Am J Physiol*. 1994;266:C1406–C1420.
35. Peng W, Michael JR, Hoidal JR, et al. ET-1 modulates K_{Ca}-channel activity and arterial tension in normoxic and hypoxic human pulmonary vasculature. *Am J Physiol*. 1998;275:L729–L739.
36. Johnson TD, Marrelli SP, Steenberg ML, et al. Inward rectifier potassium channels in the rat cerebral artery. *Am J Physiol*. 1998;274:R541–R547.