Increased Inhibition of Inward Rectifier K^+ Channels by Angiotensin II in Small-Diameter Coronary Artery of Isoproterenol-Induced Hypertrophied Model

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Objective—We investigated the effects of angiotensin II (Ang II) on inward rectifier K^+ (Kir) channels in small-diameter coronary arterial smooth muscle cells (SCASMCs) of control and isoproterenol (Iso)-induced hypertrophied rabbits.

Methods and Results—Kir current amplitude and Kir channel protein expression were definitely lower in the Iso-induced hypertrophied model than in the control. In a pressurized arterial experiment, 15 mmol/L K^+-induced vasodilation was greater in the control arteries than in the arteries of Iso-induced hypertrophied model. Ang II reduced the Kir current in a concentration-dependent manner, and this inhibition was greater in SCASMCs from Iso-induced hypertrophied model than from control. Although, there was no difference in the expression of Ang II type 2 (AT_2) receptor between SCASMCs of control and Iso-induced hypertrophied model, the expression of Ang II type 1 (AT_1) receptor and phosphorylated PKCα were greater in SCASMCs of Iso-induced hypertrophied model than of control.

Conclusion—Ang II inhibits Kir channels more prominently in SCASMCs of Iso-induced hypertrophied model owing to increases in the expression of AT_1 receptor and the activation of PKCα. Our findings about the differential expression of Kir channels and different modulation of Kir channels by a vasoconstrictor (Ang II) in a hypertrophy model are important for better understanding the responsiveness of small-diameter arteries during hypertrophy. (***Arterioscler Thromb Vasc Biol.*** **2007;27:1768-1775.)**

Key Words: inward rectifier K^+ channel ■ hypertrophy ■ angiotensin II ■ PKCα ■ microcirculation

A reduced coronary reserve in response to various factors is a recognized feature of cardiac hypertrophy and is often accompanied by a high incidence of sudden cardiac death.[1,2] Most of the studies on reduced coronary reserve have demonstrated endothelial dysfunction, increased arteriolar tone, increased perivascular fibrosis, increased extracellular forces, abnormal angiogenesis, and structural alterations to the arterioles.[3–6] Furthermore, ion channels of the coronary arteries are also altered in cardiac hypertrophy. For example, Ca^{2+}-activated K^+ channels of the coronary arteries are significantly reduced in left ventricular hypertrophy (LVH),[7] and voltage-dependent K^+ current is also decreased without changes in the channel kinetics in LVH.[8]

Inward rectifier K^+ (Kir) channels have been identified in small-diameter (microvessel, not conduit) coronary and cerebral arteries; they may play an important role in the regulation of resting membrane potential and therefore arterial tone.[9–11] Although little is known about the modulation of Kir channels in smooth muscle, the activation of Kir channels induces vasodilation of small-diameter arteries, which can cause the extracellular K^+ concentration to rise to as high as 15 mmol/L[9], and exposure to hypoxic condition.[11] During pathological conditions such as hypertrophy and hypertension, in which vessels would be exposed to elevated pressure (>120 mm Hg), changes in the properties of the Kir channels have not been fully studied. Considering the fact that small-diameter arteries play a major role in the control of systemic blood pressure and local blood flow, it is essential to identify and characterize the changes in Kir channels under pathological conditions.

Angiotensin II (Ang II) modulates various types of ion channels. For example, Ang II activates a nonselective cation channel[12] and Ca^{2+} channels in vascular smooth muscle cells.[13,14] Ang II also inhibits the voltage-dependent K^+ (Kv)

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channel and ATP-sensitive K^+ (K_{ATP}) channel by activating Ca^{2+}-independent PKCε in vascular smooth muscle cells[15–18] and inhibits the Ca^{2+}-activated K^+ (BK_{Ca}) channel independently of PKC in vascular smooth muscle cells.[19,20] Our recent report suggested that Ang II inhibits the Kir channel through the activation of Ca^{2+}-dependent PKCα by acting at the AT_1 receptor.[21] However, the Ang II effect on the Kir channel in pathological states has not been studied. Thus, in the present study, we demonstrate (1) changes in the properties of the Kir channels in small-diameter coronary arterial smooth muscle cells (SCASMCs) during LVH and (2) changes of inhibition of Kir channel by Ang II, and their mechanisms in LVH using a patch clamp technique, Western blots, and arterial dilation experiments.

Materials and Methods

Induction of Cardiac Hypertrophy in an Animal Model

Male New Zealand White rabbits (2.0 to 2.6 kg) were used as an animal model in this study. Rabbits were purchased from the Samtako Bio Korea Inc (Osan, Korea). Cardiac hypertrophy was induced in the test group by daily isoproterenol administration (300 μg/kg body weight i.v.) for 7 days. The control group was treated similarly with 0.9% saline (1 mL/kg body weight). The degree of hypertrophy was estimated by measuring the heart weight to body weight ratio, cardiac infarction size, blood pressure, and cell capacitances (supplemental Table I, available online at http://atvb.ahajournals.org). Only those test animals proven to exhibit cardiac hypertrophy (onset of hypertrophy=93%, n=78) were used for further study.

Cell Preparation

Enzymatic isolation of single coronary arterial smooth muscle cells was performed as previously described.[11,21] Briefly, the left anterior descending coronary arteries (<100 μm) were dissected out and cleaned of blood and connective tissue. The arteries were then transferred to 1 mL of 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 incubation for 25 minutes at 37°C, the arteries were transferred to 1 mL of Ca^{2+}-free normal Tyrode solution containing collagenase (2.8 mg/mL), BSA, and DTT then incubated for ≈20 minutes at 37°C. After the aforementioned enzyme treatment, the cells were isolated by gentle agitation with a fire-polished Pasture pipette in Kraft-Brühe (KB) medium.

Vessel Preparation and Measurement

The endothelium-denuded coronary arteries (from control rabbits and Iso-induced hypertrophied rabbits) with the diameter of <100 μm and 2 to 3 mm in length were isolated from branches of the left anterior descending coronary artery and cleaned of connective tissue in the physiological salt solution under a stereomicroscope. The artery was cannulated at one end with glass capillary, secured with nylon monofilament suture, and placed in chamber incubated at 35°C. The arteries were maintained in flow state and held at a constant intraluminal pressure of 60 mm Hg. The diameter of the artery was measured with the video programs (Crescent Electronics).

Solution

Normal Tyrode solution contained (in mmol/L): NaCl, 135; KCl, 5.4; NaH_2PO_4, 0.33; $CaCl_2$, 1.8; $MgCl_2$, 0.5; HEPES, 5; glucose, 16.6; adjusted to pH 7.4 with NaOH. 20, 60, and 140 mmol/L K^+ external solutions were made by substituted NaCl for KCl in the normal Tyrode solution. KB solution contained (in mmol/L): 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 mmol/L): K-aspartate, 115; KCl, 25; NaCl, 5; $MgCl_2$, 1; Mg-ATP, 4; EGTA, 0.1; HEPES, 10; adjusted to pH 7.2 with KOH. To minimize the activity of K_{ATP} channels, a high concentration of ATP (4 mmol/L) was used. The physiological salt solution contained (in mmol/L): NaCl, 119; KCl, 4.7; $NaHCO_3$, 24; KH_2PO_4, 1.2; $CaCl_2$, 1.8; $MgSO_4$, 1.2; EDTA, 0.023; glucose, 11. The solution aerated with 95% O_2/5% CO_2 to keep the pH at 7.4.

TTC Staining

Six rabbits from control and Iso-induced hypertrophied model were used to calculate the infarction ratio. The hearts were sliced into 6 sections parallel to the atrioventricular groove. The slices were incubated in a TTC solution prepared in phosphate buffer pH 7.4 for 30 minutes at 37°C. The TTC in viable myocardium was converted by lactate dehydrogenase isoenzymes (LDH, Sigma) to form a red formazan pigment that stains tissue with dark red.[22] The infarct area that did not take the TTC stain remained pale in color.

Electrophysiology

Whole-cell patch-clamp recordings were made using an Axon interface and Axopatch 1C amplifier (Axon instruments) as described previously.[11,21] To measure the resting membrane potential, we applied the perforated-patch technique using nystatin. Nystatin was added to a fresh aliquot of the above pipette solution every 2 hours to give a final concentration of 200 μg/mL.

Western Blot

Western blot of endothelium-denuded small-diameter coronary arteries was performed according to published methods.[11,21] Briefly, protein samples (15 μg from 8 rabbits) were obtained from the strips of endothelium-denuded small-diameter coronary arteries, which were homogenized in a hand-held Micro-tissue Grinder (PYREX). Membranes were probed with the β-tubulin antiserum (Sigma) at a dilution of 1:1000, antiserum for phosphorylated PKCα (Upstate Biotechnology) at a dilution of 1:1500, antiserum for total PKCα (Santa Cruz) at a dilution of 1:1000, and antiserum for AT_1 (Abcam) and AT_2 (Santa Cruz) receptors at a dilution of 1:1000 for 1 hour at room temperature. Then, membranes were incubated with secondary antibodies at a dilation of 1:1000 to 1:3000, a goat anti-mouse IgG for β-tubulin (Santa Cruz), a goat anti-rabbit IgG for PKCα (Sigma), a goat anti-mouse IgG for AT_1 receptor (Sigma), and a mouse anti-goat IgG for AT_2 receptor (Santa Cruz). Western blot for Kir2.1 also has been described previously.[11] Briefly, membranes were probed with the GAPDH antiserum at a dilution of 1:1000 and antiserum for Kir2.1 (Santa Cruz) at a dilution of 1:500. Membranes were incubated with secondary antibodies, a goat anti-mouse IgG for GAPDH (Santa Cruz), a mouse anti-goat IgG for Kir2.1 (Santa Cruz).

Statistics

Origin 6.0 software (Microcal Software, Inc) was used for data analysis. Interaction kinetics between drugs and channels was described on the basis of a first-order blocking scheme.[21]

Data are presented as the means±SEM. Statistical analyses were performed by using unpaired Student *t* test. $P<0.05$ was defined as statistically significant.

Results

Characteristics of the Experimental Model

The effects of isoproterenol (Iso) infusion are shown in supplemental Table I. The body-weight gain was not significantly different between the Iso-infused and control animals. However, the heart weight and heart to body weight ratio of Iso-infused animals were significantly increased. Left ventricular weight, infarction size, and blood pressure (systolic and diastolic) were also increased in Iso-infused animals. The

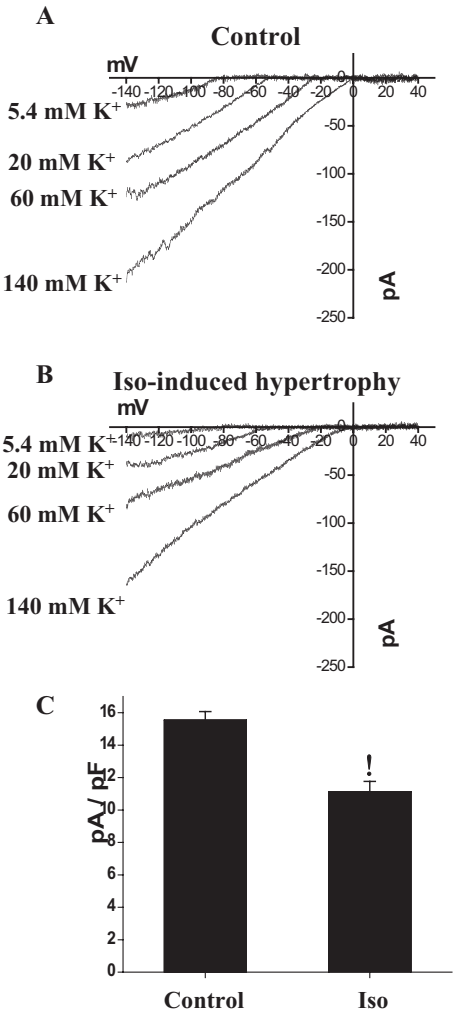

Figure 1. Inward rectifier K^+ currents in SCASMCs of control (<100 μm) and Iso-induced hypertrophied model. Current-voltage relationship of 50 μmol/L Ba^{2+}-sensitive currents in 5.4, 20, 60, 140 mmol/L K^+ in control (A) and Iso-induced hypertrophy (B). The fraction of the current that was Ba^{2+}-sensitive was determined by subtracting the current in the presence of 50 μmol/L Ba^{2+} from that in the control condition. C, Currents were normalized to cell capacitance and are 50 μmol/L Ba^{2+}-sensitive currents measured at −140 mV under extracellular 140 mmol/L K^+. All n=6. *P<0.01.

patch clamp experiment revealed that the cell volume (mean cell capacitance) of SCASMCs isolated from Iso-infused animals was 13% greater than that of control SCASMCs; the membrane potential became more positive in SCASMCs from Iso-infused animals. No serious cavity effusion or increases in lung or liver weights were observed (data not shown). From these data, this model can be considered to demonstrate hypertrophy.[23]

Comparison of Ba^{2+}-Sensitive Kir Channels

Figure 1 illustrates the Kir currents recorded from the SCASMCs of control (<100 μm) and Iso-induced hypertrophied animals. Kir currents were recorded in response to a voltage step from −60 to −140 mV for 50 ms, followed by a depolarizing voltage ramp from −140 to +40 mV at a rate of 0.5 V/s. The contribution of K_{ATP} channels to the measured current was minimized by the inclusion of ATP (4 mmol/L) in the pipette solution, and our previous reports clearly showed that K_{ATP} currents were not involved in this condition.[11] To prove the Kir currents, we examined various concentrations of extracellular K^+, 5.4, 20, 60, and 140 mmol/L with fixed intracellular K^+ concentration, 140 mmol/L. As shown in Figure 1A and 1B, Ba^{2+}-sensitive Kir currents were detected in both SCASMCs from control and Iso-induced hypertrophied model. However, the amplitude of the Kir current was greater in control than in Iso-induced hypertrophied (15.55±0.52 pA/pF in control and 11.13±0.63 pA/pF in Iso-induced hypertrophied model at −140 mV under extracellular 140 mmol/L K^+, Figure 1C). To further identify the reduction of Kir channel density, we tested the amplitudes of the Ba^{2+}-induced vasoconstriction and the 15 mmol/L K^+-induced vasodilation in small-diameter coronary arteries of control and those of Iso-induced hypertrophied model. Although most systemic arteries constrict in response to an increase in the extracellular K^+ concentration, moderate increases in extracellular K^+ (≈15 mmol/L) in small-diameter coronary and cerebral arteries lead to vasodilation and increased blood flow by the activation of Kir channels.[9,24,25] Consistent with the patch clamp data, the 50 μmol/L Ba^{2+}-induced vasoconstriction was greater in control than in Iso-induced hypertrophied model (Figure 2A and 2B, Control: 21.14±1.02%, hypertrophy: 14.13±0.79% vasoconstriction, respectively). Also, the 15 mmol/L K^+-induced vasodilation was greater in small-diameter coronary arteries of control than those of Iso-induced hypertrophied model (Figure 2C and 2D, Control: 48.89±3.45%, hypertrophy: 36.77±4.37% dilation, respectively). We also performed a Western blot experiment with an antibody specific for Kir2.1 to identify the reduction of Kir channel density. Our previous report suggested that only Kir2.1, not Kir2.2 or Kir2.3, was detected in SCASMCs.[11,25] As shown in Figure 3A, the expression level of Kir2.1 was decreased in the Iso-induced hypertrophied animals (Densitometric ration. 1.31±0.20 for control; 0.72±0.15 for hypertrophy, Figure 3B). Therefore, we concluded that the expression of Kir channels in small-diameter coronary arteries was decreased in Iso-induced hypertrophied animals.

Inhibition of Kir Current by Ang II

We investigated the effect of Ang II on Kir currents in SCASMCs from control and Iso-induced hypertrophied animal. To increase the magnitude of the Kir currents, both the extracellular and intracellular K^+ concentrations were maintained at 140 mmol/L. Ang II caused a significant reduction in the magnitude of the Kir currents (Figure 4A and 4B). Increasing the concentration of Ang II increased the level of inhibition of the Kir current in both SCASMCs from control and Iso-induced hypertrophied model. The dose-response curve indicated that the SCASMCs from Iso-induced hypertrophied model were more sensitive to the Ang II–induced inhibition of Kir currents compared with the control SCASMCs. A nonlinear least-squares fit of the Hill equation to the concentration-dependence data yielded an apparent K_d value of 253.81±7.42 nmol/L and

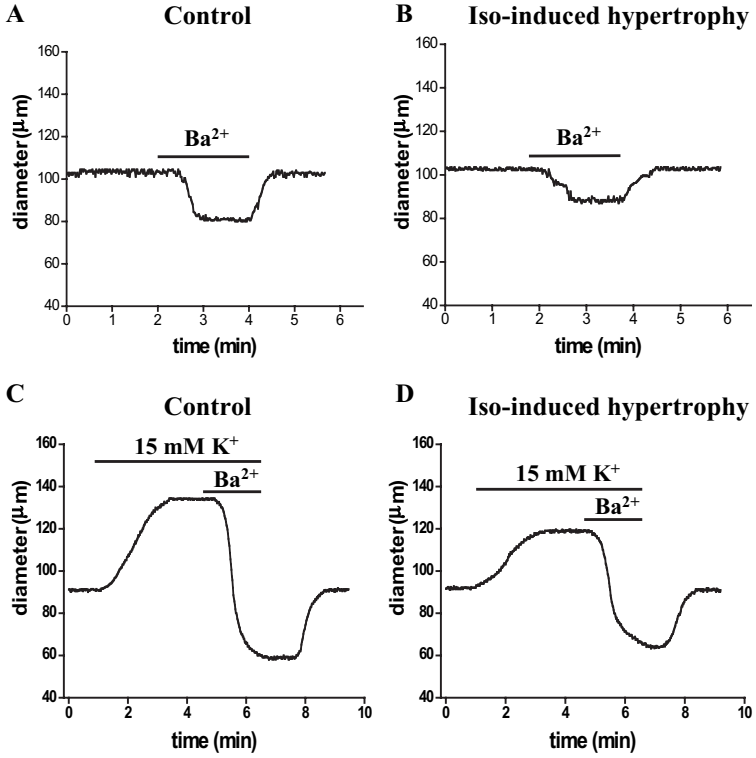

Figure 2. Change of the vascular tone in small-diameter coronary arteries from control and Iso-induced hypertrophied model by a moderate increase of K^+ (15 mmol/L K^+). Representative tracings show the effect of 50 μmol/L Ba^{2+} on vascular tone in the small-diameter coronary arteries of control (A) and Iso-induced hypertrophy (B). Typical experimental tracings show the effect of 15 mmol/L K^+ on vascular tone in the small-diameter coronary arteries from control (C) and Iso-induced hypertrophied model (D). All n=4.

74.62±5.04 nmol/L in control and in Iso-induced hypertrophy, respectively, and a Hill coefficient of 1.24±0.11 and 1.69±0.17 in control and in Iso-induced hypertrophy, respectively (Figure 4C).

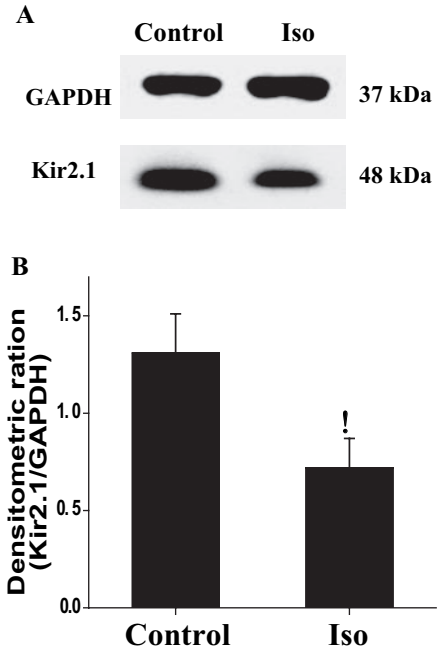

Figure 3. Kir2.1 expression level as determined by Western blot in small-diameter coronary arteries of control and Iso-induced hypertrophied model. A, Representative data showing the distribution of Kir2.1 in small-diameter coronary arteries from control and Iso-induced hypertrophied animal. B, Summary of relative intensity as shown in A. n=3. *$P<0.05$. The relative intensity was determined using Multi Gauge V2.2.

Comparison of the Expression Levels of AT_1 and AT_2 Receptor

We sought to understand why the inhibition of Kir currents by Ang II was greater in the SCASMCs from Iso-induced hypertrophied animal. Therefore, we performed Western blots on homogenized endothelium-denuded small-diameter arterial tissues to evaluate the expression of Ang II type 1 (AT_1) and Ang II type 2 (AT_2) receptor proteins in native small-diameter coronary arteries from control and Iso-induced hypertrophied animals. As shown in Figure 5B, the expression level of AT_2 receptor did not differ between the control and Iso-induced hypertrophied model (Densitometric ration. 0.42±0.04 for control; 0.39±0.05 for hypertrophy). By contrast, marked increases in the protein level of AT_1 were observed in small-diameter coronary arteries from the Iso-induced hypertrophied model (Densitometric ration. 0.36±0.06 for control; 0.59±0.07 for hypertrophy, Figure 5A).

Comparison of the Expression Levels of PKCα

Our previous study clearly showed that Ang II inhibits Kir channels through AT_1 receptors by the activation of Ca^{2+}-dependent PKCα, not Ca^{2+}-independent PKCε.[21] Thus, to test whether the expression level of PKCα was changed in the Iso-induced hypertrophied model, we conducted a Western blot analysis on homogenized endothelium-denuded small-diameter arterial tissues, using antibodies specific for phosphorylated PKCα (pPKCα) and PKCβ (pPKCβ). Although we could not detect the expression of pPKCβ in small-diameter coronary arteries from control and Iso-induced hypertrophied animal, the expression level of pPKCα was greater in small-diameter coronary arteries from Iso-induced hypertrophy (2.1-fold, Figure 5C). Furthermore, the expres-

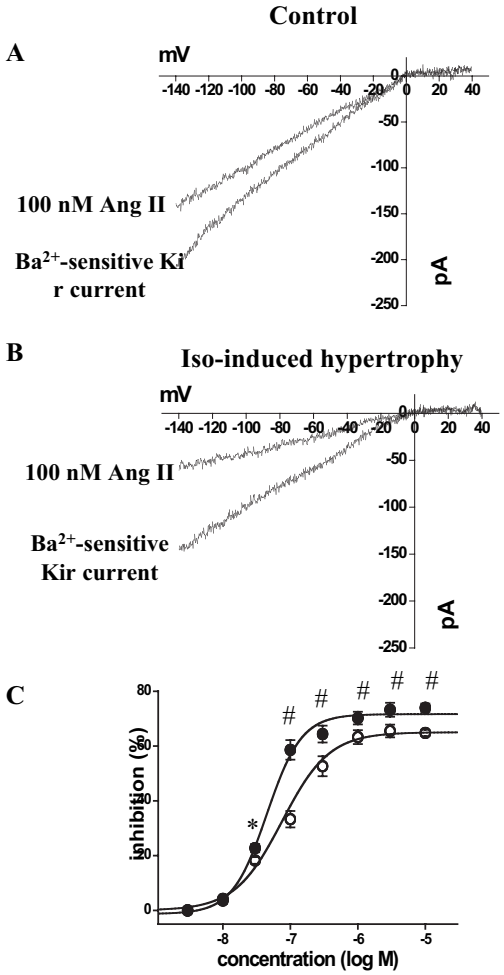

Figure 4. Ang II inhibited Kir current in SCASMCs of control and Iso-induced hypertrophy. Whole-cell current recording showing the response to 100 nmol/L Ang II of the SCASMCs from control (A) and Iso-induced hypertrophied model (B), respectively. C, Concentration-dependence of the Ang II–induced blockage of Kir currents in the SCASMCs of control (white circle, all n=5) and Iso-induced hypertrophy (black circle, all n=5). The reduction in current at −140 mV was used as an index of blockage. The smooth line represents the best fit with the Hill equation. *$P<0.05$, #$P<0.01$.

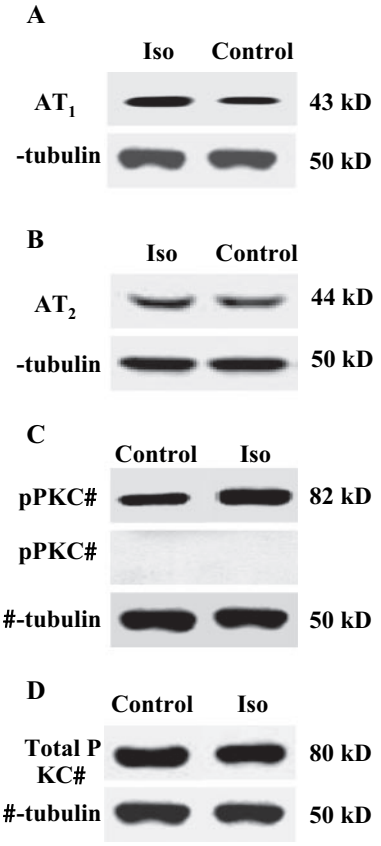

Figure 5. Representative Western blot results for AT_1, AT_2, and Ca^{2+}-dependent PKC isoforms (α and β) in endothelium-denuded small-diameter coronary arteries from control and Iso-induced hypertrophied model. Western blotting was performed using anti-AT_1 (A) and anti-AT_2 (B) receptor antibodies. All n=3. C, Western blot analysis of the expression of phosphorylated PKCα (pPKCα), PKCβ (pPKCβ), and β-tubulin (n=4). D, Western blot analysis of the expression of total PKCα and β-tubulin (n=3).

sion level of total PKCα was not different from control and Iso-induced hypertrophied animals (Figure 5D). We also tested a PKC activator, phorbol 12,13-dibutyrate (PDBu), on Kir currents in SCASMCs of control and Iso-induced hypertrophy. Application of PDBu, in the presence of an inhibitor of Ca^{2+}-dependent PKC (PKCα and PKCβ), Gö 6979 (1 μmol/L), showed no significant decrease of Kir currents in both SCASMCs of control and Iso-induced hypertrophied model (supplemental Figure IA through IC). These findings confirm that the effect of Ang II occurs through the activation of Ca^{2+}-dependent PKC isoform (α) in both SCASMCs of control and Iso-induced hypertrophied animal. Thus, to specifically activate PKCα, we included a specific inhibitor of Ca^{2+}-independent PKCε, PKCε translocation inhibitory peptide (PKCε TIP, 40 μmol/L).[26] The application of 100 nmol/L PDBu inhibited the Kir current by 32.15±3.56% in control and by 49.63±4.12% in Iso-induced hypertrophied model (Supplemental Figure ID and IE). Supplemental Figure IF summarizes the effect on the Kir current of PDBu alone and PDBu together with PKCε TIP at a concentration of 100 nmol/L. These results suggest that Kir channels in SCASMCs of Iso-induced hypertrophied animals are more strongly regulated by PKCα than those in the control SCASMCs.

Discussion

The major findings of this study were that (1) the density of the Kir current is significantly reduced in a model of Iso-induced hypertrophied small-diameter coronary arteries and (2) the sensitivity to Ang II is more prominent in Iso-induced hypertrophied small-diameter coronary arteries owing to the increased expression of AT_1 receptor and the activation of PKCα.

Several models of ventricular hypertrophy have been used in physiological studies, including catecholamine-induced hypertrophy,[7,27–29] hypertrophy occurring in spontaneously hypertensive rat strains,[30,31] and hypertrophy induced by right ventricle[32] or left ventricular pressure overload.[33,34] Among these models, catecholamine (Iso)-induced cardiac hypertrophy is a simple well-established model and provides useful information that may have a relevant application to clinically observed disease.[35] In our study, cardiac hypertrophy was

induced in rabbits by daily injections of 300 μg/kg Iso for 7 days. In this model, although the ratio of heart weight to body weight was increased by 18%, no change in the liver-to-body weight or lung-to-body weight ratio was detected (data not shown), which suggested that heart failure did not occur. Thus, this model can be classified as mild hypertrophy.[23]

Ang II can bind to at least 2 high-affinity receptors, designated AT_1 and AT_2 receptors.[36] Ang II receptors have been reported to couple to several cellular signaling pathways. For example, Ang II binds to AT_1 receptor, which induced vasoconstriction via activation of PLC and consequently PKC.[37] However, Ang II relaxes microvessel via stimulation of the AT_2 receptor with subsequent opening of BKCa channels, leading to membrane repolarization and vasodilation.[38] In our experiment, the expression level of AT_2 receptor did not differ between the control and Iso-induced hypertrophied model. Contrastingly, a marked increase in the expression level of AT_1 in small-diameter coronary arteries was observed in the Iso-induced hypertrophied model (Figure 5). Despite many previous reports insisting the absolute requirement of AT_1 receptors for the development of hypertension and cardiac hypertrophy,[39,40] most studies have focused on the reduction in left ventricular mass with AT_1 receptor blocker or angiotensin-converting enzyme inhibitor.[41,42] Therefore, this study discloses, for the first time, the role of Ang II receptors level of expression as a possible function correlated with Iso-induced hypertrophied model. The data suggest that the increased expression of AT_1 receptor, rather than AT_2 receptor, can augment the Ang II–induced Kir channel inhibition in Iso-induced hypertrophied model.

Ang II has been shown to affect several different types of ion channels in vascular smooth muscle, and various signaling pathways have been implicated in these effects. Studies have revealed that Ang II inhibits K_{ATP} current through the activation of PKCε, which is a Ca^{2+}-independent PKC isoform, and the inhibition of PKA.[16,18] Similarly, Ang II reduces the Kv current by the activation of PKCε and inhibition of PKA.[15,17] Although the mechanism of BK_{Ca} channel inhibition by Ang II is not known clearly, Ang II inhibits BK_{Ca} channels by a PKC-independent mechanism in cells cultured from pig coronary arteries.[19,20] We recently demonstrated that Ang II inhibited Kir channels by activating Ca^{2+}-dependent PKCα isoform through the AT_1 receptor,[21] and we clearly showed that pretreatment with PLC and PKC inhibitors prevented the Ang II–induced inhibition of Kir current. Although an inhibitor of Ca^{2+}-dependent PKC isoforms clearly reduced the Ang II effect on Kir channels, the inhibitory effect of Ang II on the Kir current was not affected by the inhibition of the PKCε isoform. In the current study, we confirmed that the PKCα isoform is abundant in SCASMCs, whereas PKCβ was not detectable. Moreover, there was comparatively higher level of expression of phosphorylated PKCα in SCASMCs in Iso-induced hypertrophied model than that in control. Furthermore, the inhibitory effect of Ang II in Kir channel was more prominent in SCASMCs of Iso-induced hypertrophied model. Consistent with our findings, previous reports suggested that a conduit artery contained relatively large amounts of PKCε, relatively little PKCα, and hence, showing a Ca^{2+}-independent PKC-dependent contraction, whereas the microvessels have a relative high amount of PKCα and little PKCε and hence show a Ca^{2+}-dependent PKC-dependent contraction.[43,44] Previous data also showed that PKCα is the major contributor among PKC isoforms involved in hypertrophic signaling in cardiomyocytes via ERK1/2-dependent signaling pathway.[45,46] Although our data might, at least partly, explain the active response of small-diameter coronary arteries as a function of the changes of Kir channel density as well as the sensitivity to vasoconstrictor (Ang II) in pathological condition, further study is needed to disclose the cellular benefit behind such adaptation in small-diameter coronary artery.

It has been suggested that Kir channels, which have been detected only in small-diameter cerebral and coronary arterial smooth muscle cells, contribute to the resting tone in coronary and middle cerebral artery, as increase of moderate extracellular K^+ concentration leads to vasodilation, and Ba^{2+} cause constriction of the vascular artery at resting tone.[9–11,47] 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. The significant decrease in Kir channel density in this model could be partly explained by the possibility that the membrane potential became more positive and exceeded the range for the open probability of Kir channels. The net result induced inactivation or reduced function of Kir channels during hypertrophy. Although K^+ channel function has been reported for multiple pathological conditions, pathology-associated changes in Kir channel function have received little attention in the literature. To our knowledge, only a few studies have been published. The authors reported that cerebral arteries isolated from hypertensive rats no longer dilated in response to extracellular K^+ concentrations that activated the Kir channels.[48] Furthermore, the response of Kir channels to extracellular K^+ was significantly attenuated after 2 hours of ischemia and 24 hours of reperfusion in the rat middle cerebral arteries.[49]

Although ion channels of vascular smooth muscle could be altered during hypertrophy, most studies and clinical approaches have been focused on the cardiac function that related to ion channels or receptors or even their signal transduction mechanisms. Many anti-left ventricular hypertrophy drugs eg, β-blockers, nitrates, and modulators of the rennin-angiotensin-aldosterone system have been developed to counteract ventricular remodeling.[50] In this study, however, we clearly demonstrated that the function of Kir channels in small-diameter coronary arteries was proved to be significantly altered in hypertrophy. The recorded dysfunction of Kir channel in vascular smooth muscle consequently reduces the vascular contractile response and coronary reserve, which could accelerate vascular remodeling. Therefore, the data afford new insight about possible future contribution of Kir channels in the development of therapeutic intervention especially against vascular microcirculation related cardiac hypertrophy.

In summary, we suggest for the first time that the alteration of Kir channel density limits vasodilating responses to moderate increase of extracellular K^+ in small-diameter coronary

arteries of hypertrophied rabbit heart. Considering that small-diameter coronary arteries respond actively to changes in luminal flow, intravascular pressure, and the concentration of local metabolites,[25] information about the differential expression of Kir channels and different modulation of Kir channels by a vasoconstrictor (Ang II) in a hypertrophy model is important for understanding the responsiveness of small-diameter arteries during hypertrophy.

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Disclosures

None.

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