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Altered Electrophysiological Properties of Coronary Artery in Isoprenaline-Induced Cardiac Hypertrophy

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An impaired smooth muscle cell (SMC) relaxation of coronary artery by alteration of K^+ channels would be the most potential explanation for reduced coronary reserve in left ventricular hypertrophy (LVH), however, this possibility has not been investigated. We performed morphometrical analysis of the coronary artery under electron microscopy and measured Ca^{2+}-activated K (K_{Ca}) currents and delayed rectifier K (K_{dr}) currents by whole-cell and inside-out patch-clamp technique in single coronary arterial SMCs from rabbits subjected to isoprenaline-induced cardiac hypertrophy. Coronary arterial SMCs underwent significant changes in ultrastructure. The unitary current amplitude and the open-state probability of K_{Ca} channel were significantly reduced in hypertrophy without open-time and closed-time kinetic. The concentration-response curve of K_{Ca} channel to Ca^{2+} is shifted to the right in hypertrophy. The reduction in the mean single channel current and increase in the open channel noise of K_{Ca} channel by TEA were more sensitive in hypertrophy. K_{dr} current density is significantly reduced in hypertrophy without activation and inactivation kinetics. The sensitivity of K_{dr} current on 4-AP is significantly increased in hypertrophy. This is the first study to report evidence for alterations of K_{Ca} channels and K_{dr} channels in coronary SMCs with LVH. The findings may provide some insight into mechanism of the reduced coronary reserve in LVH.

Key Words: Reduced coronary reserve, LVH, Coronary artery structure, Ca^{2+}-activated K^+ currents, Delayed rectifier K^+ currents

INTRODUCTION

The maintenance of coronary blood flow is dependent upon a certain level of tone maintained by coronary arterial smooth muscle cells (SMCs). Several ion channels in the plasma membrane of vascular SMCs are known to play a major role in the regulation of smooth muscle excitability. Among the channels, potassium channels are implicated in the genesis and regulation of resting membrane potential and in this way are critically involved in the mechanism of vascular SMCs contraction. In particular, Ca^{2+}-activated K^+ (K_{Ca}) channels, being a high density of artery, participate in the maintenance or regulation of arterial tone (Benham et al, 1986; Nelson et al, 1995; White et al, 2000) and delayed rectifier K^+ (K_{dr}) channels, being also one of dominant K^+ channels found, act mainly to limit membrane depolarization in arterial SMCs (Ishikawa et al, 1993; Leblanc et al, 1994).

Although many electrophysiological and pharmacological studies have been performed to identify and characterize these channels in normal coronary arterial SMCs from different animals, little is known about the alterations of these channels in coronary arterial SMCs with left ventricular hypertrophy (LVH) and their possible pathological roles.

With regard to LVH, several studies have reported reductions in the coronary reserve, in response to both physiological (Holtz et al, 1977; Rembert et al, 1978) and pharmacological (Johnson et al, 1978; Cimini & Weiss, 1990) stimuli. As yet, there are only limited data available on pathophysiological mechanisms of

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reduced coronary reserve in LVH, including endothelial dysfunction, increased coronary arteriolar tone, structural alterations of intramyocardial arterioles, increased perivascular fibrosis, increase extravascular compressive forces and inadequate neoangiogenesis.

Especially, an impaired SMC relaxation of coronary artery by alteration of potassium channels would be the most potential explanation for reduced coronary reserve, however, this possibility in level of single coronary SMCs has not been investigated.

We examined, for the first time, the alterations of K_{Ca} currents and K_{dr} currents in rabbit coronary arterial SMCs with LVH. In addition, we performed morphometrical analysis of, the coronary artery under electron microscopy.

METHODS

Experimental animals

New Zealand white rabbits (1 ~ 1.6 kg) underwent injection of isoprenaline (300 μg/kg/day) to produce cardiac hypertrophy (Collins et al, 1975; Gillis et al, 1996). Rabbits were studied 10 days after injection when documented cardiac hypertrophy had developed. Data were collected from 24 control and 32 cardiac hypertrophy rabbits.

Electron microscopic procedure

The samples were placed with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, for 2 hours. These samples were then washed several times with the same buffer, postfixed with 1.5% osmium tetroxide for 2 hours, and dehydrated in graded alcohol. The tissues were then embedded in Epon. They were cut with a diamond knife on an ultramicrotome; the sections were mounted on plain copper girds, stained with uranyl acetate and lead citrate, and then examined under electron microscope.

Coronary myocyte isolation

Single coronary arterial SMCs were isolated by a method described previously (Kim et al, 2001). Briefly, rabbits were anaesthetized with sodium pentobarbital (10 mg/kg I.V.) and concomitantly injected with heparin (300 I.U/ml). The heart was quickly removed and placed in a cold oxygenated Tyrode solution. The left anterior descending coronary artery was carefully removed with a portion of myocardium attached to it and the adhering ventricular myocardium and connective tissue were carefully removed under binocular examination. Thereafter, an enzymatic method was used to isolate single coronary arterial SCMs for electrophysiological experiments.

Membrane current recording

All experiments were performed at room temperature by the whole-cell and inside-out patch-clamp technique. Patch-clamp experiments were only carried out on cells that remained in a relaxed state. Electrodes had a resistance of 5 to 8 MΩ when filled with a pipette solution containing (in mM): KCl 145, HEPES 10, $MgCl_2$ 1, and $CaCl_2$ 1 (pH 7.4) for K_{Ca} currents; K-Aspartic acid 133, KCl 7, Mg-ATP 2.5, Na-ATP 2.5, tris-creatine phosphate 2.5, Na-creatine phosphate 2.5, HEPES 5 and EGTA 10 (pH 7.3) for K_{dr} currents. Bath solution for recording K_{dr} currents contained (in mM) NaCl 143, KCl 5.4, NaH_2PO_4 0.33, $MgCl_2$ 1, HEPES 5, glucose 16.6, and $CaCl_2$ 1.8 (pH 7.4) and for recording K_{Ca} currents contained (in mM): KOH 21.22, KCl 123.78, $MgCl_2$ 1, $CaCl_2$ 2.4, EGTA 3, K-ATP 2, and HEPES 10 (pH 7.4).

Membrane currents were recorded with an Axopatch-1D amplifier (Axopatch-1D, Axon Instruments, Foster City, CA, USA). These currents were digitized at a sampling rate of 5 kHz for whole-cell currents and 48 kHz for single-channel currents and stored in digitized format on digital audio tapes using a Biologic DTR-1200 recorder (Grenoble, France). For the analysis, the data were transferred to a computer with pCLAMP v 6.03 software (Axon Instruments, Burlingame, CA, USA) through an analogue-to-digital converter interface (Digidata-1200, Axon Instruments Inc.).

In single-channel currents, the open time histogram was formed from continuous recordings of more than 60 s. The open probability (P_o) was calculated using the formula:

$$P_o = \left(\sum_{j=1}^{N} t_j j\right)/(T_d N)$$

Where t_j is the time spent at current levels corresponding to j=0, 1, 2, ... N channels in the open state, T_d is the duration of the recording, and N is

the number of channels active in the patch. The number of channels in a patch was estimated by dividing the maximum current that observed by the mean unitary current amplitude. P_o was calculated over 30 s records.

Drugs

All chemicals and drugs were obtained from Sigma Chemical (St. Louis, MO, USA).

Statistical analysis

Data are presented as mean ± S.E. Cell numbers are indicated in parentheses in the figures. Student's unpaired t test was used to compare data of control and hypertrophy. Values of $P<0.05$ were considered significant.

RESULTS

Alteration of membrane capacitance in hypertrophic coronary arterial SMCs

Ten days after isoprenaline injections, heart weight and the ratio of heart weight to body weight (HW/BW) were significantly greater in hypertrophy than in control. No serous cavity effusions or increases in lung or liver weights were observed. With this 13% increment of HW/BW ratio, membrane capacitance of SMCs isolated from coronary artery increased 24%, respectively, in hypertrophy compared with control. Table 1 summaries the characteristics of the rabbit cardiac hypertrophy model used in the present study.

Alteration of ultrastructure in hypertrophic coronary arterial SMCs

Fig. 1 shows representative features of coronary arterial SMC and endothelial cell. Both of SMCs were spindle-shaped. The cytoplasm of SMC in hypertrophy was enlarged by increment of myofilaments and the nucleus was bigger and more heterochromatic compared with control. There were larger amounts of

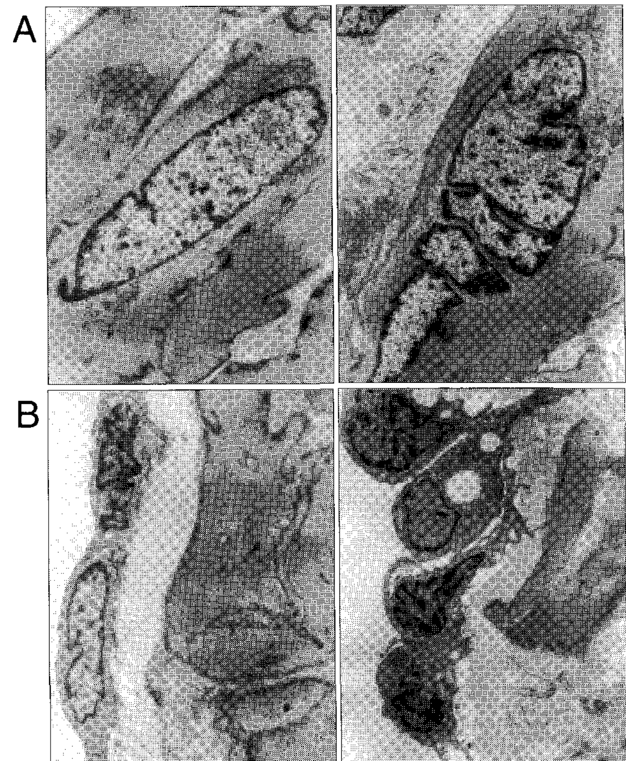

Fig. 1. Ultrastructure in coronary artery from control and hypertrophy. (A) Coronary arterial SMCs taken from control (left) and hypertrophy (right). (B) Coronary arterial endothelial cells taken from control (left) and hypertrophy (right). Magnification × 5,000.

Table 1. Characteristics of isoprenaline-induced cardiac hypertrophy

	Control	Hypertrophy
Body weight (BW), kg	1.29 ± 0.06 (n=14)	1.36 ± 0.05 (n=26)
Heart weight (HW), g	7.93 ± 0.41 (n=14)	9.66 ± 0.33* (n=26)
HW/BW, g/kg	0.62 ± 0.01 (n=14)	0.70 ± 0.01* (n=26)
Membrane capacitance of coronary arterial SMCs, pF	14.12 ± 0.01 (n=26)	17.46 ± 0.69* (n=40)

*P < 0.05, compared to control

collagen near SMC in hypertrophy compared with control. The contour on the internal elastic lamina side of endothelial cell in hypertrophy showed marked narrowing and separating compared with control. The endothelial cell in hypertrophy had increased heterochromatin of nucleus and marked protrusion compared with control.

Alteration of K_{Ca} currents in hypertrophic coronary arterial SMCs

Representative raw traces of single K_{Ca} current from +10 to +50 mV are shown in Fig. 2A. The unitary current-voltage relations for control and hypertrophy are compared in Fig. 2B. The unitary current amplitudes were reduced in hypertrophy compared with control.

The open probability of single K_{Ca} current was compared between control and hypertrophy in Fig. 2C. The open probability was also reduced in hypertrophy compared with control.

To examine the gating kinetics of K_{Ca} channels, the open- and closed-time constants were calculated at membrane potentials of +50 mV (data not shown). The parameters of open-time and closed-time kinetics were not differed between control and hyper-

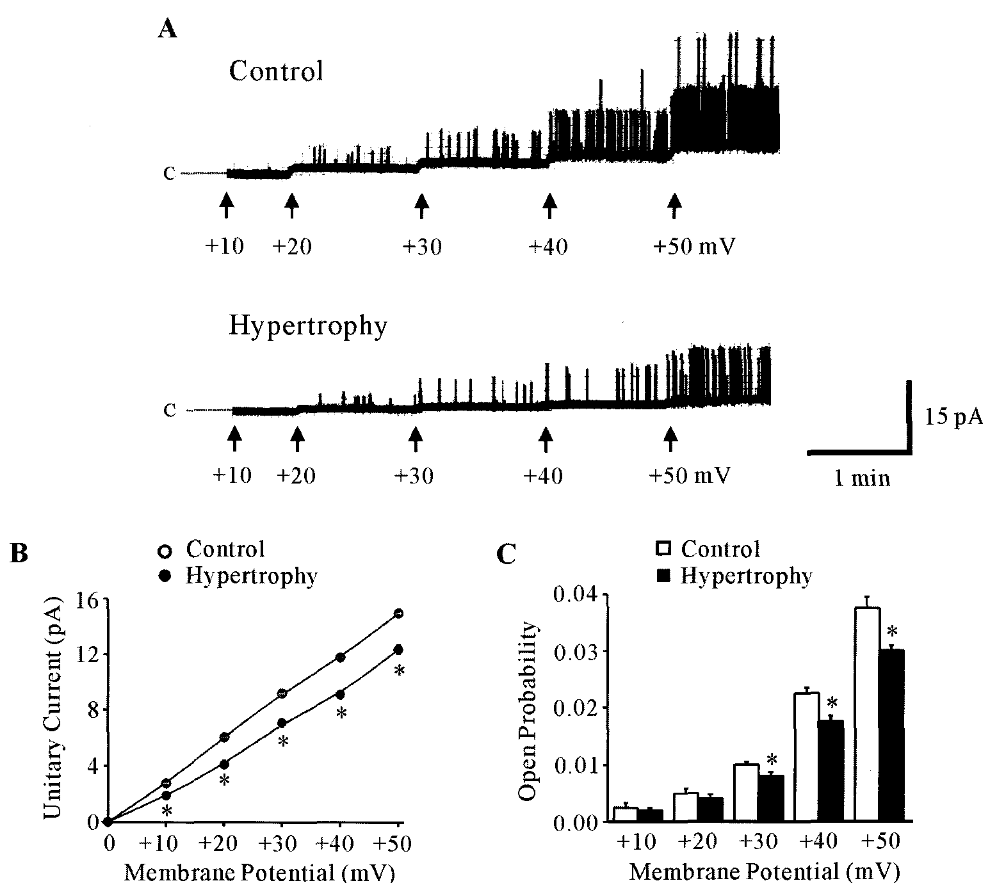

Fig. 2. Recording of single-channel K_{Ca} current from inside-out patches of control and hypertrophic coronary arterial SMCs. (A) The traces of chart recorder show that unitary amplitude of K_{Ca} channels is reduced in hypertrophy patches at 5 different membrane potentials. (B) Scatterplot of K_{Ca} current amplitude as a function of membrane potential in control (n=7) and hypertrophy (n=8) patches. Each slope ocnductances was 321 pS (Control) and 265 pS (Hypertrophy). $*P<0.05$ hypertrophy vs. control. (C) The open-state probability of K_{Ca} channels increased with membrane potential in both control (n=7) and hypertrophy (n=8). The open-state probability in hypertrophy was significantly less than that in control. $*P<0.05$ hypertrophy vs. control.

trophy.

The Ca^{2+} sensitivity of K_{Ca} channels was compared between control and hypertrophy in Fig. 3. With the increase in $[Ca^{2+}]_i$, increase in the activity of K_{Ca} channels was less steep in hypertrophy (Fig. 3B) than control (Fig. 3A). Fig. 3C shows the concentration-response relations (K_{Ca} channel activity s $[Ca^{2+}]_i$) obtained from the two cell types. Each channel activity at six selected concentrations of Ca^{2+} was normalized to the channel activity recorded at 0.1 μM Ca^{2+}.

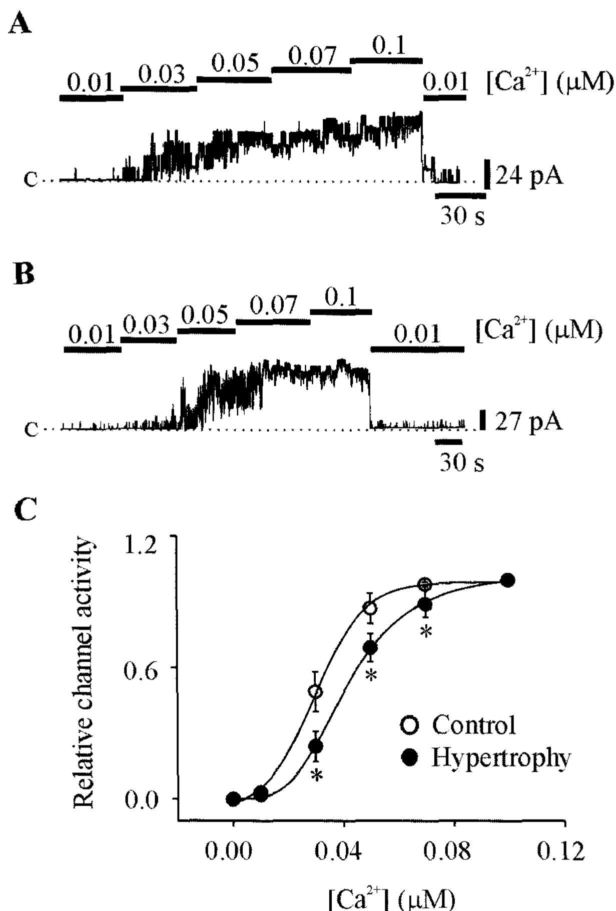

Fig. 3. Comparison of Ca^{2+} sensitivity of K_{Ca} channels in inside-out patches of control and hypertrophy. (A) Representative current traces of chart records of control. (B) Representative current traces of chart records of hypertrophy. (C) Isochronal sensitivity curves for K_{Ca} current in control (n=5) and hypertrophy (n=9). Data points are normalized channel activity. Smooth curves are best fit of mean data to a Hill equation, Relative channel activity=$1/\{1+(K_d/[Ca^{2+}]_i)^n\}$, where K_d is $[Ca^{2+}]_i$ at the half-maximal activation of the channel, and n=Hill coefficient. Control: K_d=0.03 μM, n=3.60; hypertrophy: K_d=0.04 μM, n=3.57 $*P<0.05$ hypertrophy vs. control.

As shown in Fig. 3C, the concentration-response curve of K_{Ca} channel to Ca^{2+} in hypertrophy is shifted to the right compared with that in control, indicating that Ca^{2+} has a reduced stimulatory effect on K_{Ca} channels in hypertrophy.

Fig. 4A shows unitary K_{Ca} currents recorded from patches in the absence of external TEA and from patches in presence of external 100 μM TEA at +40 mV. The reduction in the mean single channel current and increase in the open channel noise by TEA were more sensitive in hypertrophy than control. The mean current-voltage relations in control and hypertrophy with 100 μM external TEA is shown in Fig. 4B.

Alteration of K_{dr} currents in hypertrophic coronary arterial SMCs

Another significant electrophysiological change in our experimental model was a decrease in K_{dr} current density. This is shown in Fig. 5A, which compares raw currents recorded at test potentials of −20, 0, +20, and +50 mV in a control coronary SMC and a coronary SMC from hypertrophy. Fig. 5B plots the mean I-V relationships for each group of coronary SMCs and demonstrates that K_{dr} current density was markedly less in coronary SMCs from LVH than in control coronary SMCs.

To examine the kinetic properties of K_{dr} channels, the activation and inactivation kinetics of the channels were analyzed (data not shown). The parameters of activation and inactivation kinetics were not differed between control and hypertrophy.

Fig. 6 shows K_{dr} currents recorded from two type cells in the absence and presence of external 0.1 and 1 mM 4-AP. The inhibition of 4-AP on K_{dr} currents was more effective in hypertrophy than control.

DISCUSSION

Cardiac hypertrophy was induced in rabbits 10 days after injection of 300 μg/kg isoprenaline, as confirmed by significant increase in HW/BW ratio. No change was seen in the lung weight to body weight ratio or in the liver weight to body weight ratio, excluding the presence of congestive heart failure in this model. On the basis of the 13% increase in HW/BW ratio, and the absence of congestive heart failure, this model can be classified as mild cardiac hypertrophy (Cameron et al, 1983). Collins et al (19

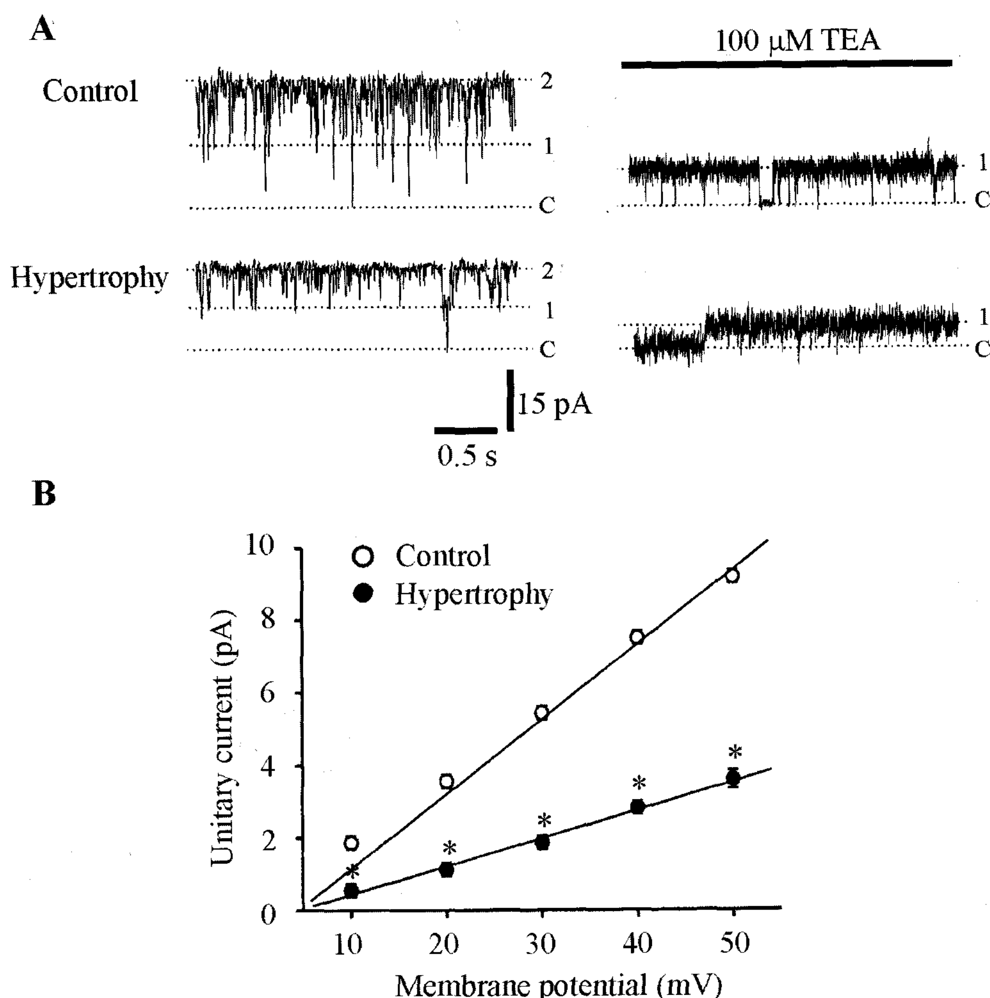

Fig. 4. Comparing of the inhibitory effect of TEA on K_{Ca} channels in coronary arterial SMCs from control (n=4) and hypertrophy (n=6). TEA (100 μM) caused profound channel block when applied to the external surface of control and hypertrophy patches (membrane potential, +40 mV; $[Ca^{2+}]_i$, 100 nM). (A) Representative current traces of K_{Ca} channels in absence and presence of TEA. (B) The current-voltage relation in the presence of TEA. $^*P<0.05$ hypertrophy vs. control.

75) showed that repeated administration of a low dose of isoprenaline caused a 13% increase in both left and right ventricular weight in the rat and the degree of hypertrophy can be controlled by administering different doses or altering duration of administration. Thus this method provides a simple well-established model with low mortality and high reproducibility, which provides useful information that may have a relevant application to clinically observed disease (Meszaros et al, 1996).

Moreover, significant changes in SMCs of coronary artery occurred in our experimental models. The membrane capacitance of SMC was 24% greater in hypertrophy than in control hearts, indicating that continuous infusion of isoprenaline may cause hypertrophy of coronary arterial SMC as well as myocardial hypertrophy. Although there are numerous ultrastructural descriptions of cardiac myocytes (Maron et al, 1975; Baandrup et al, 1981), that of coronary arterial SMCs is little in hypertrophied hearts. Our ultrastructural study showed that the significant increase in coronary arterial SMC volume is due to the increment of myofilaments, which results in increase of cross-sectional area.

The present study demonstrates for the first time that the alterations of K_{Ca} currents and K_{dr} currents

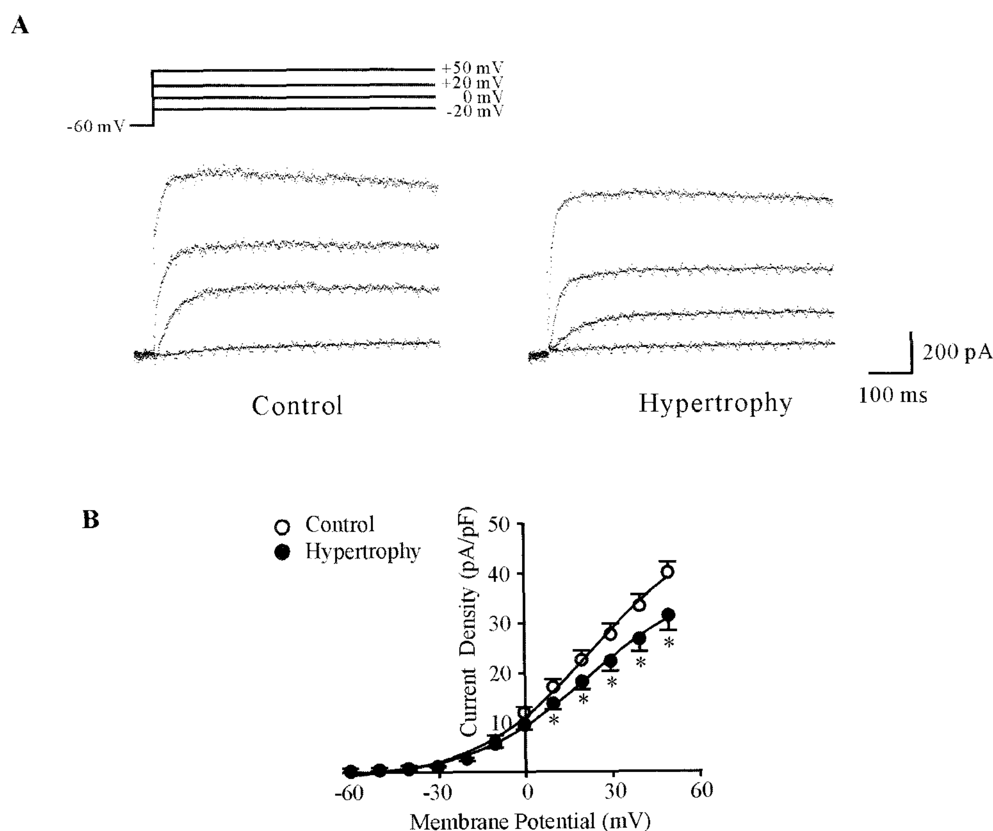

Fig. 5. Recording of K_{dr} current from whole-cell modes of control and hypertrophic coronary arterial SMCs. (A) Traces of whole-cell currents elicited by stepping from holding potential of −60 mV to −20, 0, +20, and +50 mV in presence of external TEA (1 mM) and nicardipine (10 μM). (B) K_{dr} current-voltage relations of coronary arterial SMCs from control (n=7) and hypertrophy (n=10). *$P<0.05$ hypertrophy vs control.

in coronary SMCs may be a function of impaired coronary reserve in LVH.

The major new findings are as follows:

(1) Unitary current amplitude and open-state probability of K_{Ca} channels were decreased without changes for its open-time and closed-time kinetics in coronary arterial SMCs with LVH. K_{Ca} channels are particularly abundant in coronary arterial SMCs and activation of the channels may be important buffering mechanism to counteract vessel depolarization and constriction in response to some vasoconstrictors and to increased intravascular pressure (Benham et al, 1986; Nelson et al, 1995; White et al, 2000). Altered vascular K^+ channel function under pathological conditions could be either a cause or an effect of the disease. Vasoconstriction and the compromised ability of an artery to dilate are likely consequences of defective K^+ channel function in blood vessels and may be due to a change in the number, unitary conductance, and/or open probability of the channels. The changes in unitary current amplitude and channel open-state probability may change in total membrane current. Therefore, these changes of K_{Ca} channels could contribute to the reduced K_{Ca} currents.

(2) K_{dr} current density as well as total K_{dr} currents were decreased without changes for its activation and inactivation kinetics in coronary arterial SMCs with LVH, similar to that of other studies (Volk et al, 1991). K_{dr} channels are also one of dominant K^+ channels found and are thought to serve an important buffering function against depolarization and vasoconstriction (Ishikawa et al, 1993; Leblanc et al, 1994).

Reduced K_{dr} current density would due to a decrease in the number of functional K_{dr} channels, unitary current amplitude, or open probability. Actually, recent studies have shown that openings of K_{Ca} channels and K_{dr} channels hyperpolarize the membrane potential, leading to increase coronary artery

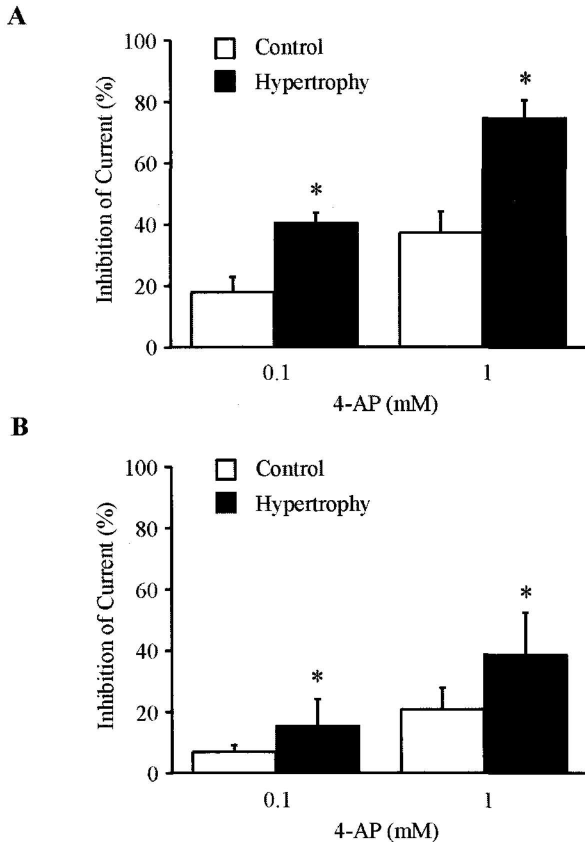

Fig. 6. Comparing of the inhibitory effect of 4-AP on K_{dr} channels in coronary arterial SMCs from control (n=3) and hypertrophy (n=4). Percent inhibition of 0.1 and 1 mM 4-AP at 2 different voltages (A; 0 mV and B; +50 mV) is shown. $^{*}P<0.05$ hypertrophy vs control.

blood flow (Node et al, 1998) or to produce vasorelaxation (Khan et al, 1998) and that alterations in K_{Ca} channels and K_{dr} channels might also initiate or aggravate pathophysiological states such as vasospasm and ischemia (Brayden & Nelson, 1992). Based on the above studies, our result provides the alterations of K_{Ca} channels and K_{dr} channels in coronary arterial SMCs as powerful possibility of reduced coronary reserve in LVH.

(3) The concentration-response curve of K_{Ca} channel to Ca^{2+} in coronary arterial SMCs with LVH is shifted to the right compared with that in control. Our results were consistent with the concept that elevation of $[Ca^{2+}]_i$, by opening of voltage-dependent Ca^{2+} channels or spontaneous Ca^{2+} release from sarcoplasmic reticulum stores, stimulated K_{Ca} channels (Hume & Leblanc, 1989; Ganitkevidh & Isenberg, 1990) and showed that the channel activity was very steeply dependent on $[Ca^{2+}]_i$. But, responsiveness of K_{Ca} channels to $[Ca^{2+}]_i$ was reduced in hypertrophy comparing to control. Several molecular mechanisms are known to modify the voltage or Ca^{2+} sensitivity of K_{Ca} channels, including the coupling of the alpha-subunit to its stimulatory beta-subunit and the generation of alternatively spliced alpha-subunit isoforms (Toro et al, 1991; Scornik & Toro, 1992; Rusch et al, 1996). In this respect, a decreased Ca^{2+} sensitivity has been suggested as a potential mechanism for the reduced open probability of K_{Ca} channels in hypertrophy. The activation of K_{Ca} channels, caused by Ca^{2+} influx, is an important negative feedback mechanism that regulates the level of vascular tone (Nelson & Quayle, 1995). This regulatory pathway is likely to influence arterial tone in many vascular beds, including coronary circulation (Khan et al, 1998). Therefore, our result suggests that negative feedback action of K_{Ca} channels against elevation of $[Ca^{2+}]_i$ may be attenuated in hypertrophy, which could not well relax coronary artery in response to various stimuli.

(4) Coronary arterial SMCs with LVH showed a stronger response to TEA and 4-AP compared with control, suggesting that coronary arterial SMCs with LVH, at least, may be more sensitive some vasoconstrictor, which are associated with TEA or 4-AP binding site, than control.

In summary, to the best of our knowledge, this is the first study to report evidence for alterations of K_{Ca} channels and K_{dr} channels in coronary SMCs with LVH. The findings may provide some insight into mechanism of the reduced coronary reserve in LVH. Our data further support the emerging concept that altered K^+ channels in coronary arteries may be associated with a diverse range of cardiovascular diseases.

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