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Regulation of the instantaneous inward rectifier and the delayed outward rectifier potassium channels by Captopril and Angiotensin II via the Phosphoinositide-3 kinase pathway in volume-overload-induced hypertrophied cardiac myocytes

Authors' Contribution:
- **A** Study Design
- **B** Data Collection
- **C** Statistical Analysis
- **D** Data Interpretation
- **E** Manuscript Preparation
- **F** Literature Search
- **G** Funds Collection

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Summary

Background: Early development of cardiac hypertrophy may be beneficial but sustained hypertrophic activation leads to myocardial dysfunction. Regulation of the repolarizing currents can be modulated by the activation of humoral factors, such as angiotensin II (ANG II) through protein kinases. The aim of this work is to assess the regulation of I_K and I_{K1} by ANG II through the PI3-K pathway in hypertrophied ventricular myocytes.

Material/Methods: Cardiac eccentric hypertrophy was induced through volume-overload in adult male rats by aorto-caval shunt (3 weeks). After one week half of the rats were given captopril (2 weeks; 0.5 g/l/day) and the other half served as control. The voltage-clamp and western blot techniques were used to measure the delayed outward rectifier potassium current (I_K) and the instantaneous inward rectifier potassium current (I_{K1}) and Akt activity, respectively.

Results: Hypertrophied cardiomyocytes showed reduction in I_K and I_{K1}. Treatment with captopril alleviated this difference seen between sham and shunt cardiomyocytes. Acute administration of ANG II (10^{-6}M) to cardiocytes treated with captopril reduced I_K and I_{K1} in shunts, but not in sham. Captopril treatment reversed ANG II effects on I_K and I_{K1} in a PI3-K-independent manner. However in the absence of angiotensin converting enzyme inhibition, ANG II increased both I_K and I_{K1} in a PI3-K-dependent manner in hypertrophied cardiomyocytes.

Conclusions: Thus, captopril treatment reveals a negative effect of ANG II on I_K and I_{K1}, which is PI3-K independent, whereas in the absence of angiotensin converting enzyme inhibition I_K and I_{K1} regulation is dependent upon PI3-K.

key words: **cardiac hypertrophy • PI3K/Akt • K channels • angiotensin converting enzyme inhibitor**

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BACKGROUND

Cardiac remodeling secondary to volume overload in conditions such as aortic and mitral regurgitation, arteriovenous shunt and anemia is characterized by a marked increase in left ventricular (LV) chamber volume with disproportionate increase in wall thickness (eccentric hypertrophy) [1–3]. Although the remodeling is initially a compensatory response that has the effect of normalizing diastolic wall stress and cardiac output, sustained volume overload induces progression from adaptive to maladaptive remodeling (LV dilatation with wall thinning) and ultimately leads to the development of heart failure [2]. Muscle cell growth associated with cardiac hypertrophy is caused by changes in gene expression controlled by cardiac signaling pathways that are mainly turned on by pro-hypertrophic humoral factors such as angiotensin II (ANG II) [4]. Angiotensin II modulates arterial pressure, regulates blood volume, and promotes growth and proliferation through the activation of specific signaling mechanisms, via its type-1 receptor (AT-1R).

Growing evidence indicates that ANG II/AT-1R signaling induces a crosstalk to the PI3-K/Akt pathway, and a few studies have shown that such changes in PI3-K/Akt activities demonstrated altered phenotypes. Yano and colleagues [5] showed that ANG II increased phosphotyrosine-associated PI3-K activity and the phosphorylation of p70S6K and Akt through AT-1R, which was abrogated by co-treatment with losartan (an AT-1R antagonist), wortmannin (a phosphoinositide 3-kinase (PI3-K) inhibitor), and/or an Akt inhibitor, and/or stable transfection of dominant negative-Akt1. Liu and colleagues [6] demonstrated that ANG II-induced Akt phosphorylation was blocked by wortmannin and that ANG II regulates PI 3-K/Akt pathways via a negative crosstalk between AT_1 and AT_2 receptors in the fibroblasts of human hypertrophic scar. Accordingly, modulation of the AT1-R activity by ANG II and losartan (AT1-R antagonist) has been also implicated in depressing the immune response through mitogen-activated proliferative response [7].

Functional down-regulation of K^+ currents is a recurring theme in hypertrophied and failing ventricular myocardium. However, the specific changes in K^+ current expression differ depending on the species and the model of heart failure. A reduction in the density of the transient outward current (I_{to}) has already been consistently found in cardiac hypertrophy and failure, but down-regulation of the inward rectifying potassium current (I_{K1}) and the delayed rectifier potassium current (I_K) have also been described [8]. Previous reports have also shown a reduction in I_{to}, I_K, and I_{K1} densities in cells isolated from failing compared with control hearts. The few electrophysiological studies available in the literature related to volume-overload induced cardiac hypertrophy showed a decrease in I_K [9,10], but an increase in I_{K1}.

Angiotensin II has emerged as a central humoral signal in the pathophysiology of cardiac hypertrophy and failure. Therefore, involvement of ANG II in the down-regulation of cardiac ion channel expression is an attractive hypothesis. In rat neonatal myocytes, both phenylephrine and ANG II caused I_{to} to decrease by 50%, but with a notably different time course: decay in response to ANG II was much more rapid. The delayed rectifier K^+ current (I_K) is the major repolarizing outward current of ventricular action potentials in mammalian species. Wang and colleagues [11] showed a marked reduction in I_K tail currents during repolarization under the influence of ANG II mediated via AT_1 activation of protein kinase C (PKC). However, Zankov and colleagues [12] showed ANG II in nanomolar concentrations markedly potentiates I_K through a mechanism involving activation of the G protein-coupled AT-1R linked to the phospholipase C (PLC)-PKC pathway.

The inward rectifier I_{K1} is the principal determinant of the resting membrane potential and is important in late repolarization of the action potential. I_{K1} density is significantly reduced in cells isolated from failing hearts compared with normal hearts at voltages more hyperpolarized than –90 mV [8]. Tsuji and colleagues [10] noticed no significant difference in the density of I_{K1} between control and failing myocytes after paced-induced heart failure. When the amplitude I_{K1} was normalized to the cell surface area, the average current density, measured on hyperpolarization to –100 mV, was significantly smaller in cells isolated from hearts of patients with terminal heart failure compared with control cells [13]. Cardiac-specific ANG II overproduction in transgenic TG1306/1R mice demonstrates blood-pressure independent cardiac hypertrophy that resulted in reduction of I_{K1} potassium current density [14]. It is noteworthy that other humoral factors involved in left ventricular hypertrophy and hypertension, such as leptin, were not correlated with diastolic dysfunction in hypertensive patients [15].

Thus, there is still a crucial lack of information regarding the intracellular signal transduction events associated with ANG II regulation of potassium channel activities, especially during the development of eccentric cardiac hypertrophy. The objective of this study was to determine the mediating ANG II effects on the functional expression of potassium channels of adult cardiomyocytes during the development of eccentric cardiac hypertrophy and the role of angiotensin-converting enzyme inhibitor (ACE-I) in modulating such effects. This study stems from our hypothesis that during cardiac hypertrophy ANG II inotropic effects are partly mediated by enhancement of I_K and I_{K1} in a PI3-K/Akt-dependent manner. Therefore, treatment with ACE-I reverses these effects in regressed cardiomyocytes. Thus we conclude that in the absence of ACE-inhibition I_K and I_{K1} regulation by ANG II is dependent upon PI3-K in the hypertrophied cardiomyocytes. Whereas, captopril treatment reveals a negative ANG II effect on I_K and I_{K1}, which is PI3-K independent.

MATERIAL AND METHODS

Animal preparation

Conformity statement: All the procedures conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH) publication No. 85-23, revised 1996. Male Sprague-Dawley rats of 200–250 g body weight were purchased from Charles Rivers (MA). The rats were allowed to recover and acquaint with their new environment upon arrival to the animal house of the college of medicine at Howard University for 1 week. The animals were kept under secure, clean and controlled room temperature (70 F-74 F) with a 6:00 h to 18:00 h light cycle and were fed food and water *ad libitum*.

Eccentric cardiac hypertrophy

Adult male Sprague-Dawley rats (200–250 g) were anaesthetized with sodium pentobarbital (30 mg/kg body weight, i.p.). A bulldog vascular clamp was placed across the aorta inferior to the renal vessels. The abdominal aorta was punctured at the union of the segment two-third caudal to the renal artery and one third cephalic to the aortic bifurcation with an 18 gauge disposable needle. The needle was advanced into the abdominal aorta and vena cava at the point of anastomosis, shunting arterial blood into the venous system. A drop of cyanoacrylate glue was used to seal the aorta-punctured point. The patency of the shunt was verified visually by swelling of the vena cava and the mixing of arterial and venous blood. As a post-operative care, the rat was administered with flunixin 2.5 mg/kg. The same procedure was performed on the age-matched sham rats, except for the insertion of the 18G needle in the abdominal aorta and vena cava. One week after surgery, shunted animals were treated with angiotensin-converting enzyme inhibitor, captopril (0.5 g/L/day in drinking water for 14 days) and the second group was given no drugs; i.e., three weeks were allowed for the cardiac hypertrophy to develop. On the experimentation day, visual inspection of the lungs did not show any signs or symptoms of pulmonary edema or pulmonary blood clots in all shunted animal used. This is relevant to the fact that these shunted rats are still in the compensated eccentric cardiac hypertrophy phase and eliminate overt decompensatory process to heart failure

Isolation of adult rat cardiomyocytes

All the reagents were purchased from Sigma Chemicals (St. Louis, Mo). Double-distilled water from MilliQ system (Millipore Corporation, MA) was used to prepare all solutions. Stock buffer solution contained (mM): 113 NaCl, 4.7 KCl, 0.6 KH_2PO_4, 0.6 Na_2HPO_4, 1.2 $MgSO_4$, 12 NaHCO3, 10 $KHCO_3$, 10 HEPES. Animals were injected with sodium heparin (1000 U/kg, i.p.) and anesthetized with pentobarbital sodium (40 mg/kg; i.p.), 20 minutes prior to removal of the heart. After excision of the heart, it was quickly transferred to a Langendorff setup for retrograde coronary perfusion through the aorta at 10 ml/min (37°C) for an initial 5 minutes equilibration with a perfusion buffer (mM): 113 NaCl, 4.7 KCl, 0.6 KH_2PO_4, 0.6 Na_2HPO_4, 1.2 $MgSO_4$, 12 NaHCO3, 10 $KHCO_3$, 10 HEPES, 1.1 D-glucose, 10.2 Butanedione Monoxime. The experimental protocol consisted of continuing the retrograde perfusion of the hearts for 12 minutes with perfusion buffer solution (Digestion Buffer) containing (mg): 25 bovine serum albumin (essentially fatty acid free), 25 collagenase (type2) and 3 Protease (Type XIV). Then the heart was perfused for 5 minutes with stop buffer solution consisting of perfusion buffer containing Fetal Calf Serum (5%) and 14 µM $CaCl_2$. The ventricles were cut, minced into the stop buffer kept in the 37°C bath and filtered into culture dishes. In a laminar flow hood calcium was re-introduced to the cells up to 1.0 mM. The dissociated cardiomyocytes were diluted and kept in Tyrode solution until experimentation. Freshly isolated myocytes showing no signs of blebs or round edges were used for up to 12 hours.

Electrophysiological studies

Whole-cell patch-clamp technique was used to study the potassium currents in the adult cardiomyocytes. Patch pipettes of 1–2 MΩ resistance were pulled from borosilicate glass capillary tubing with a 2-stage puller (David KOPF Instruments, CA). Ventricular myocytes were placed on the stage of an inverted microscope and superfused with an extracellular whole-cell K^+ current buffer containing (mM): 5 KCl, 1 $MgCl_2$, 140 NaCl, 10 HEPES, 10 D-glucose, 1 $CaCl_2$, 0.2 $CdCl_2$, and pH at 7.4. The intracellular whole-cell K^+ current solutions contained (mM): 130 K-glutamate, 20 KCl, 5 EGTA, 5 NaCl, 1 $MgCl_2$, 10 HEPES, and pH at 7.4. After the formation of a Gigaohm seal, capacitance was estimated by integrating the area of the capacitance transient due to a –10 mV voltage step from a holding potential of –80 mV. The measured currents were divided by the cell capacitance in order to normalize for cell size changes between normal and hypertrophied cardiomyocytes. The cardiomyocytes were stimulated in voltage-clamp mode using pClamp 9.0 software (Molecular Devices, CA) connected to an Axopatch 200B amplifier through a A/D converter (Digidata1320A; Molecular Devices, CA). The resulting ionic currents were displayed on a storage oscilloscope and stored on a computer for analysis with pClamp 9.0. All patch-clamp experiments were performed at room temperature (20–22°C). All whole-cell pipette-filling solutions were filtered through a 0.22-µm filter. The voltage dependency of I_K and I_{K1} activation were studied by obtaining data for the respective current-voltage (I-V) relationships. To that end, 500 msec step voltages in 10 mV increments between –40 mV and +30 mV for I_K, or –80mV and –120mV for I_{K1}, were applied from a holding potential of –80 mV, but with a 200 msec prepulse to –40 mV for I_K. Steady-state currents, measured at the end of each current response, were plotted as a function of the command potential. The action of ANG II (10 min) in the presence and absence of the PI3-K inhibitor, LY 294002 (5 min) were analyzed for their effects on the I-V relationship.

Western blotting

Activation of Akt was assessed using western blot technique. Protein samples were prepared from perfused heart tissue using a lysis buffer containing (mM): β-glycerophosphate (20), EGTA (1), $NaVO_3$ (0.5), DTT (2), benzamidine (10), Na_3VO_4 (0.2), EDTA (2), NaF (20), and 0.6% deoxycholate, 0.1% Triton X-100, and 1 tablet/10 ml of Complete protease inhibitors cocktail (Roche, CA) (pH 7.5). Cell lysates were incubated on ice for 20 min and centrifuged for 15 min at 14,000 rpm. Samples were matched for protein concentration using Bradford Assay (Bio-Rad, CA), separated by SDS-PAGE and transferred onto nitrocellulose membranes. After blocking in 5% non-fat milk in TBST (10 mM Tris, 150 mM NaCl, 0.1%Tween 20), the membranes were incubated with specific antibodies to either total or phosphorylated Akt, (Cell Signaling Technology, MA) overnight at 4°C. Afterward, membranes were washed 3 times in TBST, incubated with appropriate secondary antibody conjugated to horseradish peroxidase (Cell Signaling Technology, MA) for 2 hours, and then washed 3 times with TBST. Bands were visualized by Chemiluminescence (Renaissance, NEN Life Science Products). Films from at least three different rats were scanned and densities of the immunoreactive bands

Table 1. Structural parameters of the sham and shunt hearts.

	Heart weight (mg)	Relative heart weight (mg/100 g body weight)
Sham (12)	1081±19	317±7
Shunt (12)	2557±332	663±38*
Sham +Captopril (9)	1212±41	352±12
Shunt + Captopril (9)	1741±109	474±30$^{*,\#}$

Number of animals in parenthesis; * P<0.05 *vs.* untreated sham; $^{\#}$ P<0.05 *vs.* Captopril-treated sham.

were evaluated using NIH Image software and normalized. Akt activities were evaluated as the ratio of phosphorylated form over total Akt per experiment.

Drugs

The inhibitor for PI3-K, (1 µM) LY 294002, and angiotensin II (1 µM) were purchased from Cell Signaling Technology (MA).

Statistical analysis

All statistical analysis were performed using SigmaStat software and verified using both Microsoft Excel and Prism softwares, which all gave the same result. The paired Students't-test was used to compare data before and after drug treatment of the same animal group. The heteroscedastic two-sample unpaired Students' t-test assuming unequal variances was used when comparing the drug effects between two different animal groups (sham *vs.* shunt).

RESULTS

Structural parameters

The data on the structural parameters from sham and shunted adult rats confirmed the development of the eccentric cardiac hypertrophy within 3 weeks post-surgery as seen in Table 1. The shunted rats had greater heart weights as well as relative heart weights when compared to the sham animals. Captopril treated animals showed regression in the absolute and the relative heart weights. There was no significant difference between captopril-treated and untreated sham values. In addition, the cellular membrane capacitance was significantly greater in the cardiomyocytes from hypertrophied hearts as compared to normal ones (290±21 pF *vs.* 201±14 pF; p<0.05).

Effects of ANG II on I_K channels in sham and shunted hearts

Hypertrophied myocytes as compared to normal myocytes showed significant decrease in the basal current density levels of the delayed outward rectifier potassium channel I_K (hypertrophied 3.6±0.1 pA/pF *vs.* normal 5.7±0.7 pA/pF; P<0.05, n=4) and slope conductance g_K (hypertrophied 66.7±8.1 nS/pF *vs.* normal 114.6±11.2 nS/pF; P<0.05) (Figure 1). Superfusion with ANG II (10^{-6} M) did not affect I_K current density (control 4.1±0.4 pA/pF *vs.* ANG II 5.0±0.4 pA/pF; n=5) nor its slope conductance in normal cardiomyocytes (57.8±15.4 nS/pF *vs.* 61.3±26.8 nS/pF) (Figure 2A). In the presence of ANG II, the PI3-K inhibitor, LY294002 (10^{-6} M), had no effect on the sham I_K current density. However, the ANG II effects on I_K channels of hypertrophied cardiomyocytes caused an increase in I_K current density (control 2.1±0.4 pF/pA *vs.* ANG II 2.9±0.5 pA/pF; P<0.05, n=4) and g_K (nS/pF) (control 37.6±15.8 *vs.* ANG II 44.6±15.4; p<0.05) (Figure 2B). Interestingly, addition of LY294002 (10^{-6} M) abrogated the ANG II effect on I_K in the hypertrophied cardiomyocytes (1.7±0.4 pA/pF; P<0.05, n=5) and its slope conductance, g_K (nS/pF) (44.6±15.4 *vs.* 35.2±14.4; P<0.05).

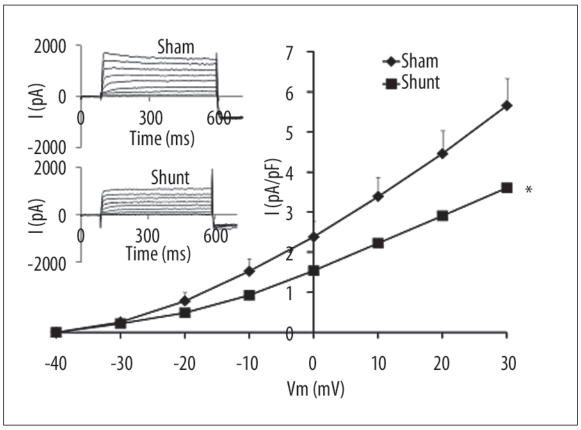

Figure 1. I_K voltage relationship for normal cardiomyocytes and volume-overload induced hypertrophied cardiomyocytes. The inset in each graph shows the respective representative current responses at +30 mV for I_K. Data are presented as average current density ±SEM with n=5–10. * P<0.05 sham *vs.* shunt.

Effects of ANG II on captopril treated I_K channels in sham and shunted hearts

Figure 3 shows that the delayed outward rectifier was significantly higher in the captopril-treated shunted cardiomyocytes *versus* the untreated shunts (4.1±0.5 pA/pF; p<0.05). Thus, treatment improved I_K current density toward sham levels. In the same line, there was no significant difference between the current density levels of captopril-treated sham and shunt cardiomyocytes. Acute administration of ANG II (10^{-6} M) to normal adult cardiomyocytes treated with captopril did not show any significant change in the delayed outward rectifier potassium current density (Figure 3A). However, Figure 3B shows that ANG II induced reduction in the outward rectifier potassium current I_K (control 4.1±0.5 pA/pF *vs.* ANG II 3.2±0.4 pA/pF, P<0.01; n=6) in hypertrophied cardiomyocytes. There was a parallel lowering in the slope conductance, g_K (nS/pF) (control 89.7±5.8 *vs.* ANG II 66.6±4.5, P<0.01). LY 294002 had no effect on I_K in captopril treated cardiomyocytes. The ANG II steady state effect was reached within 5–10 minutes.

Effects of ANG II on I_{K1} channels in sham and shunted hearts

The basal current density levels of the inward rectifier potassium channel I_{K1} was lower in hypertrophied (–8.9±0.4 pF/pA; P>0.05, n=5) *versus* normal cardiomyocytes

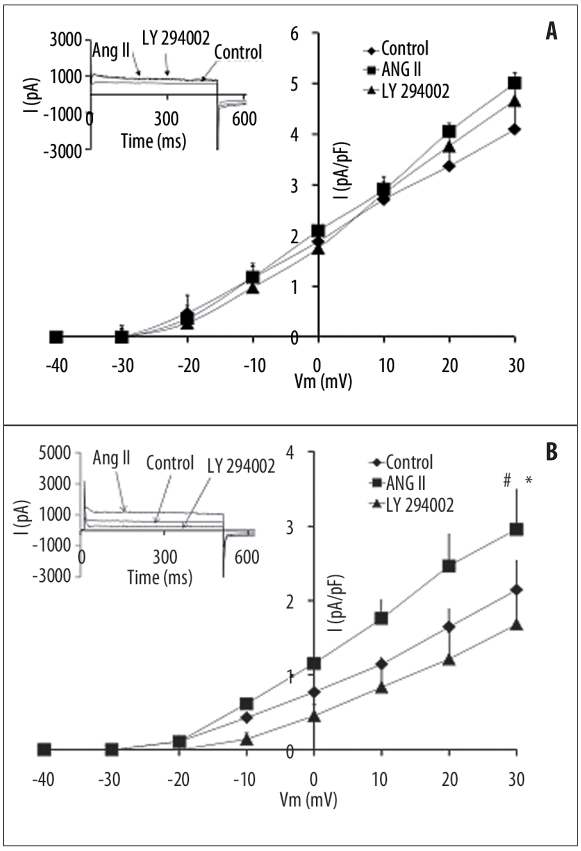

Figure 2. Effects of ANG II (10^{-6} M) and PI3-K inhibitor, LY 294002 (1 µM), on I_K voltage relationship for normal (**A**) and hypertrophied (**B**) cardiomyocytes. The inset in each graph shows the respective representative current responses at +30 mV for I_K. Data are presented as average current density ±SEM with n=5–10. * $P<0.05$ control *vs.* ANG II; # $P<0.05$ control *vs.* LY 294002.

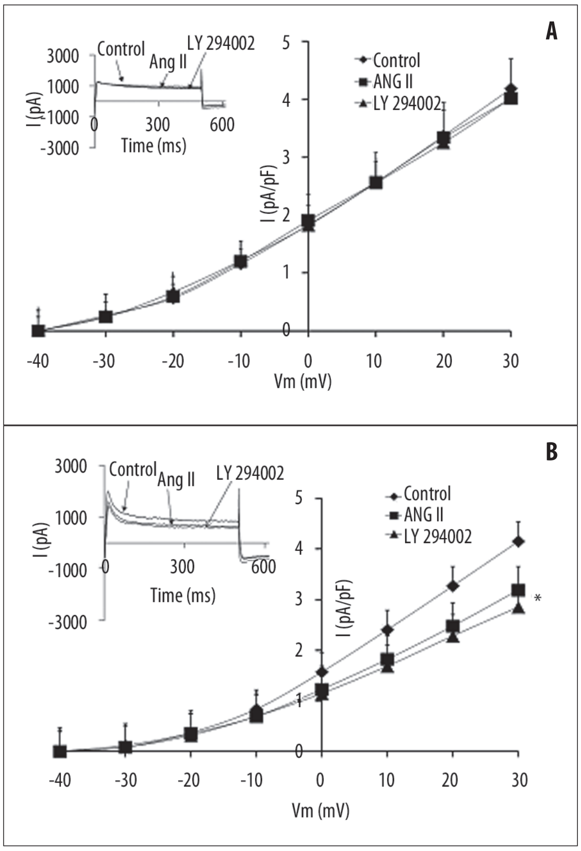

Figure 3. Effects of ANG II (10^{-6} M) and PI3-K inhibitor, LY 294002 (1 µM), on I_K voltage relationship for normal (**A**) and hypertrophied (**B**) cardiomyocytes, pretreated with captopril for 14 days. The inset in each graph shows the respective representative current responses at +30 mV for I_K. Data are presented as average current density ±SEM with n=4–6. * $P<0.05$ *vs.* control.

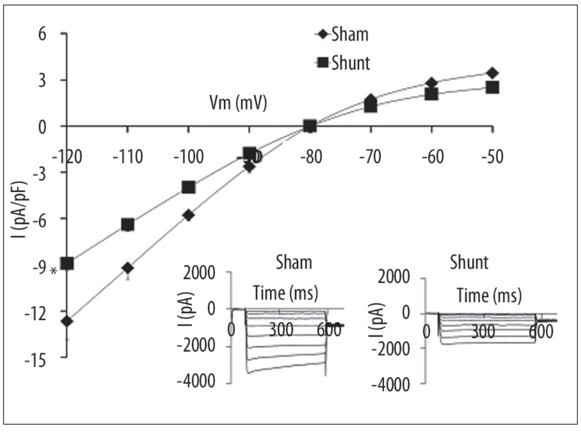

Figure 4. I_{K1} voltage relationship for normal cardiomyocytes and volume-overload induced hypertrophied cardiomyocytes. The inset in each graph shows the respective representative current responses at and -120 mV for I_{K1}. Data are presented as average current density ±SEM with n=5–10. * $P<0.05$ Sham *vs.* Shunt.

(−12.7±1.6 pF/pA; $P>0.05$, n=5) (Figure 4). Similar reduction was shown in the slope conductance of I_{K1}, g_{K1} (nS/pF) (Sham 227±75 *vs.* Shunt 129.4±58). Superfusion with ANG II (10^{-6} M) resulted in no effect on I_{K1} density of normal cardiomyocytes (Figure 5A). However, ANG II increased I_{K1} in hypertrophied cardiomyocytes from −9.3±0.1 pF/pA to −11.0±0.5 pA/pF ($P<0.05$, n=4) with improved g_{K1} (232.9±16.9 nS/pF to 267.5±24.7 nS/pF) (Figure 5B). Adding LY294002 (10^{-6} M) alleviated the ANG II effect on I_{K1} in the hypertrophied cardiomyocytes (−8.6±0.5 pA/pF; $P<0.05$, n=5) and g_{K1} (nS/pF) (232.9±15.8 *vs.* 226.4±17.7; $P<0.05$) as shown in Figure 5B.

Effects of ANG II on captopril treated I_{K1} channels in sham and shunted hearts

Acute administration of ANG II (10^{-6} M) to normal adult cardiomyocytes treated with captopril did not show any significant alterations in the inward rectifier potassium current density (Figure 6A). However in hypertrophied cardiomyocytes, Figure 6B shows that ANG II induced a reduction in the inward rectifier potassium current I_{K1} (control −8.2±0.7 pA/pF *vs.* ANG II −5.4±0.8 pA/pF, $P<0.01$; n=6) and g_{K1} (nS/pF) (control 221.4±20.8 *vs.* ANG II 144.9±18.3, $P<0.01$) was significantly reduced. PI3-Kinase inhibition did not affect the ANG II effect on I_{K1} in the captopril-treated shunted rat cardiomyocytes.

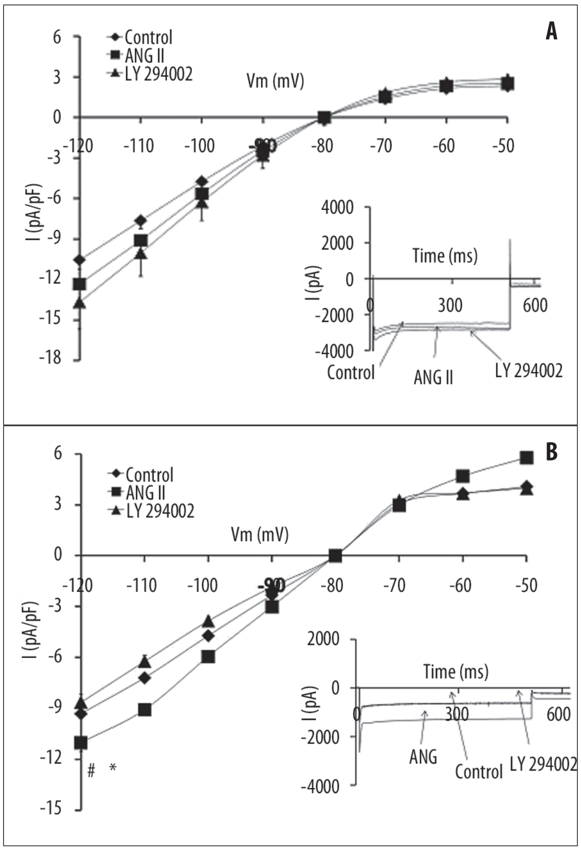

Figure 5. Effects of ANG II (10^{-6} M) and PI3-K inhibitor, LY 294002 (1 µM), on I_{K1} voltage relationship for normal (**A**) and hypertrophied (**B**) cardiomyocytes. The inset in each graph shows the respective representative current responses at and -120 mV for I_{K1}. Data are presented as average current density ±SEM with n=5–10. * $P<0.05$ control *vs.* ANG II; # $P<0.05$ control *vs.* LY 294002.

Captopril effect on the Akt activation levels in sham and shunt

Akt is a known PI3-kinase downstream effector whose activation level is dependent on PI3-K activity. Thus, we performed western blot analysis in order to assess the level of activation of the PI3-K/Akt pathway in sham and shunt hearts that have been treated with captopril *versus* untreated. The activation level of Akt was expressed as the ratio of phosphorylated Akt over total Akt protein expression. We found that the basal activation level of Akt in hypertrophied heart was significantly higher than in the sham ones (normal 1.00±0.21 *versus* hypertrophied 2.54±0.33; p<0.01), as shown in Figure 7. Treatment with captopril did not have an effect on the activity level of Akt in the normal cardiomyocytes; however it significantly down-regulated the Akt activation level in the hypertrophied cardiomyocytes toward sham levels (hypertrophied 2.54±0.33 *versus* captopril-treated 1.68±0.17; p<0.01).

DISCUSSION

In this study we have basically characterized the alterations of potassium channels during volume-overload-induced cardiac hypertrophy by the renin-angiotensin system in adult rat cardiomyocytes. We have shown a decrease in the

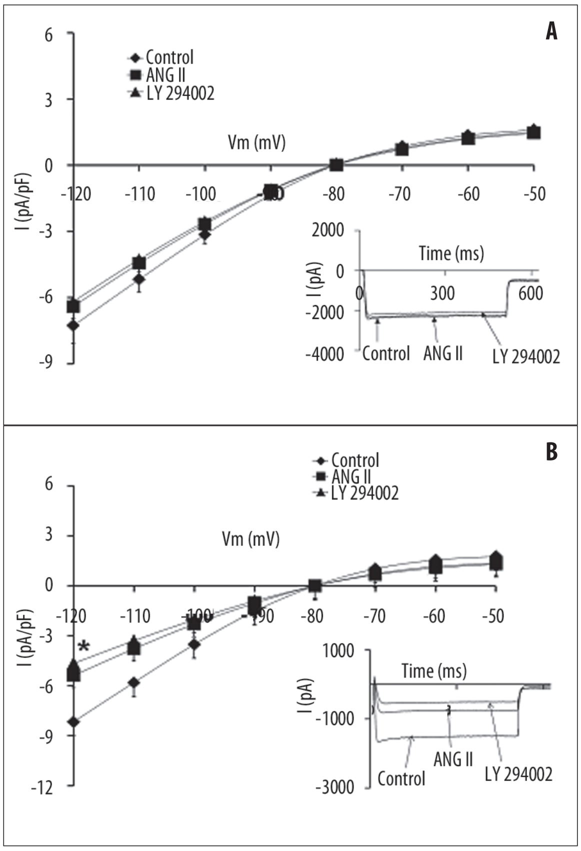

Figure 6. Effects of ANG II (10^{-6} M) and PI3-K inhibitor, LY 294002 (1 µM), on I_{K1} voltage relationship for normal (**A**) and hypertrophied (**B**) cardiomyocytes, pretreated with captopril for 14 days. The inset in each graph shows the respective representative current responses at and –120 mV for I_{K1}. Data are presented as average current density ±SEM with n=4–6. * $P<0.05$ Control *vs.* ANG II; # $P<0.05$ Control *vs.* LY 294002.

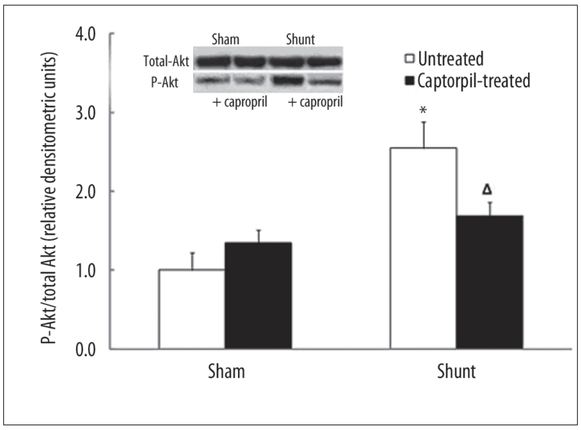

Figure 7. Effects of IGF-1 (10^{-8} M) on the activation level of Akt in untreated (white bars) *vs.* captopril-treated (black bars) of sham and hypertrophied hearts. Data are expressed as the ratio of phosphorylated over total protein normalized to control untreated hearts. The inset shows representative western blot of total and phosphorylated Akt. The data are presented as average ±SEM with n=3 (from different heart samples). * $P<0.05$ Sham *vs.* Shunt; Δ $P<0.05$ Captopril-treated *vs.* Untreated.

current density of both, the delayed outward rectifier as well as the inward rectifier potassium currents during hypertrophy. Interestingly, ANG II did not affect either current in normal cardiomyocytes; however, it increased both I_K and I_{K1} in hypertrophied cardiomyocytes through a PI3-K/Akt-dependent signaling mechanism. Treatment with an angiotensin converting enzyme inhibitor reversed ANG II effects on I_K and I_{K1} and alleviated the role of Akt on both currents.

Volume-overload-induced cardiac hypertrophy is associated with a reduction in the activity levels of the inward rectifier, I_{K1}, and the outward rectifier, I_K, potassium current densities, which is in agreement with our previous findings as well as others [16,17]. This seems to corroborate with the known lengthening of the action potential duration, a hallmark of cardiac hypertrophy [18–20]. Our new findings indicate an association between the regression of cardiac hypertrophy and an increase in the activity of I_K after captopril treatment. Thus, we speculate that normalization of the inotropic state of the hypertrophied cardiomyocytes could be related to the improvement of I_K activity; whereby higher current density would help shorten the unusually prolonged action potential duration. Interestingly, captopril treatment did not affect the basal activation level of I_{K1}, which does not seem to be readily pharmacologically altered. Thus, it is expected that captopril reduces the contractile burden on the heart without affecting its resting potential level.

Angiotensin II is a vasoactive peptide proven to evoke positive inotropic responses in cardiac function as well as increase smooth muscle contraction. We have previously reported a major role of PKC in mediating the ANG II contractile effects in the normal heart cells, through alteration of calcium channels and homeostasis in the cardiac cells [21–23]. There are very few reports found in the literature on the effects of ANG II on the potassium channels, nevertheless Gassanov and colleagues [24], showed no effect of ANG II on the normal cardiomyocytes. This corroborate well with our finding in this report. We have found no effect of ANG II as well as no effect of the converting enzyme inhibitor on I_K and I_{K1} in normal cardiomyocytes. Thus, the renin-angiotensin system does not seem to have a direct effect on the potassium channels in the normal heart [25]. Interestingly and on the contrary, in the volume-overload-induced hypertrophied cardiomyocytes we have found that ANG II increased the current densities of I_K and I_{K1} as well as their respective slope conductance. As a consequence, ANG II may shorten the action potential duration and reduces the resting potential of the hypertrophied cardiomyocytes. Such effects could exacerbate the condition of the hypertrophied heart and contribute to the progression from the compensated to the uncompensated state leading to heart failure. Treatment of hypertrophied cardiomyocytes with an angiotensin converting enzyme regressed the cardiac hypertrophy towards normal and reverted the deleterious effects of ANG II on I_K and I_{K1}. In fact, this reduction of the activities of both potassium currents, may contribute to the improvement of the inotropic state of the cardiac cells after captopril treatment. A novel and exciting finding in this study stems from our experiment showing that ANG II effects on I_K and I_{K1} in hypertrophied hearts is mediated by the PI3-K/Akt signaling pathway. This PI3-K/Akt dependency is disengaged for both channels after treatment with angiotensin converting enzyme inhibitor. This is in agreement with previous reports indicating ANG II effects may be partially mediated via the activation of the anti-apoptotic pathway PI3-K/Akt [17,26–29]. It should be noted that captopril treatment did reduce the activation level of Akt in the regressed hearts, which may indicate an uncoupling of the Akt signaling from the potassium channels.

CONCLUSIONS

In this study we have shown an important association between I_K and I_{K1} activities and the development of eccentric cardiac hypertrophy that is modulated by the renin-angiotensin system partially through PI3-K/Akt intracellular signaling pathway. This is particularly important as the degradation in the Akt signaling not only affects the electrical activity of the cardiomyocytes but also enhances apoptotic pathways [30–32]. This may play a pivotal role in the transition from the compensated to the uncompensated phase of cardiac hypertrophy and failure. Future work on the regulation of the anti-apoptotic PI3-K/Akt during eccentric hypertrophy with emphasis on its dual role may establish an interesting drug therapy which can delay/prevent the deleterious transition of the hypertrophied heart into failure.

Disclosures

None.

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