Differential ERK1/2 Signaling and Hypertrophic Response to Endothelin-1 in Cardiomyocytes from SHR and Wistar-Kyoto Rats: A Potential Target for Combination Therapy of Hypertension

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Abstract: Extracellular signal regulated kinase½ (ERK1/2) signaling is critical to endothelin-1 (ET-1)-induced cardiomyocyte hypertrophy. This study was to investigate ERK1/2 signaling and hypertrophic response to ET-1 stimulation in cardiomyocytes (CMs) from spontaneous hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Primary neonatal SHR and WKY CMs were exposed to ET-1 for up to 24 hrs. Minimal basal ERK1/2 phosphorylation was present in WKY CMs, while a significant baseline ERK1/2 phosphorylation was observed in SHR CMs. ET-1 induced a time- and dose-dependent increase in ERK1/2 phosphorylation in both SHR and WKY CMs. However, ET-1-induced ERK1/2 activation occurred much earlier with significantly higher peak phosphorylation level, and stayed elevated for longer duration in SHR CMs than that in WKY CMs. ET-1-induced hypertrophic response was more prominent in SHR CMs than that in WKY CMs as reflected by increased cell surface area, intracellular actin density, and protein synthesis. Pre-treatment with ERK1/2 phosphorylation inhibitor PD98059 completely prevented ET-1-induced ERK1/2 phosphorylation and increases in cell surface area and protein synthesis in SHR and WKY CMs. The specific PI3 kinase inhibitor LY294002 blocked ET-1-induced Akt and ERK1/2 phosphorylation, and protein synthesis in CMs. These data indicated that ERK1/2 signaling was differentially enhanced in CMs, and was associated with increased cardiac hypertrophic response to ET-1 in SHR. ET-1-induced ERK1/2 activation and cardiac hypertrophy appeared to be mediated *via* PI3 kinase/Akt signaling in SHR and WKY. The differential ERK1/2 activation in SHR CMs by ET-1 might represent a potential target for combination therapy of hypertension.

Keywords: Akt, cardiomyocyte hypertrophy, endothelin, ERK1/2, hypertension, spontaneous hypertensive rat.

INTRODUCTION

Endothelin-1 (ET-1) is a multifunctional hormone that plays an important role in the control of peripheral vascular tone and the development of hypertension, as well as cardiac hypertrophy [1, 2]. Cardiac hypertrophy is a compensatory response of the heart to stresses such as mechanical stretch and sustained neurohormonal stimulation or growth factor release. It is characterized by excessive enlargement of cardiomyocytes (CMs) and progressive interstitial fibrosis [3]. Maladaptive cardiac hypertrophy could progress to congestive heart failure, a leading cause of morbidity and mortality in the world. Under normal situation, although CMs only account for 30% of the total cell number in the heart ventricle, they represent over 70% of the total ventricular mass [4].

Therefore, ventricular CMs play a critical role in ventricular remodeling during the progression of cardiac hypertrophy [5]. ET-1 is one of important factors involved in myocardial hypertrophy. It has been demonstrated that ET-1 induces significant hypertrophic changes of CMs in cultured neonatal rat CMs [6, 7]. *In vivo* studies have shown that ET-1 level is significantly elevated in plasma and myocardium in patients with hypertension and in spontaneously hypertensive rats (SHR); while prolonged suppression of endothelin prevents the development of hypertension and cardiac hypertrophy in SHR [8, 9].

Extracellular signal regulated kinase ½ (ERK1/2) belongs to the subfamily of mitogen-activated protein kinase (MAPK). ERK1/2 is activated by a variety of extracellular stimuli including ET-1, angiotensin II (AngII), and phenylephrine (PE), and is involved in the regulation of various biological functions like cell proliferation and differentiation [2, 10]. Recent observations indicate that ERK1/2 also plays a key role in the development of cardiac hypertrophy in both *in vitro* and *in vivo* experiments [11-13]. It is reported that suppression of MAPK signaling attenuates PE-induced hypertrophic response

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in cultured rat CMs [14]. ERK activities in cardiac muscle are found to be chronically increased in hypertensive rats from the onset of hypertension to the establishment of cardiac hypertrophy, although the role of ERK1/2 signaling in cardiac hypertrophy *in vivo* still remains to be established [15, 16].

The regulatory mechanisms for ERK1/2 signaling in hypertrophic myocardium have not been fully understood. A number of factors are involved in the activation of ERK1/2 including intracellular Ca^{2+}, protein kinase C (PKC), and phosphoinositide 3-kinases (PI3 kinase) [17]. ET-1 is shown to initiate hypertrophic changes in CMs from Wistar-Kyoto rats (WKY) and Sprague-Dawley rats (SD) through ERK signaling pathway [18]. SHR rats develop sustained hypertension at their early age with massive left ventricular hypertrophy that is similar to the changes in patients with hypertensive heart diseases [19]. However, the mechanisms for cardiac hypertrophy remain largely unclear in SHR rats. In the present study, we evaluated ERK1/2 signaling and hypertrophic response to ET-1 stimulation in cultured neonatal CMs from SHR in comparison with its progenitor strain, normotensive WKY. PI3 kinase/Akt signaling was also examined in this study.

METHODS

Isolation and Culture of Cardiac Myocytes

The experimental protocol in this study followed the "Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health", and was approved by the Experimental Animal Usage Ethics Committee of Shanghai Jiao-Tong University School of Medicine (SCXK2007-0005). Primary cultures of ventricular CMs were prepared from 1-2 day old neonatal SHR and WKY rats using the method previously described with minor modifications [20, 21]. Briefly, CMs were dispersed from the neonatal rat ventricles by a series of incubation at 37 ºC in D-hanks solution containing 1.0 mg/ml trypsin (1:250, Difco Laboratories). The dispersed cells were then cultured at a density of 3×10^5 cells per cm^2 for 90 min (95% air / 5% CO_2 at 37 ºC) to eliminate the fibroblasts. The unattached cells were transferred to, and cultured on collagen-coated plates at a density of 1.25×10^5 cells per cm^2 in maintenance media consisting of 79% Modified Eagle's Medium (MEM), 20% fetal bovine serum (Hyclone), and 1% penicillin/streptomycin solution (Gibco). After 48 hrs of culture, over 99% of the cells were CMs as estimated by immunocytochemical staining for the sarcomeric protein alpha-actin. The cells were then maintained in serum-free MEM for another 24 hrs, and divided into four treatment groups: control, cells treated with ET-1 (10 nM) alone, cells treated with ET-1 in the presence of ERK1/2 inhibitor PD98059 (50 μM), and cells treated with ET-1 in the presence of PI3 kinase inhibitor LY294002 (10 μM).

Immunocytochemistry and Microscopic Measurement of Cell Surface Area

The cells seeded in 6-well plates were stimulated with ET-1 for 24 hrs in the absence or presence of PD98059 or LY294009, and then fixed for 20 min at room temperature with 4% formaldehyde after rinsing with PBS. The cells

were then permeabilized with 0.3% Triton X-100 in PBS, and exposed to the primary antibody (mouse anti-alpha actin monoclonal antibody, Calbiochem) with dilution factor of 1:200 in 1% BSA/PBS at 37 ºC for 1 hr, followed by incubation with peroxidase-conjugated secondary antibody (diluted 1:100 in 1% BSA/PBS). The nuclei of the cells were stained with hematoxylin. The microphotographs were scanned by the computerized-image analyzing system (Zeiss). The sizes of CMs were determined by measuring the areas of actin-positive cells. For each slide, the mean cell area was determined by dividing the total actin-positive area in three microphotographs (×10) by the number of independent myocyte nuclei (bi-nucleated cells counted as one cell).

Actin Staining

To visualize myofibrillar organization, fluorescent phalloidin staining was performed as described previously [18, 22]. After 24 hrs of culture in serum-free media, CMs were stimulated with ET-1 for 24 hrs. The cells on chamber slides were then prepared for staining by incubation with rhodamine-conjugated phalloidin for 1 hr in the dark. The preparations were readily examined and photographed using a confocal fluorescent microscope (Zeiss LSM 510) to determine the cytoskeleton actin organization.

[3H]-leucine Incorporation Assay

Protein synthesis was assessed using [3H]-leucine incorporation into CMs as described [23]. Briefly, the cells in 24-well plates were pretreated with PD98059 or LY294009 for 1 hr, and then exposed to ET-1 for 24 hrs. [3H]-leucine (1 μCi/ml or 3.7×10^4 Bq, Beijing Atom High Tech Co, Ltd) was added into the culture media at the same time as ET-1. The cells were then collected for the assay as described. The radioactivity incorporated into proteins of the cells in each group was determined by beta-liquid scintillation counting (Beckman; LS 6500).

Western Blot Analysis for ERK1/2 and Akt

CMs were stimulated with ET-1 (10 nM) for different time (0, 5, 10, 15, 30, 60 min) or with different doses (0, 5, 10, 20 nM) for 10 min with or without the treatment of PD98059 or LY294009, and then harvested for Western blot analysis as described previously [24]. The samples (12 μg) were separated on 10% SDS–PAGE, and transferred to a polyvinylidene difluoride membrane. The preparations were then incubated with primary antibodies (1:1000) against phosphor-ERK1/2 and Akt (Cell Signaling) for 2 hrs at room temperature. Subsequently, the preparations were exposed to horseradish peroxidase-conjugated IgG (1:2000 dilution, Protein Tech Group, Inc) for 1 hr at room temperature. The phosphorylated- and total- ERK1/2 or Akt were visualized by Western Lighting Chemiluminescence Reagent Plus (PerkinElmer Life Sciences, Inc). The intensity of individual band was analyzed using Image Qwin Software (Leica).

Statistical Analysis

All the data were presented as means ± SEM of "n" independent experiments. The data on cell surface measurement

and [3H]-leucine incorporation assay were analyzed using ANOVA followed by Tukey post hoc multiple comparison tests. Western blot data were analyzed with Student's t-test. A p value of 0.05 or less was considered statistically significant.

RESULTS

ERK1/2 Signaling was Enhanced at Baseline and in Response to ET-1 in CMs from SHR

There was a low basal level of ERK1/2 phosphorylation in CMs from WKY rats as determined with Western blot (Fig. **1A**). When exposed to ET-1, a time- and dose-dependent increase in ERK1/2 phosphorylation was observed in these cells. ERK1/2 phosphorylation reached its maximal level after 10 min incubation with ET-1. Exposure to ET-1 beyond 10 min or ET-1 concentration over 10 nM resulted in a rapid decline in ERK1/2 phosphorylation, and returned to baseline by 60 min (Fig. **1A & B**). In contrast, a significant basal ERK1/2 phosphorylation was observed in CMs from SHR. When treated with ET-1, ERK1/2 phosphorylation increased rapidly, and reached its peak level by 5 min of ET-1 exposure. The elevated level of ERK1/2 activation dwindled slowly after 10 min, and remained at a high

level after 60 min. The dose-response curve revealed that ERK1/2 activation started to occur at lower ET-1 dose in CMs from SHR as compared with the cells from WKY (Fig. **1B**). The maximal level of ET-1-induced ERK1/2 activation in CMs from SHR was almost twice that in CMs from WKY. These results indicated that ET-1 induced an enhanced and sustained ERK1/2 phosphorylation in CMs from SHR compared to the ones from WKY.

ET-1 Induced an Increased Hypertrophic Response in CMs from SHR

As expected, when CMs were exposed to ET-1, the cells exhibited a symmetric growth with a significant increase in cell surface area (Fig. **2A & B**). The cell surface area was increased by 40% and 30% for CMs from SHR and WKY rats, respectively, after ET-1 treatment (n=3, $p < 0.01$). The ET-1-induced increase in cell surface area was accompanied by an enhanced density of intracellular actin in the cells as evaluated by fluorescent phalloidin staining (Fig. **2C**). The hypertrophic response to ET-1 was supported by increased protein synthesis as evaluated using [3H]-leucine incorporation assay. There was a low level of basal protein synthesis in the cells from both SHR and WKY rats without ET-1
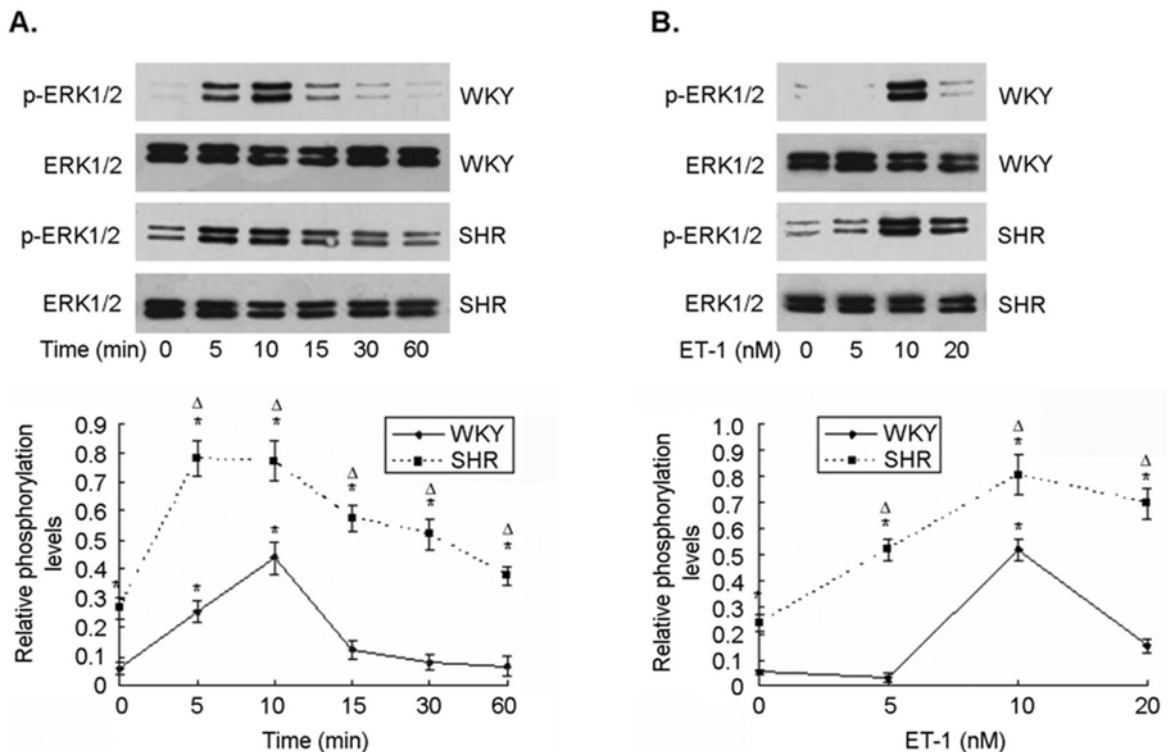

Fig. (1). ERK1/2 activation was elevated in CMs from SHR at both baseline and in response to ET-1 stimulation. Primary neonatal SHR and WKY CMs were prepared, and exposed to different concentrations of ET-1 for up to 24 hrs. There was minimal basal ERK1/2 phosphorylation in CMs from WKY. In contrast, a significant level of basal ERK1/2 phosphorylation was observed in CMs from SHR. ET-1 induced a time- and dose-dependent increase in ERK1/2 phosphorylation in CMs from both SHR and WKY. However, ET-1-induced ERK1/2 phosphorylation occurred significantly earlier with higher maximal level of activation, and stayed elevated for longer time in CMs from SHR as compared with WKY. A representative Western blotting was shown in the top panel. The relative ERK1/2 phosphorylation in the cells from three independent experiments was summarized in the bottom panel.
*$p < 0.05$ *vs* baseline in the same group; Δ$p < 0.05$ *vs* WKY rats.
(A) Time-dependent activation of ERK1/2 in CMs by ET-1 (10 nM).
(B) Dose-dependent activation of ERK1/2 in CMs by ET-1 for 10 min.
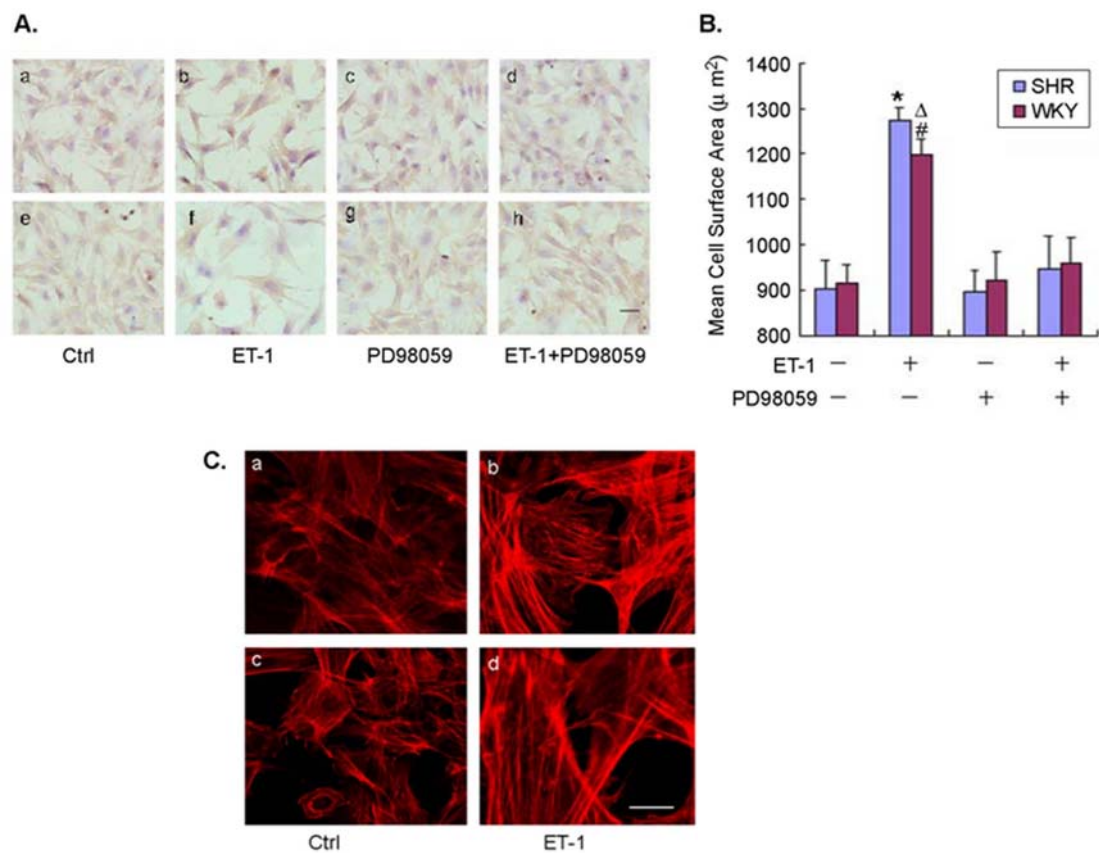
Fig. (2). Enhanced hypertrophic response of CMs from SHR to ET-1 stimulation.

(A) Morphological changes of CMs to ET-1 stimulation. Cultured neonatal myocardial cells from SHR (**a-d**) and WKY rats (**e-h**) were stained with antibody against alpha-actin with nuclei stained with hematoxylin. The size of the cells especially in the ones from SHR was enlarged after ET-1 treatment in both SHR and WKY rats (**b**, **f**) compared to control (**a**, **e**). When pre-treated with PD98059, no cell enlargement was observed in response to ET-1 exposure (**d**, **h**). PD98059 had no impact on the cell size without ET-1 stimulation (**c**, **g**). Scale bar in "h" represented 20 μm. Each microscopic view represented a typical field of three independent experiments that were very reproducible.

(B) Summarized cell surface area for CMs after treatment with ET-1. The cell surface area was significantly increased by ET-1 stimulation in CMs from both SHR and WYK. However, the cell surface area induced by ET-1 was increased to a greater extent in CMs from SHR than that in CMs from WKY. Data were presented as mean ± SEM (n=3). *$p < 0.01$ *vs* SHR control; #$p < 0.01$ *vs* WKY control; $^{\Delta}$$p < 0.05$ *vs* WKY rats.

(C) Myofibrillar organization in CMs after stimulation by ET-1. The actin density was significantly increased in the cells treated with ET-1 especially in CMs from SHR (b). Scale bar in "d" represented 30 μm. Each microscopic view represented a typical field of three independent experiments that were easily reproducible.

(**a**) Untreated SHR myocardial cells;

(**b**) SHR myocardial cells treated with 10 nM ET-1;

(**c**) Untreated WKY myocardial cells;

(**d**) WKY myocardial cells treated with 10 nM ET-1.

stimulation that was significantly increased by 80% and 62% for CMs from SHR and WKY rats (p < 0.05, n=3, Fig. **4**), respectively. These data demonstrated that ET-1 induced a greater hypertrophic response with increased cell surface area and protein synthesis in CMs from SHR than that in the cells from WKY rats.

Inhibition of ERK1/2 Signaling Prevented ET-1-Induced Cardiomyocyte Hypertrophy

To further investigate the role of ERK1/2 signaling in ET-1-induced cardiomyocyte hypertrophy, the cells were pre-treated with the specific ERK1/2 phosphorylation inhibitor PD98059. As expected, treatment with PD98059 completely prevented the ET-1-induced ERK1/2 phosphorylation in CMs

from both SHR and WKY rats as analyzed with Western blot (Fig. **3**). PD98059 treatment had no effect on the cell surface area in the control cells (no exposure to ET-1) (Fig. **2B**). However, pre-treatment with PD98059 totally blocked the ET-1-induced increase in cell surface area in CMs from both SHR and WKY rats (Fig. **2B**). The surface area of the cells treated with ET-1 in the presence of PD98059 was the same as that of control cells. Similarly, when the cells were pre-treated with PD98059, the ET-1-induced increase in [^{3}H]-leucine incorporation was completely reversed in CMs from both SHR and WKY rats (Fig. **4**). Of note, treatment with PD98059 had no impact on the basal [^{3}H]-leucine incorporation in CMs from either SHR or WKY rats. These results further suggested that ET-1-induced hypertrophy was mediated through ERK1/2 signaling in both SHR and WKY rats.
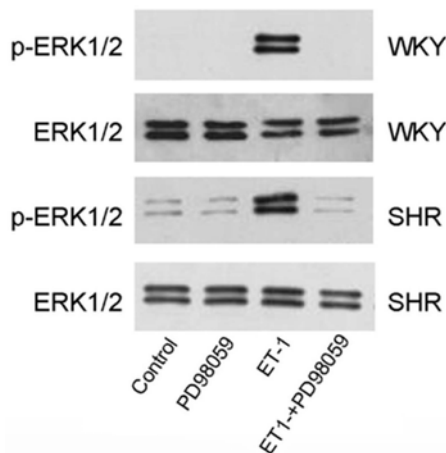
Fig. (3). ET-1-induced phosphorylation of ERK1/2 was blocked by PD98059.
ERK1/2 phosphorylation was significantly increased by ET-1 in the neonatal CMs from both SHR and WKY rats. Pretreatment of the cells with PD98059 (50 μM) for 30 min completely prevented ET-1-induced increase in ERK1/2 phosphorylation as evaluated with Western blotting.
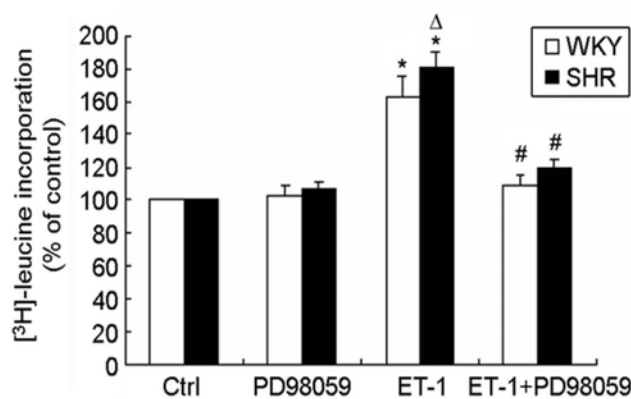

Fig. (4). The specific ERK1/2 kinase inhibitor PD98059 suppressed ET-1-induced protein synthesis in CMs. Enhanced protein synthesis by ET-1 was observed in CMs in SHR and WKY rats, especially in the cells from SHR. Pre-treatment of the cells with PD98059 completely blocked ET-1-induced protein synthesis as evaluated with [^{3}H]-leucine incorporation assay. Data were presented as means ± SEM of three different experiments (each in triplicate).
*$p < 0.05$ *vs* control; #$p < 0.05$ *vs* ET-1-treated cells; Δ $p < 0.05$ *vs* WKY rats.

Inhibition of PI3 kinase/Akt Signaling Attenuated ET-1-induced ERK1/2 Activation and Protein Synthesis

To determine the involvement of PI3 kinase/Akt signaling in ET-1-induced ERK1/2 phosphorylation and protein synthesis, CMs from both SHR and WKY rats were pre-treated with specific PI3 kinase inhibitor LY294002 for 30 min prior to exposure to ET-1. There was detectable level of Akt phosphorylation in CMs from both SHR and WKY rats at baseline that was significantly increased when exposed to ET-1 in a time-dependent manner (Fig. **5**). The maximal
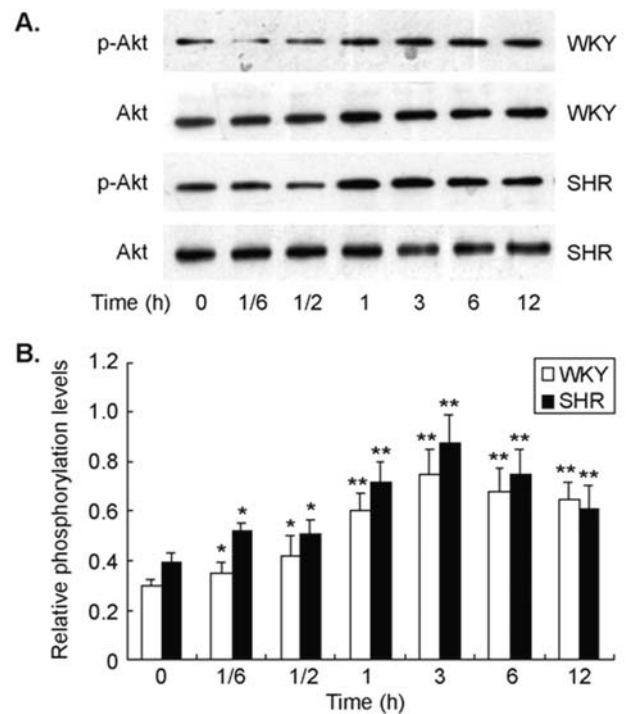

Fig. (5). ET-1 stimulated Akt phosphorylation in cultured CMs.
There was a low level of Akt phosphorylation in CMs that was significantly increased by ET-1 stimulation in a time-dependent manner in both SHR and WKY rats. ET-1-induced Akt phosphorylation reached maximal level at 3 hrs after stimulation in the cells.
Top panel: representative Western blots; Bottom: band density presented as mean ± SEM of three independent experiments. *$p < 0.05$; **$p < 0.01$ *vs* control.

ET-1-induced Akt activation occurred after 3 hrs of stimulation, and slowly declined afterwards. The pattern of ET-1-induced Akt activation was similar in the cells from both SHR and WKY rats. When the cells were pre-treated with LY294002, the baseline Akt phosphorylation was not affected. However, the ET-1-induced increase in Akt phosphorylation was completely reversed to the baseline level (Fig. **6A**). Interestingly, pre-treatment of CMs with LY294002 also completely prevented ET-1-induced ERK1/2 phosphorylation in both SHR and WKY rats (Fig. **6B**), suggesting that PI3 kinase / Akt signaling is crucial to ET-1-induced ERK1/2 activation in these cells. In parallel to ERK1/2 activation, pre-treatment of CMs with LY294002 also inhibited ET-1-induced protein synthesis as evaluated with [^{3}H]-leucine incorporation assay (Fig. **6C**). These data indicate that PI3 kinase / Akt /ERK1/2 signaling plays an important role in ET-1-induced hypertrophy of CMs in both SHR and WKY rats.

DISCUSSION

ET-1 is a 21-amino acid peptide that is abundantly expressed in CMs, vascular endothelial cells, and vascular smooth muscle cells primarily as a local paracrine/autocrine hormone [25]. ET-1 plays an important role in the patho-
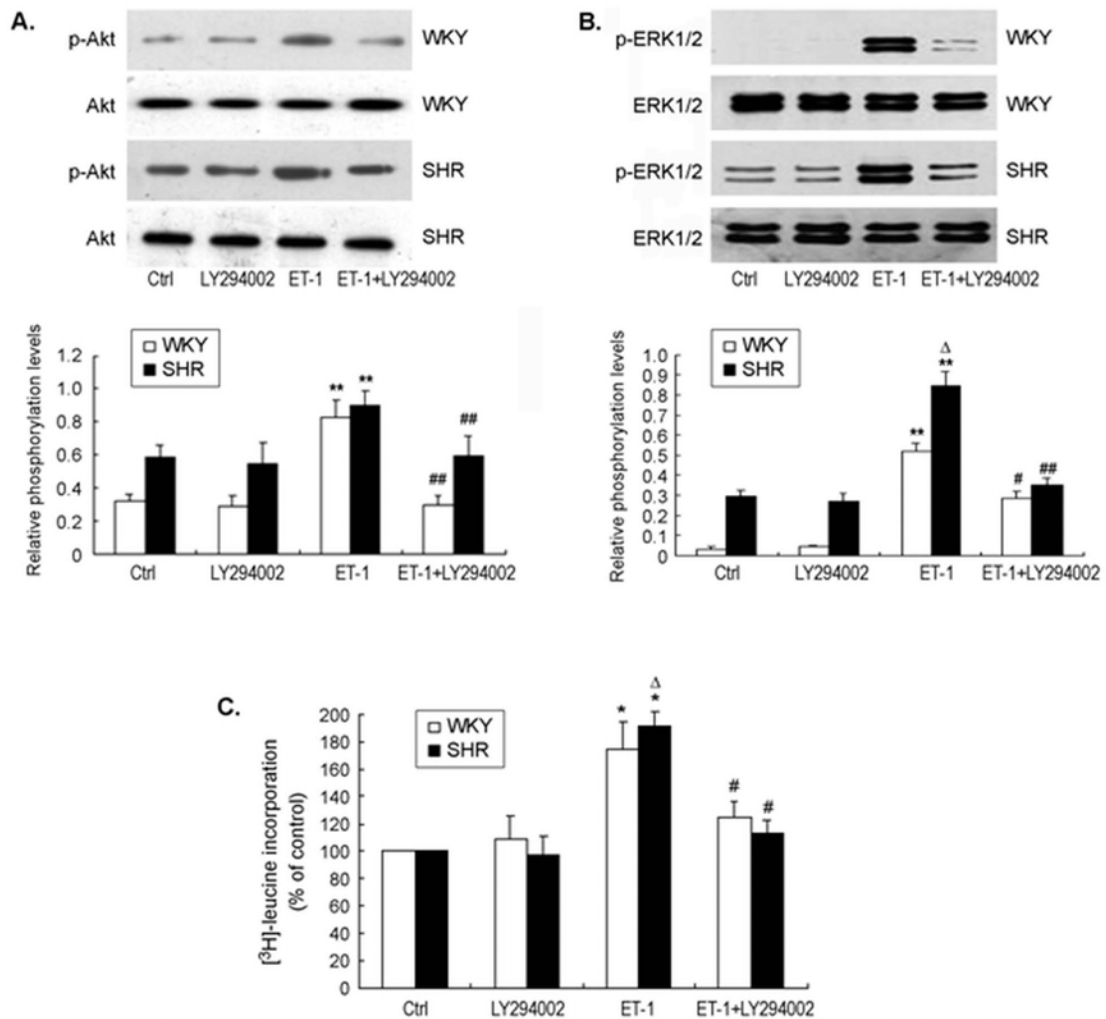
Fig. (6). Inhibition of PI3 kinase / Akt signaling prevented ET-1-induced ERK1/2 phosphorylation and protein synthesis in CMs from SHR and WKY rats.

A. ET-1-induced Akt phosphorylation was blocked by the specific PI3 kinase inhibitor LY294002 (10μM) in CMs from both SHR and WKY. Top: representative Western blot of Akt phosphorylation; Bottom: summarized band density presented as means ± SEM of three experiments. [**]$p < 0.01$ *vs* control, [##]$p < 0.01$ *vs* ET-1-treated group.

B. ET-1-induced ERK1/2 phosphorylation in CMs from both SHR and WKY was suppressed by the specific PI3 kinase inhibitor LY294002. Top: representative Western blot of ERK1/2 phosphorylation; Bottom: summarized band density presented as means ± SEM of three experiments. [**]$p < 0.05$, *vs* control; [#]$p < 0.05$; [##]$p < 0.01$ *vs* ET-1-treated cells; [Δ]$p < 0.05$ *vs* WKY rats.

C. ET-1-induced increase in [³H]-leucine incorporation in CMs from both SHR and WKY was completely inhibited by pre-treatment of the cells with LY294002 for 30 min. [*]$p < 0.05$ *vs* control; [#]$p < 0.05$ *vs* ET-1- treated cells; [Δ]$p < 0.05$ *vs* WKY rats.

genesis of a variety of diseases such as hypertension, atherosclerosis, and acute myocardial infarction [26-28]. ET-1 also contributes to the development of cardiac hypertrophy [6]. This was confirmed in the present study as evidenced by increased cell surface area, enhanced intracellular actin density, and protein synthesis in the neonatal CMs from both SHR and WKY rats. We also demonstrated that ET-1-induced myocardial cell hypertrophy was mediated through PI3 kinase / Akt / ERK1/2 signaling pathway in neonatal cardiac myocyte preparations.

It is well established that ERK1/2 signaling is important in ET-1-induced cardiac hypertrophy [13, 17, 29]. However, there is no previous report that showed ERK1/2 signaling was involved in cardiac hypertrophy induced by ET-1 in

SHR. For the first time, we observed that the level of ERK1/2 phosphorylation was significantly enhanced in CMs from SHR at baseline, and remained at high level after ET-1 stimulation compared to the cells from WKY rats. The enhanced ERK1/2 activation in response to ET-1 stimulation was associated with augmented ET-induced hypertrophy as reflected with increased cell surface area and protein synthesis in CMs from SHR as compared with WKY. This may provide a new mechanism for the development of cardiac hypertrophy in SHR and possibly in patients with essential hypertension. Of note, the blood pressure in the neonatal SHR is not elevated until a few weeks after birth in these animals [30, 31]. The changes in ERK1/2 signaling occurred much earlier than the development of left ventricle hypertro-

phy and hypertension. This may provide a window for the prevention of ventricular hypertrophy in SHR and hypertensive patients by modifying ERK1/2 signaling pathway. Interestingly, increased activation of ERK1/2 by AngII stimulation was also observed in vascular smooth muscle cells from SHR as compared with WKY [32, 33]. Inhibition of ERK1/2 activity with PD98059 improved endothelial function and reduced arterial reactivity to Ang II in SHR *in vivo*, and blocked vascular smooth muscle cell growth responses to AngII *in vitro* [32, 34].

The role of Akt signaling in ET-1-induced cardiac hypertrophy is controversial although it is important to physiological cardiac growth and cardiac hypertrophy induced by other growth factors like connective tissue growth factor [35, 36]. It has been recently shown that isolated CMs exhibit an enhanced protein synthesis in response to ET-1 stimulation with increased incorporation of [^{3}H]-leucine into CMs from the Akt1 knock-out mice. Interestingly, the Akt1 knock-out animals develop an exacerbated form of cardiac hypertrophy in the setting of aortic constriction [35], indicating that Akt1 inhibits cardiac hypertrophy in this animal model. The level of phosphorylated Akt was not increased in isolated neonatal CMs from WKY rats [37]. In our study, however, the data clearly showed that ET-1 stimulated Akt activation in CMs from both SHR and WKY rats. Our results also indicated that PI3 kinase / Akt signaling was involved in ET-induced cardiac hypertrophy *in vitro* as evidenced by the fact that the specific PI3 kinase inhibitor LY294002 prevented ET-induced protein synthesis. The reason(s) for the apparent opposite roles of Akt signaling in cardiac hypertrophy is very likely due to the different models used. Further studies are needed to clarify the difference in the role of Akt signaling in ET-1-induced cardiac hypertrophy.

One of the major findings in the present study is that inhibition of Akt signaling with LY294002 also led to the suppression of ET-1-induced ERK1/2 activation in CMs from both SHR and WKY rats. It is very likely that the hypertrophic response of CMs to ET-1 is through PI3 kinase / Akt / ERK1/2 signaling cascade in both SHR and WKY rats. It is known that PI3 kinase is involved in the regulation of a number of cell functions including membrane trafficking, actin rearrangement and adhesion, as well as cell survival [38]. PI3 kinase also plays a critical role in cardiac growth and contractility [39, 40]. Recent studies demonstrate that PI3 kinase is involved in maladaptive cardiac hypertrophy triggered by neurohormonal mediators and biomechanical stress [41]. The mechanisms for PI3 kinase-mediated cardiac growth and hypertrophy are believed to be associated with the activation of heterotrimeric G-protein-coupled receptors, growth factors or hormones [40, 41]. PI3 kinase / Akt signaling is another major pathway for PI3 kinase function including cell survival and proliferation [42, 43]. There are extensive cross-talking and interactions between PI3 kinase / Akt pathway and ERK pathways on the regulations of a variety of biological events [44-47]. ERK pathway is frequently co-activated along with PI3 kinase / Akt pathway, and functioning synergistically [44, 47]. In our study, the specific PI3 kinase inhibitor LY294002 blocked ET-1-induced Akt phos-

phorylation and ERK1/2 activation, as well as protein synthesis in CMs, suggesting that ET-1-induced cardiac hypertrophy is mediated through PI3 kinase Akt / ERK1/2 signaling pathway in SHR and WKY rats. However, the mechanism for ET-1-induced PI3 kinase activation is unclear. It is possible that ET-1 stimulates PI3 kinase directly [19].

In conclusion, this study demonstrated that ERK1/2 signaling was significantly enhanced both at baseline and in response to ET-1 stimulation in isolated neonatal CMs in SHR as compared with WKY rats. The elevated ET-1 signaling was associated with increased hypertrophic response to ET-1 stimulation in CMs in SHR. PI3 kinase / Akt signaling was involved in ET-1-induced ERK1/2 activation and cardiac hypertrophy in CMs from both SHR and WKY rats. The data from this study might provide a molecular explanation for the development of cardiac hypertrophy in SHR, and help to identify potential molecular targets for prevention and treatment of cardiac hypertrophy and combination therapy of hypertension.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

LIST OF ABBREVIATIONS

AngII	=	Angiotensin II
BSA	=	Bovine Serum Albumin
CMs	=	Cardiomyocytes
ERK1/2	=	Extracellular Signal Regulated Kinase½
ET-1	=	Endothelin-1
MAPK	=	Mitogen-Activated Protein Kinase
PE	=	Phenylephrine
PI3	=	Phosphoinositide 3-Kinases
PKC	=	Protein Kinase C
SHR	=	Spontaneous Hypertensive Rat
WKY	=	Normotensive Wistar-Kyoto Rat

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