Angiotensin II activates myostatin expression in cultured rat neonatal cardiomyocytes via p38 MAP kinase and myocyte enhance factor 2 pathway

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Abstract

Angiotensin II (AngII) plays a critical role in cardiac remodeling and promotes cardiac myocyte hypertrophy. Myostatin, a negative regulator of muscle growth, is increased in hypertrophied and infarcted heart. The direct effect of AngII on cardiac myocyte myostatin expression has not been previously investigated. We hypothesized that myostatin may act as a cardiac endocrine inhibitor for AngII. AngII-induced myostatin protein expression in cultured rat neonatal cardiomyocytes was dose-dependent. AngII significantly increased myostatin protein and mRNA expression in a time-dependent manner. Addition of losartan, SB203580, or p38 siRNA 30 min before AngII stimulation significantly

blocked the increase of myostatin protein by AngII. AngII significantly increased phosphorylation of p38 while SB203580 and losartan attenuated the phosphorylation of p38 induced by AngII. AngII increased, while myostatin-Mut plasmid, SB203580, losartan, and myocyte enhance factor 2 (MEF-2) antibody abolished the myostatin promoter activity. Co-stimulation with myostatin and AngII significantly inhibited the protein synthesis induced by AngII. In conclusion, AngII enhances myostatin expression in cultured rat neonatal cardiomyocytes. The AngII-induced myostatin is mediated through p38 MAP kinase and MEF-2 pathway.

Journal of Endocrinology (2008) **197**, 85–93

Introduction

Angiotensin II (AngII) plays a critical role in cardiac remodeling and promotes cardiac myocyte hypertrophy (Schnee & Hsueh 2000). Excess of AngII can lead to cardiac dysfunction and failure. Myostatin is a transforming growth factor- β family member that plays an essential role in regulating skeletal muscle growth (McPherron *et al.* 1997, Lee & McPherron 2001). Like other transforming growth factor- β , myostatin is translated into a precursor protein that is proteolytically cleaved to yield the N-terminal, latency-associated peptide (LAP) and the C-terminal, mature processed peptide before secretion from the cell (Thies *et al.* 2001). Myostatin is expressed in fetal and adult hearts and its expression is upregulated in cardiomyocytes after infarction (Sharma *et al.* 1999). Recently, myostatin was found to be dramatically upregulated in hypertrophied hearts with transgenic overexpression of Akt (Cook *et al.* 2002). Since myostatin plays a role in limiting skeletal muscle growth, the upregulation of myostatin in hypertrophic heart may represent a negative feedback mechanism of myostatin in cardiac myocytes to inhibit the overgrowth of cardiac

myocytes. We have demonstrated that the myostatin gene is mechanically responsive in cardiac myocytes (Shyu *et al.* 2005). Mechanical stretch increases the release of AngII from cardiac myocytes (Sadoshima *et al.* 1993, Shyu *et al.* 2001). AngII mediates cardiac myocyte growth and myostatin is secreted by cardiac myocytes and accumulates until it reaches a threshold causing inhibition of cardiac growth. The direct effect of AngII on cardiac myocyte myostatin expression has not been previously investigated. An endocrine inhibitor is secreted by specific tissues and provides a negative feedback mechanism to control the size of the tissue producing it (Gaussin & Depre 2005). We hypothesized that myostatin may act as a cardiac endocrine inhibitor for AngII.

Myocyte enhance factor 2 (MEF-2) transcription factors are critically involved in the regulation of inducible gene expression during myocardial hypertrophy and MEF-2–DNA-binding activity is increased in the rat hearts by pressure or volume overload (Akazawa & Komuro 2003). The transactivation activity of MEF-2A and MEF-2C is stimulated by p38 MAP kinase (Zetser *et al.* 1999, Zhao *et al.* 1999). The myostatin gene upstream region contains MEF-2 site, and muscle-specific expression of myostatin appears to be regulated by MEF-2

(Spiller *et al.* 2002). Mechanical stretch induced myostatin expression via p38 MAP kinase and MEF-2 pathway (Shyu *et al.* 2005). Since AngII is secreted from cardiac myocytes by mechanical stretch, we hypothesized that myostatin-induced by AngII is via p38 MAP kinase and MEF-2 pathway.

Materials and Methods

Primary cardiac myocyte culture

Cardiac myocytes were obtained from Wistar rats, aged 2–3 days old, by trypsinization as previously described (Shyu *et al.* 2001). Cultured myocytes thus obtained were >95% pure as revealed by observation of contractile characteristics with a light microscope and stained with anti-desmin antibody (Dako Cytomation, Glostrup, Denmark). The study conforms to Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Various concentrations of AngII were added to the culture medium. To determine the roles of c-Jun N-terminal kinase (JNK), p38 MAP kinase, p42/p44 MAP kinase, or phosphatidylinositol-3 (PI-3) kinase in the expression of AngII-induced myostatin expression, myocytes were pretreated with SP600125 (20 μ M, Calbiochem, San Diego, CA, USA), SB203580 (3 μ M, Calbiochem), PD98059 (50 μ M, Calbiochem), or wortmannin (5 nM, Sigma Chemical) for 30 min, respectively, followed by addition of AngII. The SP600125 is a potent, cell-permeable, selective, and reversible inhibitor of JNK. The SB203580 is a highly specific, cell-permeable inhibitor of p38 kinase. The PD98059 is a specific and potent inhibitor of p42/p44 MAP kinase. Wortmannin is a potent and specific inhibitor of PI-3 kinase. In experiments involving the AngII receptor antagonist, losartan at 100 nM was added 30 min before AngII stimulation.

Western blot analysis

Western blot was performed as previously described (Shyu *et al.* 2004). Rabbit polyclonal anti-myostatin antibody (Chemicon, Temecula, CA, USA), polyclonal anti-p38 MAP kinase, monoclonal anti-phospho p38 MAP kinase antibodies (Cell Signaling, Beverly, MA, USA), goat polyclonal antibody against total MEF-2 (Santa Cruz Biotechnol Inc., Santa Cruz, CA, USA), and rabbit polyclonal antibody against phosphorylated MEF-2 (Santa Cruz Biotechnol Inc.) were used. Equal protein loading of the samples was verified by staining monoclonal antibody GAPDH or α -tubulin. All western blots were quantified using densitometry.

Northern blot analysis

Total RNA was prepared by solubilizing myocytes in Ultraspec RNA kit (Biotecx Laboratory Inc., Houston, TX, USA). Aliquots of 20 μ g total RNA were fractionated in formaldehyde agarose gels, transferred to Hybond-N⁺ nylon

membrane, and hybridized with [α ³²-P]dCTP-labeled cDNA probes, generated from mouse myostatin cDNA. The northern blot was performed as previously described (Shyu *et al.* 2001).

Electrophoretic mobility shift assay

Nuclear protein concentrations from cultured cardiomyocytes were determined by Bio-Rad protein assay. Consensus and control oligonucleotides (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) were labeled by polynucleotides kinase incorporation of [γ ³²P]-dATP. The oligonucleotides sequences included the MEF-2 consensus 5'-GATCGCTCTAAAAATA-ACCCTGTCG-3'. The MEF-2 mutant oligonucleotides sequences were 5'-GATCGCTGTAAACATAACCCTGTCG-3'. After the oligonucleotide was radiolabeled, the nuclear extracts (4 μ g protein in 2 μ l nuclear extract) were mixed with 20 pmol of the appropriate [γ ³²P]-dATP-labeled consensus or mutant oligonucleotide in a total volume of 20 μ l for 30 min at room temperature. The samples were then resolved on a 4% polyacrylamide gel. Gels were dried and imaged by autoradiography. Controls were performed in each case with mutant oligonucleotides or cold oligonucleotides to compete with labeled sequences.

Promoter activity assay

A-1977 to +32 bp mouse myostatin promoter construct (a gift from Prof WS Yang National Taiwan University, Taiwan) was generated. The myostatin promoter contains MEF-2 conserved sites (CTAAAAATAA) at -637 to -646 bp. For the mutant, the MEF-2 binding sites were mutated using the mutagenesis kit (Stratagene, La Jolla, CA, USA). Site-specific mutations were confirmed by DNA sequencing. Plasmids were transfected into cardiomyocytes using a low pressure-accelerated gene gun (BioWare Technologies, Taipei, Taiwan, ROC) essentially following the manufacturer's protocol. In brief, 2 μ g plasmid DNA were suspended in 5 μ l PBS and were delivered to the cultured myocytes at a helium pressure of 15 psi. The transfection efficiency using this method is 25%. Following 6 h of AngII stimulation, cell extracts were prepared using Dual-Luciferase Reporter Assay System (Promega) and measured for dual luciferase activity by luminometer (Turner Designs, Sunnyvale, CA, USA).

RNA interference

Neonatal cardiomyocytes were transfected with 800 ng p38 annealed siRNA oligonucleotide according to the manufacturer's instructions (Santa Cruz Biotechnology Inc.). The p38 siRNA is a target-specific 20–25 nt siRNA designed to knock down gene expression of p38 α and p38 β . As a negative control, a non-targeting siRNA (control siRNA) purchased from Dharmacon (Dharmacon Inc., Lafayette, CO, USA) was used. Neonatal cardiomyocytes were transfected with siRNA oligonucleotides using Effectene Transfection Reagent as

suggested by the manufacturer (Qiagen Inc). After incubation at 37 °C for 24 h, cardiac myocytes were stretched for 18 h, and subjected to analysis of western blot. The effect of p38 siRNA transfection was verified by the downregulation of p38 protein when compared with control siRNA.

Protein synthesis assay

Cardiomyocytes were cultured with serum-free medium in ViewPlate for 60 min (Packard Instrument Co., Meriden, CT, USA). AngII (10 nM) and myostatin (100 ng/ml) were added to the medium. The cells were then labeled with 100 µCi/ml [³⁵S]-methionine for various periods of time. Losartan, a selective antagonist of the AngII receptor, was added to the medium 30 min before addition of AngII. The cells were washed with PBS twice. Then, 50 µl MicroScint-20 were added and the plate was read with TopCount (Packard Instrument Co).

Statistical analysis

The data were expressed as mean ± s.d. Statistical significance was performed with ANOVA (GraphPad Software Inc., San Diego, CA, USA). The Tukey–Kramer comparison test was used for pairwise comparisons between multiple groups after ANOVA. $P < 0.05$ was considered to denote statistical significance.

Results

AngII enhances myostatin protein expression in cardiomyocytes

The western blot showed the three forms of myostatin detected by the polyclonal anti-myostatin antibody and the relative sizes of precursor, LAP, and processed myostatin in cardiomyocytes (Fig. 1). These data indicate that myostatin protein is synthesized in neonatal cardiomyocytes and that the precursor myostatin is processed in cardiomyocytes. AngII induced both precursor and processed myostatin. The levels of processed myostatin were used to represent the myostatin protein expression in the present study. The AngII-induced myostatin protein expression was dose-dependent. AngII at 10 nM showed the maximal effect to enhance myostatin protein expression in neonatal cardiomyocytes. Therefore, the concentration of AngII used for the following experiments is 10 nM. Addition of losartan (100 nM, an antagonist of the AngII receptor), 30 min before AngII treatment completely inhibited the increase of myostatin induced by AngII.

AngII increases myostatin protein and mRNA expression in cardiomyocytes

As shown in Fig. 2, exogenous addition of AngII at 10 nM for 18 h significantly increased the myostatin protein expression and the myostatin expression remained elevated up to 48 h.

Figure 1 Angiotensin II (AngII) increases myostatin expression in cardiomyocytes. (A) Representative western blots for myostatin in cardiomyocytes treated with different concentrations of AngII for 18 h. Precursor, latency-associated peptide (LAP), and processed forms of myostatin are indicated. (B) Quantitative analysis of myostatin protein levels. The processed myostatin protein was measured. The values from treated myocytes have been normalized to values in control cells ($n = 4$ per group). * $P < 0.001$ versus control.

The northern blots showed that myostatin message to RNAs began to increase as early as 6 h of AngII stimulation at 10 nM, reached a maximum of 3.3-fold over the control by 18 h and remained elevated up to 48 h (Fig. 3).

AngII increases myostatin protein expression mainly through p38 MAP kinase

AngII at 10 nM significantly increased the myostatin protein expression when compared with control ($P < 0.01$, Fig. 4A and B). The western blot demonstrated that the increase of AngII-induced myostatin protein was almost completely attenuated after the addition of SB203580 (a highly specific, cell-permeable inhibitor of p38 kinase), 30 min before AngII stimulation. Addition of PD98059 (a specific and potent inhibitor of p42/p44 MAP kinase) significantly, but partially inhibited the increase of myostatin protein induced by AngII. The myostatin protein induced by AngII was not affected by the addition of SP600125 (a potent, cell-permeable, selective, and reversible inhibitor of JNK) or wortmannin (a potent and

Figure 2 AngII increases myostatin protein expression in cardiomyocytes. (A) Representative western blots for myostatin in cardiomyocytes subjected to 10 nM AngII stimulation for various periods of time. (B) Quantitative analysis of myostatin protein levels. The values from stimulated myocytes have been normalized to values in control cells ($n=4$ per group). * $P<0.001$ versus control.

Figure 3 AngII increases myostatin mRNA expression in cardiomyocytes. (A) Representative northern blot for myostatin mRNA in cardiomyocytes subjected to 10 nM AngII stimulation at 10 nM. (B) Quantitative analysis of myostatin mRNA levels. The values from stimulated myocytes have been normalized to matched GAPDH measurement and then expressed as a ratio of normalized values to mRNA in control cells ($n=4$ per group). * $P<0.001$ versus control.

Figure 4 The p38 MAP kinase is an important regulator that mediates AngII-induced myostatin expression in myocytes. (A and C) Representative western blots for myostatin protein levels in myocytes subjected to AngII stimulation for 18 h or control cells without stimulation in the absence or presence of inhibitors, and siRNA. (B and D) Quantitative analysis of myostatin protein levels. The values from stimulated myocytes have been normalized to values in control cells ($n=4$ per group). * $P<0.001$ versus control, + $P<0.01$ versus AngII.

specific inhibitor of PI-3 kinase). To test the specific effect of p38 MAP kinase pathway mediating the expression of myostatin, p38 siRNA was transfected to neonatal cardiomyocytes before AngII stimulation. As shown in Fig. 4A and B, p38 siRNA also completely blocked the myostatin expression induced by AngII ($P<0.001$). The control siRNA did not affect the myostatin protein expression induced by AngII. The SB203580 and p38 siRNA did not affect the myostatin expression in control cells without AngII treatment (Fig. 4C and D). As shown in Fig. 5A and B, phosphorylated p38 protein was induced by AngII stimulation for 6 and 18 h. The phosphorylated p38 proteins induced by AngII was abolished by p38 siRNA, SB203580, and losartan. These findings implicate that p38 MAP kinase is an important regulator that mediates the induction of myostatin protein by AngII in cardiomyocytes. Exogenous addition of p38 MAP kinase activator, anisomycin (50 μ M),

Figure 5 Expression of p38 MAP kinase and MEF-2 in myocytes. (A) Representative western blots for phosphorylated and total p38 MAP in myocytes after treatment with AngII at 10 nM for 6–18 h with or without inhibitor. (B) Quantitative analysis of phosphorylated protein levels. (C) Representative western blots for phosphorylated and total MEF-2 protein in myocytes after treatment with AngII at 10 nM for 6–18 h with or without inhibitor. (D) Quantitative analysis of phosphorylated MEF-2 protein levels. The values from treated myocytes have been normalized to matched GAPDH and corresponding total protein measurement and then expressed as a ratio of normalized values to each phosphorylated protein in control cells ($n=4$ per group). * $P<0.001$ versus control, ** $P<0.001$ versus 6 h.

to the myocytes without any treatment also increased the myostatin protein expression (data not shown). AngII induced the phosphorylated MEF-2 protein in a time-dependent manner (Fig. 5C and D). The MEF-2 siRNA, losartan, and SB203580 attenuated the phosphorylated MEF-2 protein induced by AngII treatment. Addition of anisomycin to the control cells without AngII treatment significantly increased the phosphorylated MEF-2 protein. These data indicate that p38 MAP kinase was activated before the MEF-2 stimulation and p38 MAP kinase stimulated the phosphorylated MEF-2

protein expression, clarifying the sequence of the p38 MAP kinase activation and the MEF-2 stimulation.

AngII increases MEF-2-binding activity

Stimulation of myocytes with AngII at 10 nM for 4–18 h significantly increased the DNA–protein-binding activity of MEF-2 (Fig. 6). An excess of the unlabeled MEF-2 oligonucleotide competed with the probe for binding the MEF-2 protein, whereas an oligonucleotide containing a 2 bp substitution in the MEF-2 binding site did not compete for binding. Addition of SB203580 or losartan 30 min before AngII stimulation abolished the DNA–protein-binding activity induced by AngII. DNA-binding complexes induced by AngII could be supershifted by a specific MEF-2 monoclonal antibody and IgG antibody did not affect the DNA-binding activity, indicating the presence of this protein in these complexes.

AngII increases myostatin promoter activity through MEF-2

To study whether the myostatin expression induced by AngII is regulated at the transcriptional level, we cloned the promoter region of rat myostatin (–1977 ~ +32), and constructed a luciferase reporter plasmid (pGL3-Luc). The myostatin promoter construct contains HIF-1 α , CREB, MEF-2, Myc–Max, Smad3/4, GATA, and Ets-binding sites. As shown in Fig. 7, transient transfection experiment in cardiomyocytes using this reporter gene revealed that AngII stimulation for 6 h significantly caused myostatin promoter activation. This result indicated that myostatin expression is induced at the transcriptional level by AngII. When the MEF-2 binding sites were mutated, the increased promoter activity induced by AngII was abolished. Moreover, addition

Figure 6 AngII increases MEF-2-binding activity. Representative EMSA showing protein binding to the MEF-2 oligonucleotide in nuclear extracts of cardiomyocytes after AngII treatment in the presence or absence of inhibitors. Arrow indicates the mobility of the complex. Similar results were found in another two independent experiments. Cold oligo means unlabeled MEF-2 oligonucleotides. A significant supershifted complex (S) after incubation with MEF-2 antibody was observed.

Figure 7 Effect of AngII on myostatin promoter activity in cardiomyocytes. (A) Constructs of myostatin promoter gene. Positive +1 demonstrates the initiation site for the myostatin transcription. (B) Quantitative analysis of myostatin promoter activity. Cardiomyocytes were transiently transfected with pGL3-Luc by gene gun. The luciferase activity in cell lysates was measured and was normalized with *Renilla* activity ($n=3$ per group). * $P < 0.001$ versus control, + $P < 0.001$ versus 6 h.

of SB203580, losartan, and MEF-2 siRNA caused an inhibition of transcription. These results indicate that MEF-2-binding site in the myostatin promoter, AngII receptor, and p38 pathways are essential for the transcriptional regulation by AngII.

AngII increases and myostatin inhibits protein synthesis

To study the functional consequences of myostatin expression by cardiomyocytes, [35 S]methionine incorporation assay was performed. Stimulation with AngII at 10 nM for 6–48 h increased protein synthesis for 2- and 2.2-fold, respectively, in cardiomyocytes when compared with control cells without treatment (Fig. 8). Exogenous addition of myostatin at 100 ng/ml alone did not increase the protein synthesis. Co-stimulation with myostatin at 100 ng/ml and AngII at 10 nM significantly inhibited the protein synthesis induced by AngII. Addition of losartan 30 min before AngII stimulation significantly attenuated the protein synthesis by AngII. The protein synthesis was similar between addition of SB203580, 30 min before AngII stimulation and AngII stimulation alone. Protein synthesis was lower in the group of co-stimulation of

AngII with myostatin than in the group of AngII with SB203580 ($P < 0.05$). This result indicates that the primary effect of the p38 MAP kinase activation is stimulation of myostatin expression, but not stimulation of cell growth. Our data indicate that the catabolic effect might be induced by increased myostatin in cardiomyocytes.

Discussion

In this study, we demonstrated that AngII upregulates myostatin expression in cardiomyocytes, and p38 MAP kinase and MEF-2 transcription factor are involved in the signaling pathway of myostatin induction. AngII plays a critical role in cardiac remodeling and promotes cardiac myocyte hypertrophy (Schnee & Hsueh 2000). Cardiac myocytes respond to increased mechanical load and hormonal stimulation by hypertrophic growth, but mechanical stress or hormonal factor is also an important stimulus for triggering the initial steps toward cardiac myocytes degeneration and death, which play a critical role in the maladaptive myocardial remodeling and heart failure (Torsoni *et al.* 2003). Myostatin has been shown to regulate

Figure 8 Effect of AngII and myostatin on protein synthesis by [35 S]methionine incorporation assay. * $P < 0.001$, + $P < 0.01$ ($n = 3$ per group).

cardiomyocyte growth through the modulation of Akt signaling (Morissette *et al.* 2006). More recently, two studies reported that myostatin is an inhibitor of cardiomyocyte proliferation and reduces cardiac mass (Atraza *et al.* 2007, McKoy *et al.* 2007), while one study reported that myostatin does not regulate cardiac hypertrophy or fibrosis (Cohn *et al.* 2007). Therefore, the effect of myostatin on cardiac hypertrophy is controversial. The functional role of myostatin in cardiac remodeling is unclear and understanding its regulatory mechanism under hormonal stimulation may help to reveal more insights. Given the role of myostatin in limiting skeletal muscle growth, the upregulation of myostatin in AngII-treated cardiomyocytes may represent a negative feedback mechanism to counteract the pathologic hypertrophy effect of AngII. Actually, in the present study, we have demonstrated that the AngII-induced protein synthesis was inhibited by co-stimulation with myostatin. Angiotensin receptor blocker inhibited the cardiac hypertrophy effect of AngII. Without AngII stimulation, exogenous addition of myostatin did not have effect on cardiac hypertrophy. These results indicate that the actions of AngII on hypertrophy and myostatin expression are counter-intuitive.

Sadoshima *et al.* (1993) and our group have demonstrated that mechanical stretch enhances AngII secretion from cardiomyocytes (Shyu *et al.* 2001). In the present study, we have demonstrated that losartan significantly blocked the increase of myostatin protein by AngII. These data indicated that AngII increases myostatin expression in cardiomyocytes through AngII type 1 receptor. The response of myostatin in cardiomyocytes to AngII may strongly suggest that myostatin represents an endocrine inhibitor of the AngII pathway in the heart. Zou *et al.* (2004) have demonstrated that mechanical stress activates

AngII type 1 receptor without the involvement of AngII. However, in that study, short period (5–8 min) of mechanical stress was applied. Previous study and our group used longer period (more than 4 h) of mechanical stress. Different periods of stress may have different activated mechanisms in cardiac hypertrophy. We have demonstrated that mechanical stretch enhances myostatin expression in cultured rat neonatal cardiomyocytes through insulin-like growth factor (IGF-1; Shyu *et al.* 2005). The interaction of AngII and IGF-1 in ventricular myocardium has been previously investigated in animal models (Brink *et al.* 1999, Leri *et al.* 1999). The AngII infusion has been demonstrated to stimulate cardiac IGF-1 gene expression but reduces circulating IGF-1 levels (Brink *et al.* 1999). Constitutive overexpression of IGF-1 in myocytes downregulates AngII formation (Leri *et al.* 1999). IGF-1 is synthesized by almost all tissues and is an important mediator of cell growth, differentiation, and transformation (Delafontaine *et al.* 2004). IGF-1 is an important growth and survival factor for cardiac muscle cell (Wang *et al.* 1998). IGF-1 is induced in pathological myocardium such as left ventricular hypertrophy and myocardial infarction, and in normal myocardium such as mechanical stress (Donohue *et al.* 1994, Loennechen *et al.* 2001, Palmieri *et al.* 2001). Our study confirms the autocrine or paracrine production of cardiomyocytes in response to AngII stimulation. Cachexia has been observed in patients with chronic severe heart failure (Anker *et al.* 2004). The AngII infusion causes weight loss and skeletal muscle wasting in rats (Song *et al.* 2005). Excess myostatin could induce cachexia in mice and myostatin may be involved in human cachexia (Zimmers *et al.* 2002). AngII is implicated in pathophysiological processes associated with cardiac hypertrophy and remodeling,

and the induction of myostatin by AngII may serve to ameliorate the effects of excess hypertrophy. The role of myostatin in human cachexia due to chronic heart failure needs further study.

The MEF-2 transcription factors are critically involved in the regulation of inducible gene expression during myocardial hypertrophy and MEF-2-DNA-binding activity is increased in the rat hearts of pressure or volume overload (Akazawa & Komuro 2003). The myostatin gene upstream region contains MEF-2 site and muscle-specific expression of myostatin appears to be regulated by MEF-2 (Zhao *et al.* 1999). Although p38 MAP kinase is an important transducer of growth and stress stimuli in virtually all eukaryotic cell types, the role of p38 MAP kinase signaling in cardiac hypertrophy is controversial (Liang & Molkentin 2003). The p38 MAP kinase has been shown to play a critical role in stretch-induced cardiomyocyte hypertrophy (Aikawa *et al.* 2002). Our results suggest that AngII is responsible for MEF-2-DNA binding in cardiomyocytes. In this study, we demonstrated that AngII stimulation of MEF-2-DNA-binding activity required at least phosphorylation of the p38 since p38 inhibitor, SB203580, abolished the MEF-2-binding activity. The SB203580, a potent and specific inhibitor of p38 MAP kinase, completely inhibited the myostatin expression induced by AngII, while inhibitor of PI-3 kinase did not have the inhibitory effect and inhibitors of JNK and p42/p44 MAP kinase had partial inhibitory effect. These data implicated that the p38 MAP kinase pathway, but not the JNK, p42/p44 MAP, and PI-3 kinase pathway is an important signaling pathway that mediates the increased transcriptional activity of MEF-2. Recently, Yang *et al.* (2007) reported that myostatin inhibits skeletal muscle proliferation through PI-3 kinase pathway. The regulation pathway for myostatin may be different in different cell types.

In summary, AngII enhances myostatin expression in cultured rat neonatal cardiomyocytes. The AngII-induced myostatin is mediated at least in part, through p38 MAP kinase and MEF-2 pathway.

Acknowledgements

This study was supported in part by Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan and National Science Council, Taiwan. No conflict of interests for all authors.

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Received in final form 2 February 2008

Accepted 4 February 2008

Made available online as an Accepted Preprint

4 February 2008