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Endogenous BNP attenuates cardiomyocyte hypertrophy induced by Ang II *via* p38 MAPK/Smad signaling

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Previous studies suggest that B-type natriuretic peptide (BNP) exerts inhibitory effects on cardiac hypertrophy. Our studies have shown that long-term treatment of rats with BNP attenuated cardiac hypertrophy *via* down-regulation of TGF-β1 and up-regulation of smad7. However, the mechanisms have not been fully elucidated. In the present study, we examined the role of endogenous BNP on cardiomyocyte hypertrophy and the related molecular mechanisms. Cardiomyocytes from neonatal rats were cultured and a cardiomyocyte hypertrophy model was established with angiotensin II (Ang II). The effects of blockade of endogenous BNP by its receptor antagonist, HS-142-1, on cell hypertrophy were investigated. Cardiomyocyte hypertrophy indices, including cell surface area, protein content and [^{3}H] incorporation were measured. Smad and mitogen-activated protein kinase (MAPK) protein expressions were detected using Western blot analysis. We found that HS-142-1 increased Ang II-stimulated cardiomyocyte hypertrophy and Smad activation. In addition, the increase of cardiomyocyte hypertrophy and the activation of Smad caused by HS-142-1 were not altered by the ERK inhibitor, PD98059, but were decreased by the p38 MAPK inhibitor, SB203580. These results demonstrate that endogenous BNP attenuates cardiomyocyte hypertrophy, and this may be mediated through p38 MAPK/Smad, but not ERK/Smad signaling pathway.

1. Introduction

Cardiac hypertrophy is implicated in the development of heart failure and is also a risk factor for myocardial infarction and sudden death (Shende et al. 2011; Chang et al. 2009; Shen and Qian 2006).

Natriuretic peptides comprise a family of three structurally related hormones, containing atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) (Silberbach and Roberts 2001). The actions of natriuretic peptides are mediated by the specific binding of these peptides to three cell surface receptors: type A natriuretic peptide receptor (NPR-A, GC-A), type B natriuretic peptide receptor (NPR-B) and type C natriuretic peptide receptor (NPR-C) (Huntley et al. 2006). NPR-A and NPR-B are guanylyl cyclase receptors that mediate most of the known effects of natriuretic peptides. NPR-A binds ANP and BNP, whereas NPR-B preferentially binds CNP (Huntley et al. 2006). HS-142-1, an antagonist of NPR-A and NPR-B, can block the function of natriuretic peptides (Huntley et al. 2006; Horio et al. 2000). BNP is produced by ventricular and atrial myocytes and is a marker of cardiac hypertrophy (Gardner 2003). It has diuretic, natriuretic, vasodilator, and antifibrotic properties (Kapoun et al. 2004; Tamura et al. 2000; Tsuruda et al. 2002; Alter et al. 2008). Recent studies suggest that BNP exerts inhibitory effects on cardiac hypertrophy (Kapoun et al. 2004; Nishikimi et al. 2006; Yu et al. 2012). However, the underlying molecular mechanisms have not yet been elucidated.

Multiple signaling pathways agonize and antagonize the hypertrophic growth of cardiomyocytes (Muslin 2008). We reported that proteasome inhibition attenuates cardiac hypertrophy and heart failure through alterations in collagen expression (Ma et al. 2013). Moreover we demonstrated that long-term treatment with BNP prevented ventricular remodeling associated with the inhibition of transforming growth factor (TGF)-β1/Smad2 signaling and BNP attenuates cardiac hypertrophy *via* TGF-β1/smad7 pathway *in vivo* and *in vitro* (He et al. 2009, 2010). Previous studies have shown that BNP regulates cardiac remodeling by inhibiting both cardiomyocyte hypertrophy and cardiac fibrosis (Ellmers et al. 2007). However, whether endogenous BNP inhibits cardiomyocyte hypertrophy *via* Smad signaling is unclear. In addition, multiple lines of evidence have suggested the importance of the mitogen-activated protein kinase (MAPK) pathway in the development of pathologic cardiac hypertrophy (Muslin 2008; Qu et al. 2013). Three subfamilies of MAPK such as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38MAPK have been reported to be involved in cardiac hypertrophy (Qu et al. 2013). It has been shown that BNP affected fibrotic responses of cardiac fibroblasts through ERK signaling (Tamura et al. 2000). However, little is known

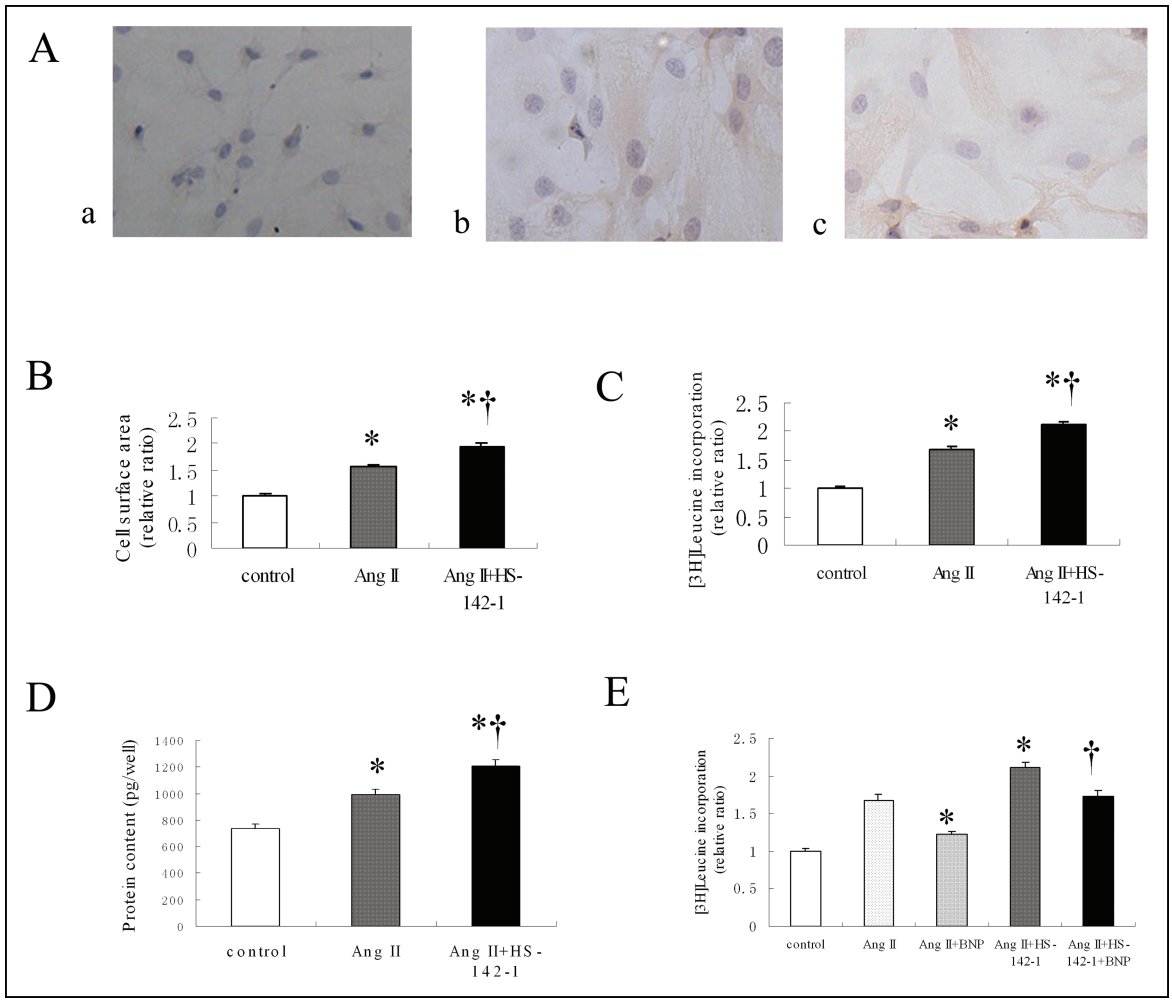

Fig. 1: Effect of HS-142-1 (100 ug/mL) on Ang II (10^{-7} mol/L)-induced increases in the hypertrophy indices of cultured cardiomyocytes (A to D). (A, B) Effect of HS-142-1 on the surface area of cardiomyocytes (n = 100). (C) Effect of HS-142-1 on [^{3}H]-leucine incorporation in cardiomyocytes (n = 5). (D) Effect of HS-142-1 on protein content in cardiomyocytes (n = 5). Data are expressed as mean ± SE, *: $P < 0.01$ vs unstimulated cardiomyocytes (control group), † $P < 0.01$ vs Ang II group. (E) Effect of exogenous BNP (10^{-6}mol/L) on [^{3}H]-leucine incorporation in cardiomyocytes (n = 5). Data are expressed as mean ± SE, *: $P < 0.01$ vs Ang II group, † $P < 0.01$ vs Ang II + HS-142-1 group.

about whether BNP attenuates cardiomyocyte hypertrophy *via* the MAPK pathway.

In the present study, we used HS-142-1 to examine the role of inhibition of endogenous BNP by blockade of BNP binding to NPR-A. We used a neonatal rat cardiomyocyte hypertrophy model induced by angiotensin II (Ang II) to examine the molecular mechanisms of the endogenous BNP-stimulated inhibitory effect on cardiomyocyte hypertrophy. We investigated whether Smad and MAPK signaling are involved in BNP-mediated effects.

2. Investigations and results

2.1. Effect of HS-142-1 on Ang II-induced cardiomyocyte hypertrophy

As shown in Fig. 1, cell surface area, protein content and [^{3}H]-leucine incorporation, were increased significantly in Ang II-stimulated cardiomyocytes compared with unstimulated cardiomyocytes (control group) ($p < 0.01$). The changes in these three indices indicate that Ang II caused significant cardiomyocyte hypertrophy. The BNP receptor antagonist, HS-142-1, significantly increased all of the hypertrophy indices of the Ang II-stimulated cardiomyocytes ($p < 0.01$). HS-142-1 had no effect on the hypertrophy indices of unstimulated cardiomyocytes (results not shown).

2.2. Effect of exogenous BNP on Ang II-induced cardiomyocyte hypertrophy

As shown in Fig. 1, the increase in [^{3}H]-leucine incorporation of cardiomyocyte induced by Ang II was reduced significantly by treatment with exogenous BNP ($p < 0.01$). In addition, the elevated [^{3}H]-leucine incorporation caused by HS-142–1 was suppressed by treatment with exogenous BNP ($p < 0.01$).

2.3. Effect of HS-142-1 on Ang II-induced Smad protein overexpression

As shown in Fig. 2 and Fig. 3, Smad2 and Smad4 protein expression was significantly higher in Ang II-stimulated cardiomyocytes compared with unstimulated cardiomyocytes ($P < 0.01$). HS-142–1 up-regulated this increase in phosphorylated-Smad2 protein expression ($P < 0.01$), but did not modify the increase in total Smad2 and Smad4 protein expression ($P > 0.05$). This result indicates that HS-142–1 increased the activation of the Smad protein, but could not modify the overexpression of the Smad protein.

2.4. Effect of HS-142-1 on Ang II-induced MAPK activation

As shown in Fig. 4, the activation of ERK and P38MAPK protein was significantly higher in Ang II-stimulated cardiomyocytes

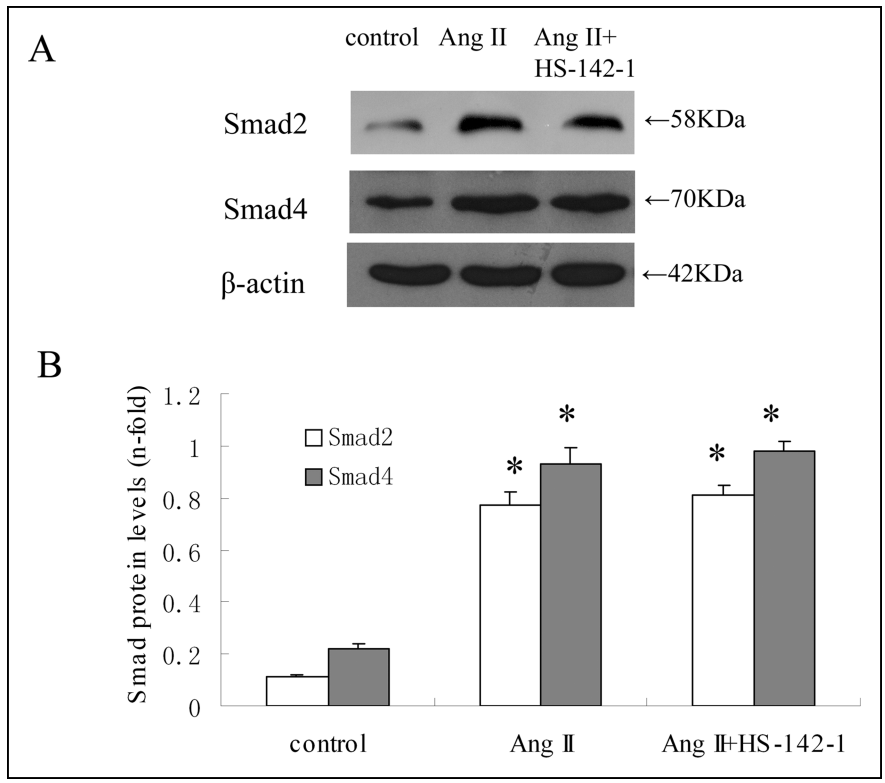

Fig. 2: Effect of HS-142-1 on expression of total Smad2, Smad4 protein in cultured cardiomyocytes (n = 5). Representative image of Western blot in cells incubated with or without HS-142-1 (100 ug/mL) and/or Ang II (10^{-7} mol/L). Smad levels were obtained from densitometric analysis and expressed as the Smad/β-actin ratio and as n-fold over control. Data are expressed as mean ± SE, *: $P < 0.01$ vs control group, † $P < 0.01$ vs Ang II group.

compared with unstimulated cardiomyocytes ($P < 0.01$). HS-142–1 treatment did not modify the activation of ERK protein ($P > 0.05$), but up-regulated the elevation in p38MAPK protein activation ($P < 0.05$). In addition, the elevated [^{3}H]-leucine incorporation and the activation of Smad caused by HS-142-1 was not altered by the ERK inhibitor, PD98059 ($P > 0.05$), but was decreased by the p38 MAPK inhibitor, SB203580 ($P < 0.05$). These data suggest that Smad pathway activation is dependent on p38 MAPK activation.

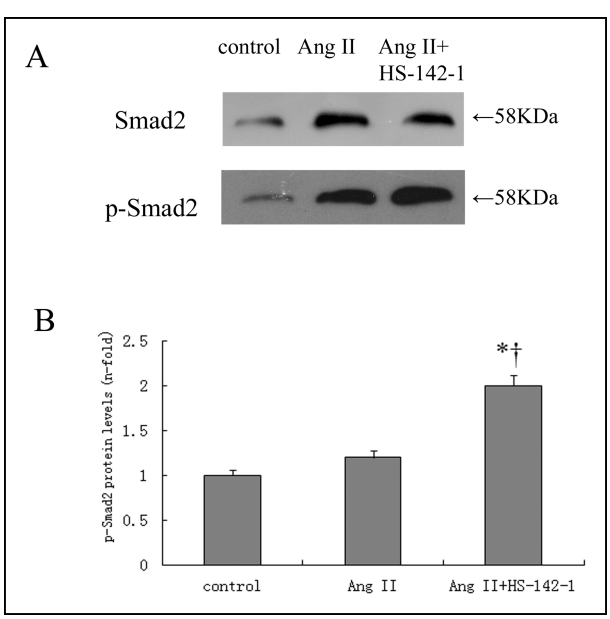

Fig. 3: Effect of HS-142-1 on expression of phosphorylated-Smad2 (p-Smad2) protein in cultured cardiomyocytes (n = 5). P-Smad2 levels were obtained from densitometric analysis and expressed as the p-Smad2/Smad ratio and as n-fold over control. Data are expressed as mean ± SE, *: $P < 0.01$ vs control group, † $P < 0.01$ vs Ang II group.

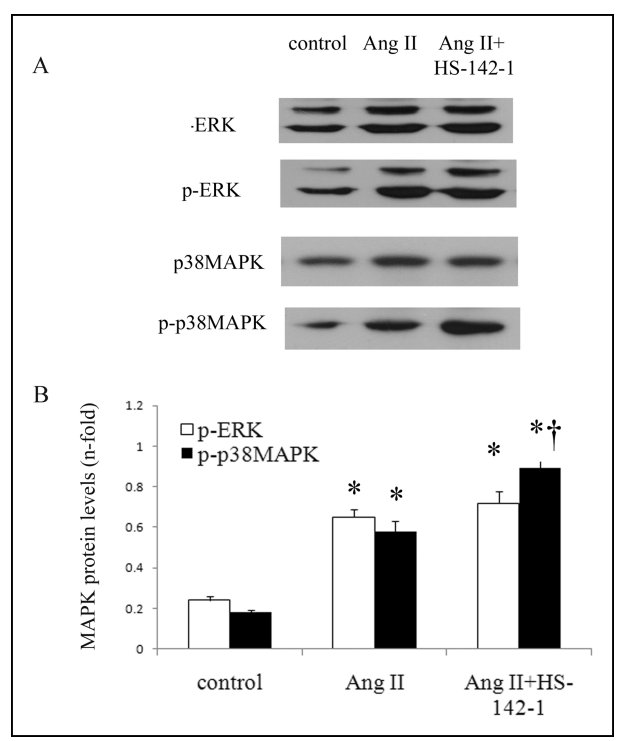

Fig. 4: Effect of HS-142-1 on expression of phosphorylated-ERK (p-ERK) and phosphorylated-p38MAPK (p-p38MAPK) protein in cultured cardiomyocytes (n = 5). Data are expressed as mean ± SE, *: $P < 0.01$ vs control group, † $P < 0.01$ vs Ang II group, ‡ $P < 0.05$ vs Ang II group.

3. Discussion

In the present study, we used a cultured neonatal rat cardiomyocyte model of hypertrophy induced by Ang II to determine the effects of endogenous BNP on cardiomyocyte hypertrophy and the related molecular mechanisms. The rat model showed repro-

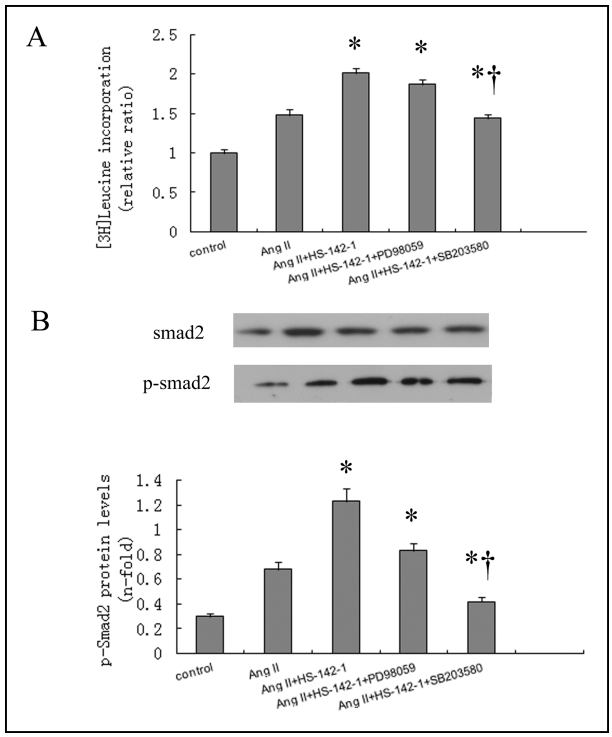

Fig. 5: Effect of PD98059 (ERK inhibitor, 10^{-6}mol/L) and SB203580 (p38 MAPK inhibitor, $2*10^{-5}$mol/L) on [^{3}H]-leucine incorporation (A) and phosphorylated-Smad2 (p-Smad2) protein (B) in cultured cardiomyocytes. Data are expressed as mean ± SE, *: $P < 0.01$ vs Ang II group, † $P < 0.01$ vs Ang II + HS-142-1 group, ‡ $P < 0.05$ vs Ang II + HS-142-1 group.

ducible cardiomyocyte hypertrophy as indicated by increases in cell surface area, protein content and [^{3}H]-leucine incorporation. Using this model, we showed that the blockade of endogenous BNP by HS-142-1, a NPR-A/B antagonist, significantly increased cell surface area, protein content and [^{3}H]-leucine incorporation induced by Ang II. Moreover, the enhanced [^{3}H]-leucine incorporation caused by HS-142–1 was significantly suppressed by treatment with a high dose of exogenous BNP. These results suggest that HS-142-1 abrogates the responses of endogenous BNP. Moreover, endogenous BNP could significantly inhibit cardiomyocyte hypertrophy.

Recent studies have indicated that BNP is a modulator of cardiac hypertrophy (Kapoun et al. 2004; Nishikimi et al. 2006). As reported previously, NPR-A is the major receptor for BNP that is expressed in a wide variety of tissues, including kidneys, blood vessels, adrenal glands, and heart. Binding of BNP to NPR-A leads to the generation of cyclic guanosine monophosphate (cGMP), which mediates most biological effects of the peptide (Kishimoto et al. 2001). Recent reports demonstrated that the BNP/ NPR-A (GC-A)/cGMP system plays an important role in modulating the molecular program of cardiac hypertrophy as an antihypertrophic factor (Nishikimi et al. 2006; Yu et al. 2012). Knowles et al. (2001) showed that mice lacking NPR-A (GC-A) develop cardiac hypertrophy and fibrosis independent of their blood pressure. However, whether BNP could inhibit cardiac hypertrophy is controversial. Tamura et al. reported that disrupting BNP in mice did not induce ventricular hypertrophy (Rodriguez-Vita et al. 2005). Our study demonstrated that the blockade of endogenous BNP by HS-142-1 increased cardiomyocyte hypertrophy, as shown by increases in cell surface area, protein content and [^{3}H]-leucine incorporation induced by Ang II. This study establishes endogenous BNP as a cardiomyocyte-derived antihypertrophy factor, which is in agreement with the observations of Knowles et al. (2001).

To determine the molecular factors that mediate the endogenous BNP-induced reduction of cardiomyocyte hypertrophy, we focused on the Smad pathway. Recent studies indicated that Smad signaling involved in cardiac hypertrophy. Our present study showed that total Smad2, Smad4 and phosphorylated-Smad2 protein expression was markedly increased in cardiomyocytes treated with Ang II. The blockade of endogenous BNP by HS-142-1 elevated the activation of Smad2, but could not modify the overexpression of Smad2 and Smad4. It is demonstrated that endogenous BNP prevents cardiomyocyte hypertrophy probably through activation of Smad protein.

Previous studies demonstrated that the MAPK family played a pivotal role in the development of cardiac hypertrophy, including ERK1/2, p38MAPK and JNK (Muslin 2008; Qu et al. 2013). It is well known that many responses elicited by Ang II are mediated by MAPK and MAPK influences on Smad activation. Rodriguez-Vita et al. (2005) reported that P38MAPK was necessary for Smad activation induced by Ang II. Euler-Taimor and Heges (2006) demonstrated that ERK could inhibit, P38MAPK and JNK enhance Smad activity. Our present work showed that the activation of ERK and P38MAPK was significantly increased in Ang II-stimulated cardiomyocytes, which is consistent with previous studies (Muslin 2008; Qu et al. 2013). In addition, the blockade of endogenous BNP with HS-142-1 did not modify the activation of ERK, but suppressed the activation of p38MAPK. Moreover, treatment of cardiomyocytes with the p38 MAPK inhibitor, SB203580, markedly diminished the elevated [^{3}H]-leucine incorporation and the activation of Smad caused by HS-142-1, whereas the ERK inhibitor, PD98059, had no effect. These data suggest that activation of the Smad pathway is dependent on p38 MAPK activation. Therefore, the antihypertrophic actions of endogenous BNP may be partly mediated by p38 MAPK/Smad signaling.

The present study demonstrates that endogenous BNP inhibited cardiomyocyte hypertrophy, and this may be mediated in part through the p38 MAPK/Smad, but not the ERK/Smad signaling pathway. These data may contribute to our knowledge of the mechanisms underlying cardiac hypertrophy in cardiovascular diseases.

4. Experimental

4.1. Cell culture

Neonatal cardiomyocytes from the hearts of 1- to 3-day-old Sprague-Dawley rats were isolated under germ-free conditions. The cardiac ventricles were cut into 1- to 2-mm cubes and then dissociated by 0.125% trypase and 0.05% collagenase type I (Worthington Biochemical Corp, USA). Cell suspensions obtained in this way were pelleted by centrifugation, and then the cells were resuspended and inoculated in a 50 ml culture flask with Dulbecco's Modified Eagle Medium (DMEM) (Sigma Chemical Co, Spain) containing 10% fetal bovine serum (FBS) (Sigma). Subsequently, the adherent cells were cultured in a humidified incubator at 37 °C containing a mixture of 95% O_2 and 5% CO_2 for 1 h to separate out the cells that were not cardiomyocytes. The cardiomyocytes that were obtained were seeded at 2×10^5/ml in a 6-well culture cluster. During the first two days, 0.1 mmol/L 5-Brdu (Sigma) was used to inhibit the growth of other cell types. Assessment was performed by observing pulsations of the cells under a microscope and by immunohistochemical staining with β-actin monoclonal antibody. Using these methods, the purity of cardiomyocytes reached more than 90% (data not shown). After 48 h, cells were switched to a serum-free medium. Twenty-four hours later, cells were treated with Ang II (10^{-7} mol/L, Sigma) and/or HS-142-1 (100 μg/ml, American Peptide Company, Sunnyvale, CA). In selected experiments, pharmacological inhibitors (PD98059, an ERK inhibitor, SB203580 and a p38 MAPK inhibitor, SB600125) and exogenous BNP (10^{-6} mol/L, American Peptide Company) were added 1 h before treating the cells with Ang II and/or HS-142-1 for 6 or 24 h. PD98059 (10^{-6}mol/L) and SB203580 ($2*10^{-5}$mol/L) were purchased from Calbiochem (San Diego, CA, USA).

4.2. Cardiomyocyte surface area

To determine changes in cell size, the boundaries of cell images captured by a charge-coupled device camera (Olympus, Japan) were traced and analyzed using NIH image software. These values were doubled to account for the portion of the cell surface in contact with the dish. All cells from randomly selected fields in five dishes were examined for each experimental group. A total of 100 cells were examined from each experimental group.

4.3. Cardiomyocyte protein content

Cardiomyocytes from each experimental group were cultured for 24 h. On the second day, cardiomyocytes were washed with D-Hank's solution and scraped off the well. After the cellular lysates were prepared, the cardiomyocyte protein contents were measured by Lowry's modified assay (Singh et al. 1992).

4.4. [^{3}H]-leucine incorporation

Cultured neonatal ventricular myocytes, plated at 5×10^5 cells/ml in 12-well plates (1.5 ml/well), were treated with BNP and coincubated with 37kBq 3H-leucine (Beijing high tech Co., China) for 48 h. The precipitated protein was collected on a fiberglass membrane. 3H-leucine incorporation was determined to evaluate cardiomyocyte growth by counting the protein samples in a LS-6500 liquid scintillometer.

4.5. Western blot

ERK and P38MAPK protein expressions were detected using cardiomyocytes treated for 6 h. Smad protein expressions were measured using cardiomyocytes treated for 24 h. Cardiomyocytes were plated at a field density of 2×10^6 cells/cm^2 on 60-mm culture dish with 2 ml of culture medium. Cardiomyocytes from each experimental groups were lysed on ice with buffer (10 mM Tris·HCl, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.1% sodium deoxycholate, and 0.1% SDS). Total protein concentration was determined using the bicinchoninic acid protein assay (Pierce, Rockford, IL). The crude protein extracts (30 μg) were then loaded onto a 10% SDS-polyacrylamide gel and transferred to a PVDF membrane. The membrane was blocked for 2 h at room temperature in blocking solutions and incubated for 1 h with anti-smad2 (1:1000 dilution, Cell Signal Technology, Beverly, MA.), anti- p-smad2 (1:1000 dilution, Cell Signal Technology, Beverly, MA.) anti-smad4 (1:500 dilution, Sigma), anti-p-smad4 (1:500 dilution, Sigma), anti-ERK (1:500 dilution, Santa Cruz), anti-p-ERK (1:500 dilution, Santa Cruz), anti-P38MAPK (1:500 dilution, Santa Cruz), anti-p-P38MAPK (1:500 dilution, Santa Cruz) antibodies diluted in TBS-T. After washing, membranes were incubated for 1 h with rabbit anti-mouse (Cell Signal Technology) or mouse anti-rabbit secondary antibodies (Cell Signal Technology) diluted 1:5000 in TBS-T. The blots were visualized with an enhanced chemiluminescence detection system (Amersham, Pharmacia Biotech), exposed to X-ray film, and analyzed using NIH Image software. β-actin was used as a control.

4.6. Statistical analysis

All values were expressed as mean ± standard error (SE). The statiSstical analysis of differences among the groups was done by one-way ANOVA. It was followed by a Student Newman-Keul's test for multiple group comparisons. Differences were judged to be significant when $P < 0.05$. Statistical analyses were performed using SPSS 11.0 statistics software (SPSS Inc., Chicago, IL, USA).

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References

Alter P, Rupp H, Rominger MB, Vollrath A, Czerny F, Figiel, JH, Adams P, Stol, F, Klose KJ, Maisch B (2008) B-type natriuretic peptide and wall stress in dilated human heart. Mol Cell Biochem 4: 179–191.

Chang SA, Kim YJ, Lee HW, Kim DH, Kim HK, Chang HJ, Sohn DW, Oh BH, Park YB (2009) Effect of rosuvastatin on cardiac remodeling, function, and progression to heart failure in hypertensive heart with established left ventricular hypertrophy. Hypertension 54: 591–597.

Ellmers LJ, Scott NJ, Piuhola (2007) Npr1-regulated gene pathways contributing to cardiac hypertrophy and fibrosis. J Mol Endocrinol 38: 245−257.

Euler-Taimor G, Heger J (2006) The complex pattern of SMAD signaling in the cardiovascular system. Cardiovasc Res 69: 15−25.

Gardner DG (2003) Natriuretic peptides: markers or modulators of cardiac hypertrophy? Trends Endocrinol Metab 14: 411−416.

He J, Chen Y, Huang Y, Yao F, Wu Z, Chen S, Wang L, Xiao P, Dai G, Meng R, Zhang C, Tang L, Huang Y, Li Z (2009) Effect of long-term B-type natriuretic peptide treatment on left ventricular remodeling and function after myocardial infarction in rats. Eur J Pharmacol 602: 132−137.

He J, Chen Y, Chen B, Huang YY, Yao FJ, Chen SL, Dong YG (2010) B-type natriuretic peptide attenuates cardiac hypertrophy via transforming growth factor-β1/smad7 pathway in vivo and in vitro. Clin Exp Pharmacol Physiol 37: 283–289.

Horio T, Nishikimi T, Yoshihara F, Matsuo H, Takishita S, Kangawa K (2000) Inhibitory regulation of hypertrophy by endogenous atrial natriuretic peptide in cultured cardiac myocytes. Hypertension 35: 19−24.

Huntley BK, Sandberg SM, Noser JA, Cataliotti A, Redfield MM, Matsuda Y, Burnett JC Jr. (2006) BNP-induced activation of cGMP in human cardiac fibroblasts: interactions with fibronectin and natriuretic peptide receptors. J Cell Physiol 209: 943−949.

Kapoun AM, Liang F, O'Young G, Damm DL, Quon D, White RT, Munson K, Lam A, Schreiner GF, Protter AA (2004) B-type natriuretic peptide exerts broad functional opposition to transforming growth factor-beta in primary human cardiac fibroblasts: fibrosis, myofibroblast conversion, proliferation, and inflammation. Circ Res 94: 453−461.

Kishimoto I, Rossi K, Garbers DL (2001) A genetic model provides evidence that the receptor for atrial natriuretic peptide (guanylyl cyclase-A) inhibits cardiac ventricular myocyte hypertrophy. Proc Natl Acad Sci USA 98: 2703−2706.

Knowles JW, Esposito G, Mao L, Hagaman JR, Fox JE, Smithies O, Rockman HA, Maeda N (2001) Pressure-independent enhancement of cardiac hypertrophy in natriuretic peptide receptor A-deficient mice. J Clin Invest 107: 975−984.

Ma Y, Chen Y, Yang Y, Chen B, Liu D, Xiong Z, Zhang C, Dong Y (2013) Proteasome inhibition attenuates heart failure during the late stages of pressure overload through alterations in collagen expression. Biochem Pharmacol 85, 223–233.

Muslin AJ (2008) MAPK signalling in cardiovascular health and disease: molecular mechanisms and therapeutic targets. Clin Sci (Lond) 115: 203−218.

Nishikimi T, Maeda N, Matsuoka H (2006) The role of natriuretic peptides in cardioprotection. Cardiovasc Res 9: 318−328.

Qu H, Tong D, Zhang Y, Kang K, Zhang Y, Chen L, Ren L (2013) The synergistic antitumor activity of arsenic trioxide and vitamin K2 in HL-60 cells involves increased ROS generation and regulation of the ROS-dependent MAPK signaling pathway. Pharmazie 68: 839–845.

Rodriguez-Vita J, Sanchez-Lopez E, Esteban V, Rupérez M, Egido J, Ruiz-Ortega M (2005) Angiotensin II activates the Smad pathway in vascular smooth muscle cells by a transforming growth factor-beta-independent mechanism. Circulation 111: 2509−2517.

Shen XC, Qian ZY (2006) Effects of crocetin on antioxidant enzymatic activities in cardiac hypertrophy induced by norepinephrine in rats. Pharmazie 61: 348–352.

Shende P, Plaisance I, Morandi C, Pellieux C, Berthonneche C, Zorzato F, Krishnan J, Lerch R, Hall MN, Rüegg MA, Pedrazzini T, Brink M (2011) Cardiac raptor ablation impairs adaptive hypertrophy, alters metabolic gene expression, and causes heart failure in mice. Circulation 123: 1073–1782.

Silberbach M, Roberts J CT (2001) Natriuretic peptide signalling: molecular and cellular pathways to growth regulation. Cell Signal 13: 221−231.

Tamura N, Ogawa Y, Chusho H, Nakamura K, Nakao K, Suda M, Kasahara M, Hashimoto R, Katsuura G, Mukoyama M, Itoh H, Saito Y, Tanaka I, Otani H, Katsuki M (2000) Cardiac fibrosis in mice lacking brain natriuretic peptide. Proc Natl Acad Sci USA 97: 4239−4244.

Tsuruda T, Boerrigter G, Huntley BK, Noser JA, Cataliotti A, Costello-Boerrigter LC, Chen HH, Burnett JC Jr. (2002) Brain natriuretic peptide is produced in cardiac fibroblasts and induces matrix metalloproteinases. Circ Res 91: 1127−1134.

Yu L, Rao C, Shi X, Li Y, Gao K, Li X, Wang J (2012) A novel bioassay for the activity determination of therapeutic human brain natriuretic peptide (BNP). PLoS One 7: e49934.