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Master's Thesis

**The Role of Angiotensin Type 1
Receptor and Src Kinase in Stretch
Induced Cardiac Fibroblast Injury
Model**

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Jeju National University

February, 2016

신전손상으로 유도된 심장 섬유모세포에서의
안지오텐신 1형 수용체와 Src kinase의 역할

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The Role of Angiotensin Type 1 Receptor and Src Kinase in Stretch Induced Cardiac Fibroblast Injury Model

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(Supervised by Professor **Ki-Seok Kim**)

A thesis submitted in partial fulfillment of the requirement for
degree of Master of Science in Biomedicine and Drug Development

2016.02

This thesis has been examined and approved.

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**The Role of Angiotensin Type 1 Receptor and
Src Kinase using Stretch-Induced Cardiac
Fibroblast Injury Model**

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LIST OF ABBREVIATIONS

| | |
|---------------|--|
| Ang II | Angiotensin II |
| AT1R | Angiotensin II type 1 receptor |
| ARB | Angiotensin II type 1 receptor blocker |
| ERK | Extracellular-signal-regulated kinase |
| EGFR | Epidermal growth factor receptor |
| Src | Proto-oncogene tyrosine-protein kinase Src |
| Akt | Protein kinase B (PKB) |
| p38 | p38 mitogen-activated protein kinase |
| MAPK | Mitogen-activated protein kinase |
| JNK | c-Jun N terminal kinase |
| PI3K | Phosphoinositide 3-kinase |
| Lo | Losartan |
| AZD | AZD0530 |
| ECM | Extracellular matrix |
| μM | $\mu\text{mol/L}$ |
| min | minute |
| hrs | hours |

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1. ABSTRACT

Cardiac hypertrophy is a central risk factor of cardiac morbidity and mortality. Mechanical stretch activated Angiotensin II type I (AT1) receptor without the involvement of Angiotensin II (AngII) during the development of cardiac hypertrophy and causes secretion of Ang II, which plays a central role in mechanical stretch-induced hypertrophy. Here, we attempted to show the role of AT1 receptor and Src kinase in mechanical stretch-induced cardiac fibroblasts injury. Western blot analysis showed the differentiation of stimulation by Ang II and mechanical stretch triggered ERK1/2 phosphorylation was suppressed by Losartan, Src phosphorylation was suppressed by AZD0530 or both inhibitors on Akt phosphorylation. The phosphorylation of ERK and Src were increased by Ang II (100nM) and by mechanical stretch in 1hour. The effect of Ang II and stretch on ERK was blocked by Losartan and Src phosphorylation was suppressed by AZD0530. However, both Losartan and AZD0530 did not effect on Akt phosphorylation induced by Ang II, but they are could inhibit in stretch-induced Akt phosphorylation. We investigated that Losartan and AZD0530 can be took part in effect of mechanical stretch and Ang II induced cardiac fibroblast dysfunction. Moreover, mechanical stretch and Ang II induced all of three kinases phosphorylation that involves AT1 receptor. But they are might be located in different pathways.

Key words: Angiotensin II, Mechanical stretch, AT1 receptor, Losartan, AZD0530.

2. INTRODUCTION

Cardiac fibroblasts are the most abundant cell type in the myocardium which plays a pivotal role in the maintenance of cardiac structure and functions (Qang *et al.*, 2013; Wang *et al.*, 2003; Fan *et al.*, 2012). They are the essential cell type responsible for secreting components of the extracellular matrix (ECM) in the heart (Atance *et al.*, 2004; Cheng *et al.*, 2003; Tian *et al.*, 2003; Wang *et al.*, 2013). The activation of cardiac fibroblasts and their differentiation contribute to cardiac fibrosis and hypertrophy (Wang *et al.*, 2003). Cardiac hypertrophy is one of the independent risk factor responsible for myocardial injury and remodeling (Wang *et al.*, 2014). Mechanical stretch can induce cardiac hypertrophy by activating angiotensin II (Ang II) type 1 (AT1) receptor through an Ang II-independent pathway (Balakumar and Jagadeesh, 2010; Dostal *et al.*, 2014; Malhotra *et al.*, 1999; S. Wang *et al.*, 2014; Zablocki and Sadoshima, 2013) and by secretion of Ang II, which plays a central role in cardiovascular diseases including stimulates cardiac fibroblast proliferation, collagen synthesis and ECM production (Chen *et al.*, 2004; Heandeler and Ber, 2000; Malhotra *et al.*, 1999; Wang *et al.*, 2013; Zou *et al.*, 1998). AT1 receptor is a well-known receptor, which significant contribution for the development of cardiac hypertrophy (Dostal *et al.*, 2014). On binding to AT1 receptor, Ang II activates an array of protein kinases which is important for its growth-promoting effects such as Src family kinase, epidermal growth factor receptor (EGFR), ERK1/2, p38 MAPK, and Akt/protein kinase B (Ruwhof and Laarse, 2000; Schiffrin and Touyz, 2004; Taniyama *et al.*, 2004; Yamazaki *et al.*, 1998). The ERK1/2 plays a key role in cardiac hypertrophy and failure (Balakuma and Jagadeesh, 2010). However, it is still unclear whether

ERK1/2 is a critical mediator of hypertrophic responses (Bernardo *et al.*, 2010). In cardiac fibroblasts, Ang II activates ERK1/2 through a pathway including the G β γ subunit of Gi protein and Src family tyrosine kinases (Yamazaki *et al.*, 1998). Blockade of ERK1/2 signaling pathways prevented the progression of cardiac hypertrophy (Balakuma and Jagadeesh, 2010). Although, the pathological and physiological mechanisms were associated with pressure overload-induced cardiac hypertrophy that focus about intense scientific investigation for over 3 decades, the detail molecular mechanisms of how mechanical stress-induced AT1 receptor activation and its inhibition by angiotensin II receptor blockers (ARBs) still remain elucidation (Dostal *et al.*, 2014).

To elucidate the role of AT1 receptor and Src kinase in stretch induced human cardiac fibroblast injury, we examined the inhibition effect of mechanical stretch and Ang II by using Losartan (AT1 receptor antagonist) including AZD0530 (Src inhibitor). Using mechanical stretch machine, Flexcell Tension System (FX-5000T), as a stress from disease such as high blood pressure. The human cardiac fibroblasts were stretched with/without inhibitors, which block the pathology signaling making hypertrophy.

3. MATERIALS AND METHODS

3.1. Cells culture

Human cardiac fibroblasts were purchased from Cell Applications Inc. Cells were culture in Fibroblast Growth Medium in a humidified incubator with 5% CO₂ at 37°C. For all experiments, five passages cells were cultured to 80% - 90% confluence were used for treatment and mechanical stretch experiment.

3.2. Incubation of agonist and antagonists

Cells were seeded in 6-wells plate until the cells were cultured to 70% - 80% confluence. The medium was changed to serum starvation for 4-12 hour or without serum starvation prior to experiment treatment. The serum-free media was changed before the cells were treated with Ang II (#A9525) from sigma-Aldrich. Some cells were pretreated with/without the selective antagonists Losartan (#61188) from sigma-aldrich or/and AZD0530 (#A2133) from Apexbio before stimulated with Ang II or take mechanical stretch, as indicated.

3.3. Mechanical stretch

Bio Flex culture plates-collagen are 6-weels cultures plate consisting of Flexible rubber membrane percolated with collagen I (BF-3001C, Flexcell international corp., US). After plated cells are confluence of 70-80%, the media were changed and transferred to serum starvation for 4-12 hours (or no serum starvation). In some experiment, cells were also stimulated with Ang II 100nM. Both mechanical stretch and Ang II were applied to cells in the absence and presence inhibitors, including Losartan (20µM), AZD0530 (20µM). These

concentrations were used base on previous studies that demonstrated selectivity and sensitivity. For all inhibitor studies, cells were treated with the inhibitors 10-30 minutes before stimulation with Ang II or stretch. Mechanical stretch was applied to cells using the Flexcell FX-5000 tension system (Flexcell international corp., US). For the experiment in this study, cyclic sinusoidal stretch of 20% elongation and 0.4 frequencies was used for 5 minutes to 4 hours for our investigation.

3.4. Western Blotting

After incubated with Ang II or/and applied for mechanical strain, the cells were washed with PBS (Biosesang, KR), scrapped with lysis buffer (RIPA, Biosesang, KR) contained protease inhibitor (protease inhibitor cocktail, Sigma, US) and collected into micro tubes (1.5ml) in ice. The collected cells were kept in ice for 30minutes with vortex very often. Lysate was centrifuged at 15 000 rpm at 4°C for 15 minutes and transferred only the supernatant into new micro tube. Protein was measured using BCA protein assay kit (thermo scientific, Rockford, US). Following stimulation, proteins (5-10 μ g) of total proteins were extracted from cardiac fibroblasts, separated by 10% polyacrylamide gel electrophoresis and transferred onto a nitrocellulose (NC) or PVDF transfer membrane (Whatman TM, a part of GE Healthcare Life Science Ltd, UK) in transfer buffer. Membranes were incubated with 3-5% non-fat dry milk or 3-5% albumin traction V (BSA) (biobasac canada inc, CA) in Tris-buffered saline with Tween (TBS-T) for 1-2 hours was used to block nonspecific binding sites. The membranes were then incubated for overnight at 4 °C with phosphor-specific or total antibodies diluted

in TBS-T. The primary antibodies were used as follows: Anti- Phospho-44/42 MAPK (ERK 1/2) (#4377), Anti-44/42 MAPK (ERK 1/2), Akt (#9272), Anti-Phospho-Akt (Ser473) (#9271), Src (#2108), Anti-Phospho-Src (Tyr416) (#2101) were purchased from Cell Signaling Technology, US. Then the membranes are fully washed with TBS-T buffer, and incubated with secondary antibody. The secondary antibody; goat anti-rabbit IgG HRP (SC-2030) from Santa Cruz Biotechnology, US. After washed with TBS-T, western lightning Plus-ECL reagents (NEN Life Science Products Inc, Boston, US) were used to detection immunoreactive protein bands of membrane and exposed on to an X-ray film blue.

3.5. Statistical analysis

Image J software were used to transform images into numerical values. Student's t-test was used to determine the statistical significance of differences compare to control. Data represent as the mean of relative to control \pm standard deviation from at least three replications.

4. RESULTS

4.1. Effect of Angiotensin II on human cardiac fibroblasts

Ang II is known as an activator which has multiple effects in cardiac fibroblasts, which induces cardiac fibroblast proliferation, fibrosis and hypertrophy (Janet et al. 2000). In previous study showed that Ang II activates an array of protein kinases important for its promoting effects such as Src, ERK1/2, and Akt/protein kinase B via binding to AT1 receptor (Taniyama et al., 2004). To examine that Ang II stimulates cardiac fibroblast activation via AT1 receptor, we treated Ang II in proportion of its concentration for 10 minutes. According to western blot analysis, we investigated that phosphorylation of ERK1/2 was started to increase by Ang II 10nM and significantly increased by 100nM to 1 μ M before starting to decrease by 10 μ M (Fig. 2). Furthermore, the effect of time courses of Ang II was also determined. Cells were stimulated with Ang II 100nM in different time courses. Ang II induced a rapid phosphorylation of ERK1/2 at 5 min and peaked at 10 or 15 min. The activity was returned to the basal level after 30min (Fig. 3A). These results showed the same in phosphorylation of Src. The activation of Src phosphorylation was started to increase at 5 min until 15min and it was decreased at 20min (Fig. 3B). Similarly, we also found that ERK1/2, Src and Akt phosphorylation significantly increased by Ang II 100nM at 10min (Fig. 4A-C). These results suggested that Ang II stimulated ERK1/2, Src and Akt phosphorylation depended on dose and time dependent maybe involved to AT1 receptor.

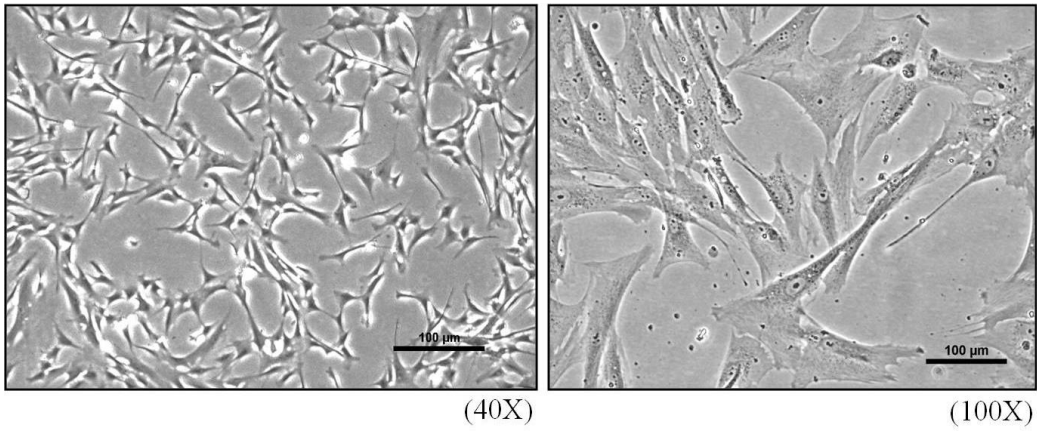


Figure 1. Photography of Human cardiac fibroblast (HCF) derived from normal human heart at second passage

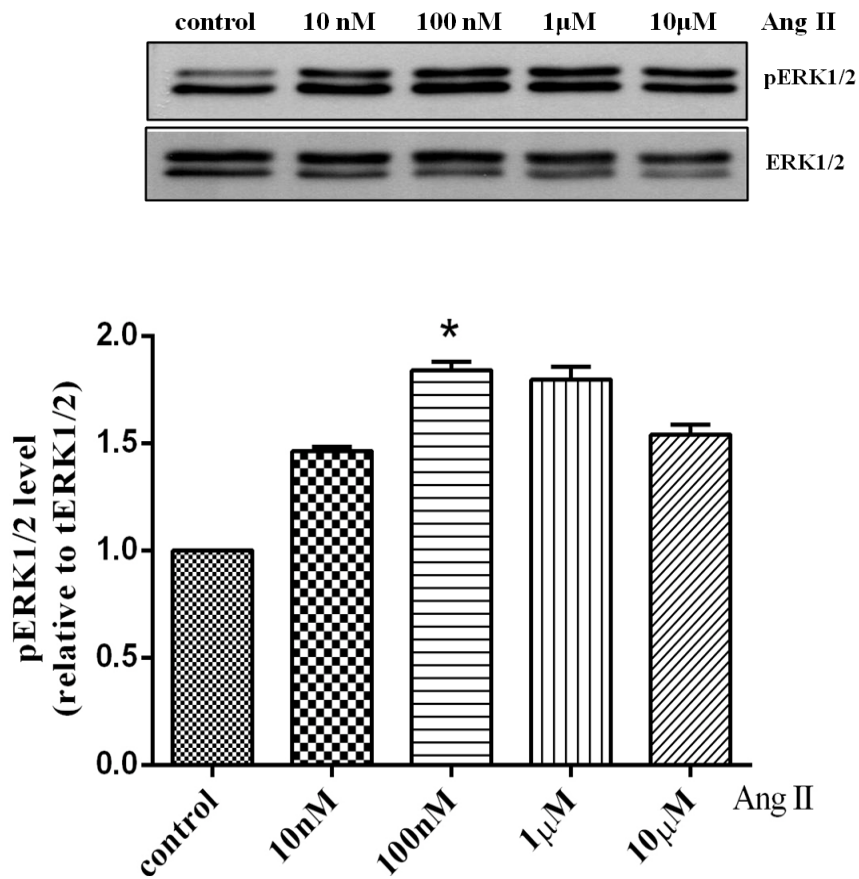


Figure 2. Effect of Angiotensin II dose-dependent on ERK1/2 activation.

Human cardiac fibroblasts were stimulated with Ang II (10nM -10μM) for 10min and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibody to detect ERK1/2 phosphorylated. Values are mean \pm SD compared to control. * $P < 0.005$.

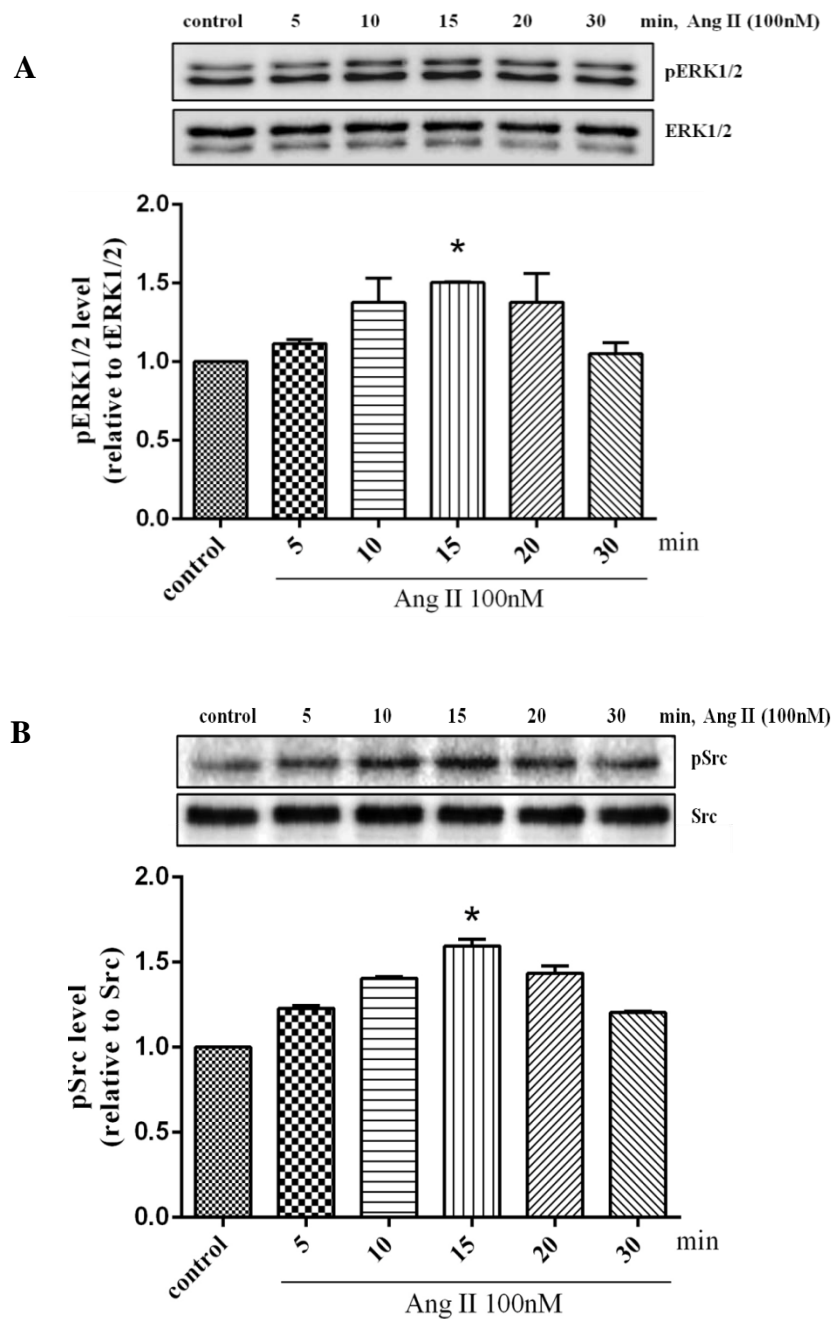


Figure 3. Time course of ERK1/2 and Src activated by Ang II.

Human cardiac fibroblasts were stimulated with Ang II 100nM for indicated times and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibodies to detect ERK1/2 and Src phosphorylated. Values are mean \pm SD compared to control. * P < 0.005.

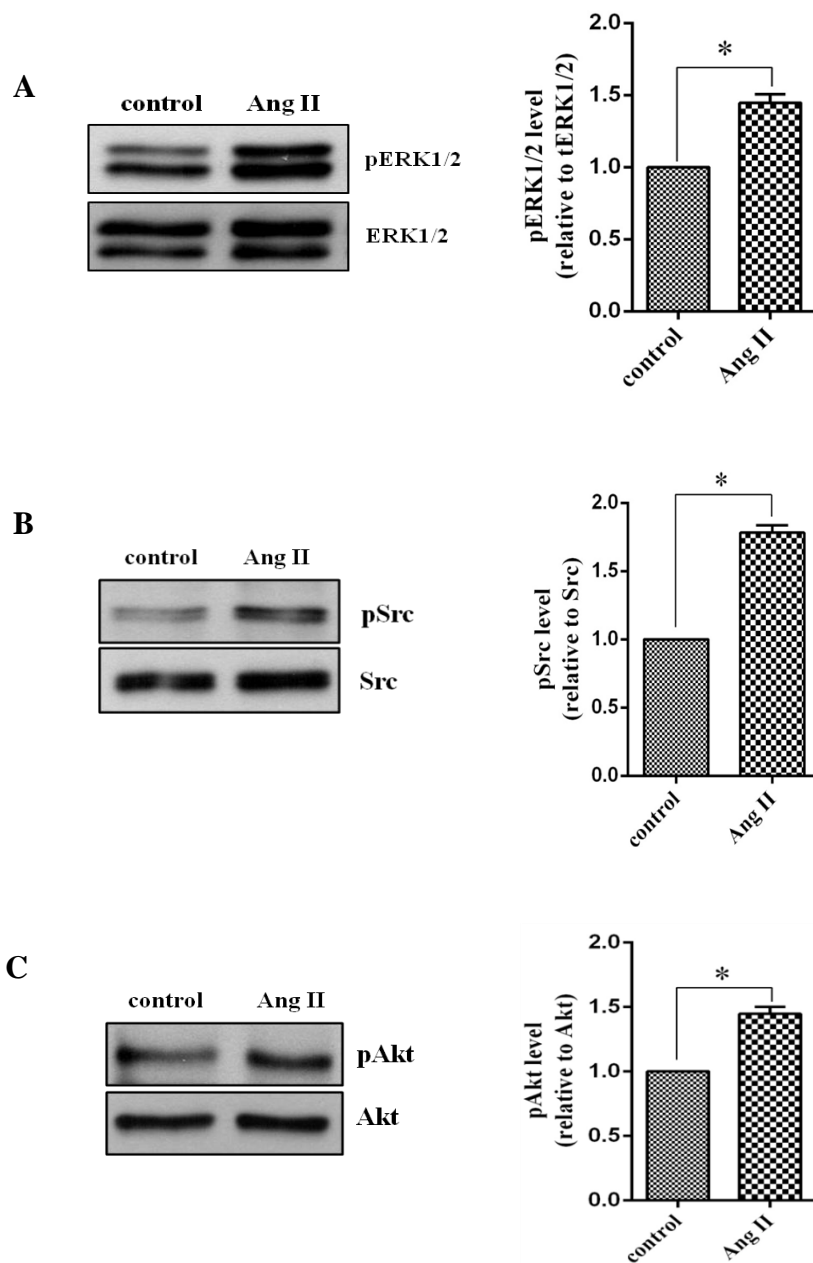


Figure 4. Effect of Angiotensin II on ERK1/2, Src and Akt activation.

Human cardiac fibroblasts were stimulated with Ang II 100nM for 10min and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibodies to detect ERK1/2, Src and Akt phosphorylated. Values are mean \pm SD compared to control. * $P < 0.05$.

4.2. Effect of Losartan dose-dependent on ERK1/2 phosphorylation

To examine the best concentration of Losartan for use to inhibit AT1 receptor, cells were treated with Losartan (10 μ M to 30 μ M), whereas some cells were stimulated with Ang II 100nM for 10min at the same time with Losartan. In this result indicated that Losartan 20 μ M significantly blocked the ERK1/2 phosphorylation compared to 10 μ M. So, we assumed that Losartan 20 μ M is the best concentration for used to block AT1 receptor-stimulated by Ang II compared to 10 μ M (Fig.5).

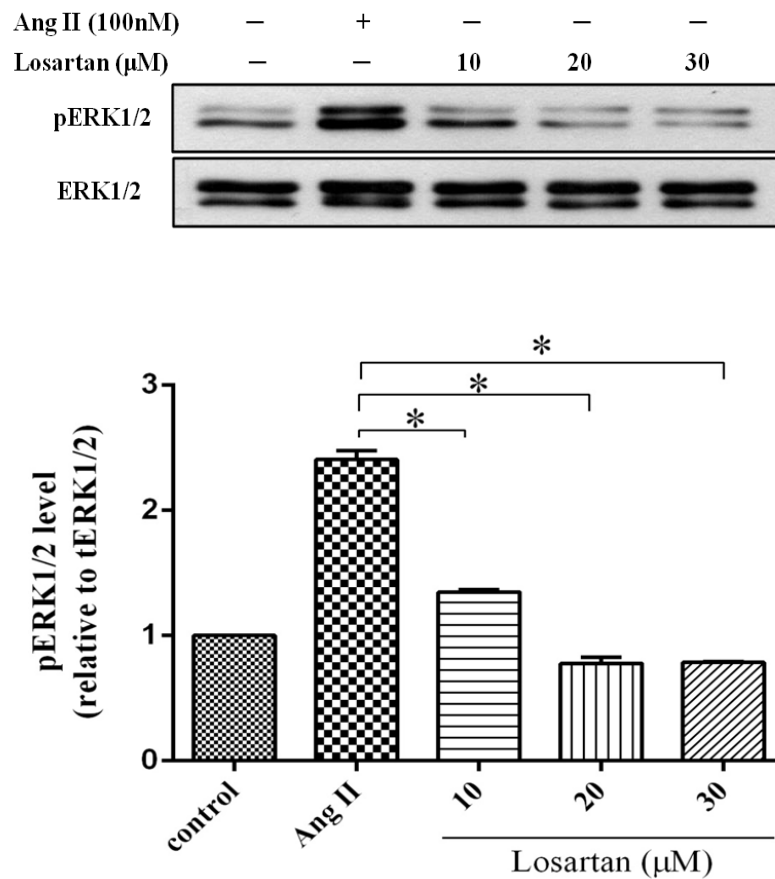


Figure 5. Effect of Losartan dose-dependent on ERK1/2 activation.

Human cardiac fibroblasts were stimulated with Ang II 100nM and Losartan (10 μ M-30 μ M) for 10min and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibody to detect ERK1/2 phosphorylated. Values are mean \pm SD compared to Ang II. * $P < 0.005$.

4.3. Effect of Losartan and AZD0530 on Ang II-induced ERK1/2, Src, and Akt phosphorylation

In previous study, we have demonstrated that Ang II stimulates ERK1/2, Src and Akt activation in cardiac fibroblasts maybe via AT1 receptor. To conform that Losartan and AZD0530 can block AT1 receptor and inhibit Src pathway induced by Ang II, we treated Losartan 20 μ M, AZD0530 20 μ M. Losartan 20 μ M or AZD0530 20 μ M and Losartan 20 μ M or AZD0530 20 μ M plus Ang II 100nM were treated in the same time for 10minutes. Other cells we stimulated with Ang II 100nM for 10 minutes same. From these experiments, we found that Ang II still induced ERK1/2, Src including Akt phosphorylation. The phosphorylation of ERK1/2 was decreased in Losartan plus Ang II and more attenuated when we treated with Losartan alone (Fig. 6A). However, Losartan had no effect on Src and Akt phosphorylation. (Fig. 6B, C). Conversely, AZD0530 has inhibited Ang II induce phosphorylation of Src (Fig. 7B), but it could not inhibit ERK1/2 and Akt phosphorylation (Fig.7A, C). These results illustrated that Ang II induced ERK1/2 phosphorylation via AT1 receptor, whereas Src and Akt phosphorylation induced by Ang II were not involved AT1 receptor.

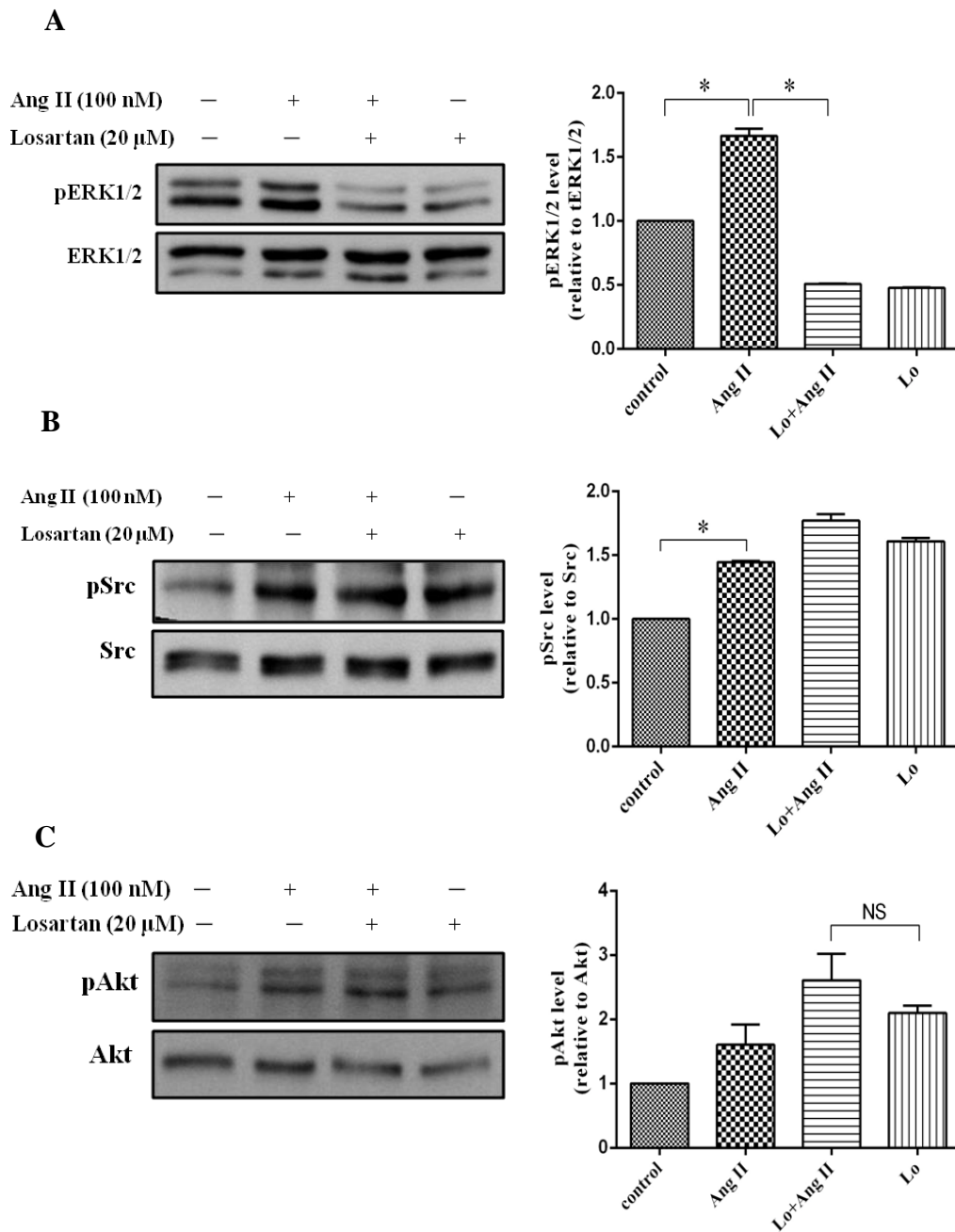


Figure 6. Effect of Losartan on Ang II induced ERK1/2, Src and Akt activation.

Human cardiac fibroblasts were treated with Losartan 20 μ M then stimulated with Ang II 100nM for 10min in the same time and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibodies to detect phosphorylated of ERK1/2, Src and Akt phosphorylated. Values are mean \pm SD compared to Ang II. * $P < 0.005$

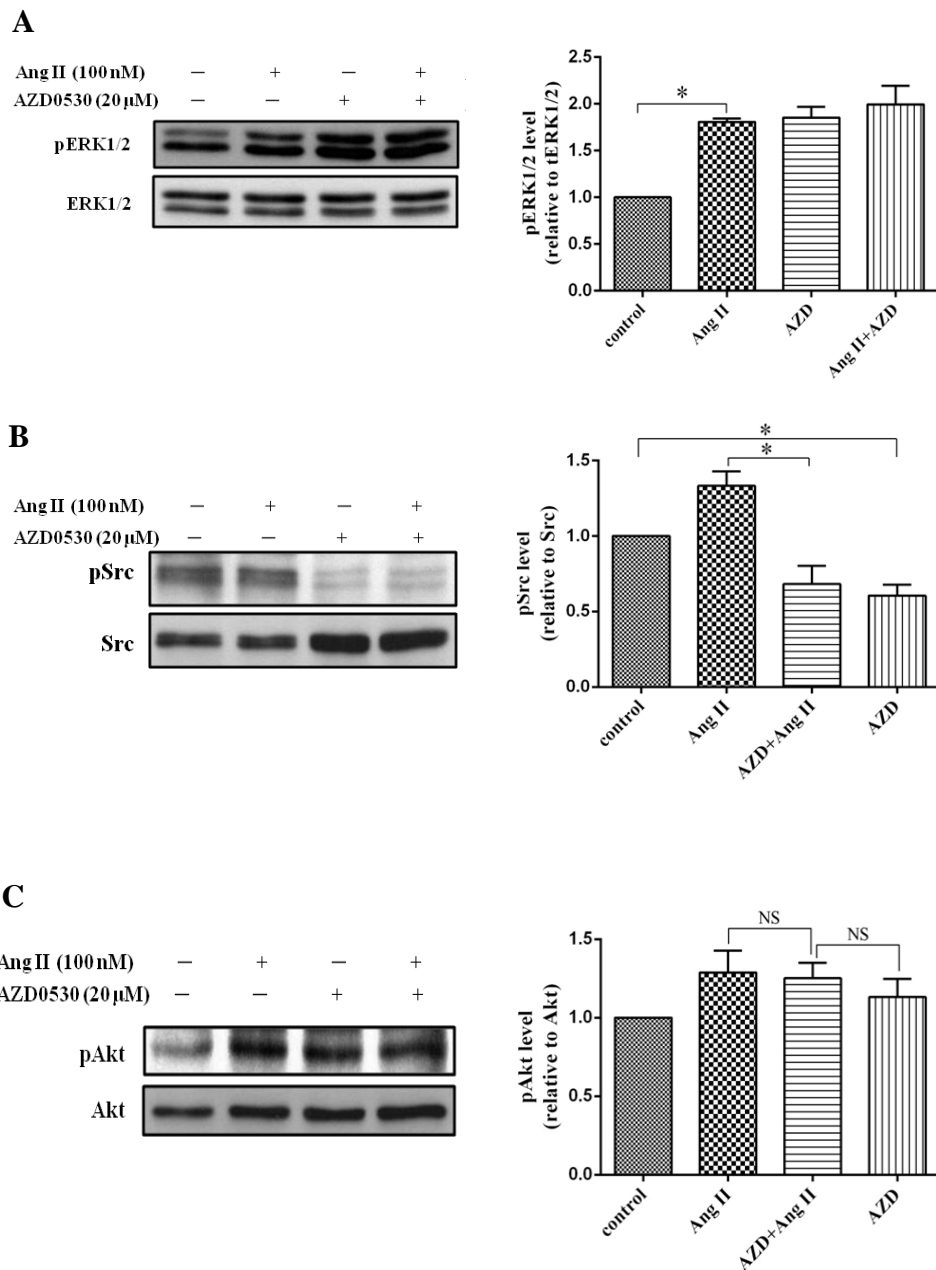


Figure 7. Effect of AZD0530 on Ang II induced ERK1/2, Src and Akt activation.

Human cardiac fibroblasts were pretreated with AZD0530 20 μ M then stimulated with Ang II 100nM for 10min in the same time and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibodies to detect ERK1/2, Src and Akt phosphorylated. Values are mean \pm SD compared to Ang II. * $P < 0.05$.

4.4. Effect of mechanical stretch on ERK1/2, Src and Akt phosphorylation

To conform whether mechanical stretch effect on ERK1/2, Src and Akt phosphorylation in human cardiac fibroblasts. Cells were stretched without present of Ang II at 20% elongation in different times (15min, 30min, 1h, and 4h). The acute cyclic stretch in human cardiac fibroblast induced phosphorylation of ERK1/2, Src and Akt were started increased at 15 minutes. Then it decreased at 30 minutes. But the activity was increased again in 60 minutes and decrease again at 4hr (Fig.9A-C). These results suggested that without involvement of Ang II, mechanical stretch also effect on ERK1/2, Src and Akt phosphorylation. However, the effect of mechanical stretch was depending on time dependent.

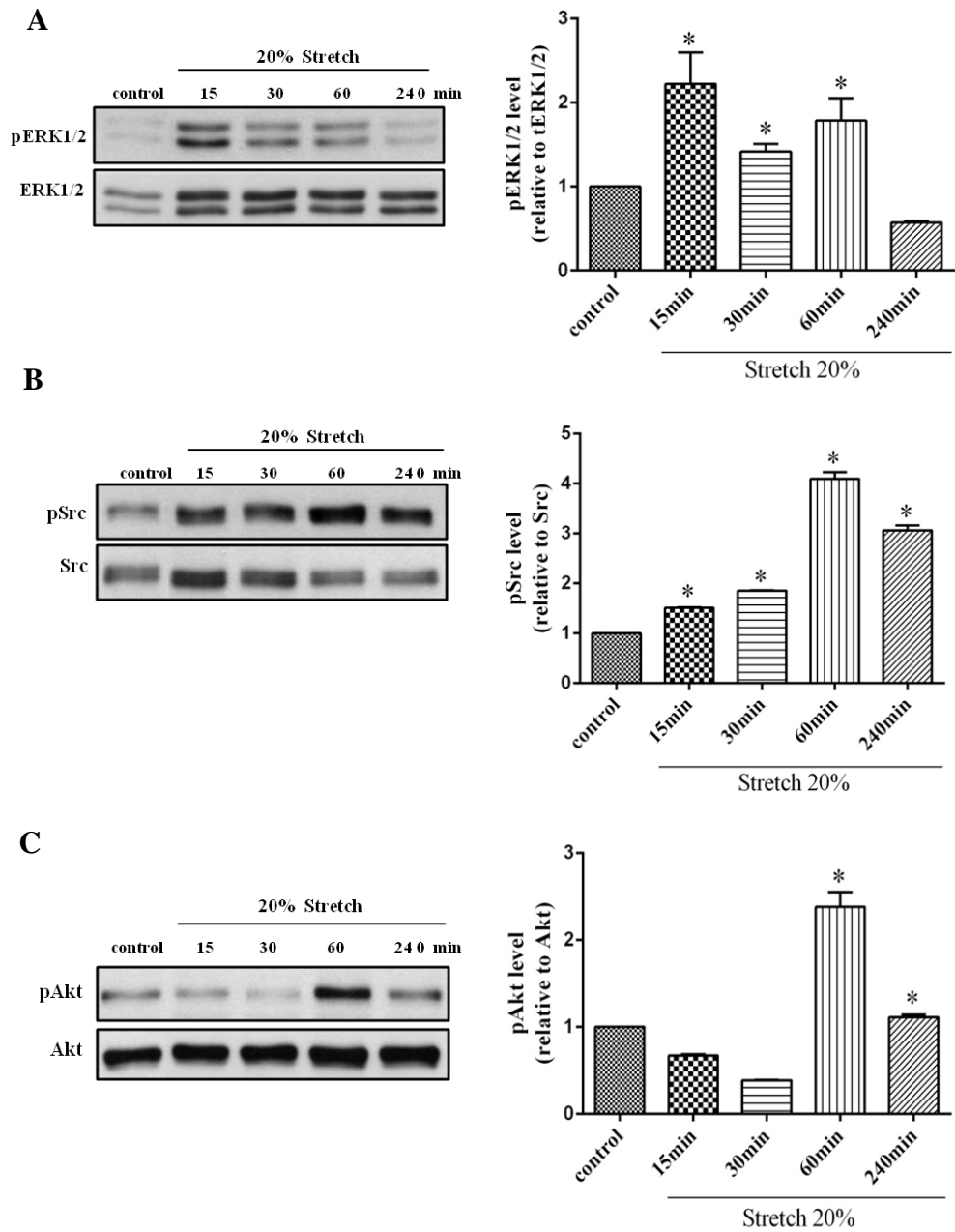


Figure 8. Effect of mechanical stretch on ERK1/2, Src and Akt activation.

Human cardiac fibroblasts were taken mechanical stretch in different times and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibodies to detect ERK1/2, Src and Akt phosphorylated. Values are mean \pm SD compared to Ang II alone. * $P < 0.05$.

4.5. Effect of Losartan dose-dependent on mechanical stretch induced ERK1/2, Src and Akt phosphorylation

Dostal *et al* reported that pretreatment with AT1 receptor blockers can attenuate mechanical stretch induced hypertrophic responses (Dostal *et al.*, 2014). To verify whether AT1 receptor was involved in mechanical stretch induced ERK1/2, Src and Akt phosphorylation in cardiac fibroblasts, we treated the cells with Losartan in order concentration (10 μ M and 20 μ M) for 10minutes before took mechanical stretch 20% elongation for 1hour. We investigated that Losartan 20 μ M is the best concentration for use to block AT1 receptor in mechanical stretch induced ERK1/2, Src and Akt phosphorylation (Fig. 10A-C). This result demonstrated that mechanical stretch induced ERK1/2, Src and Akt phosphorylation was involved to AT1 receptor, which could be block by Losartan.

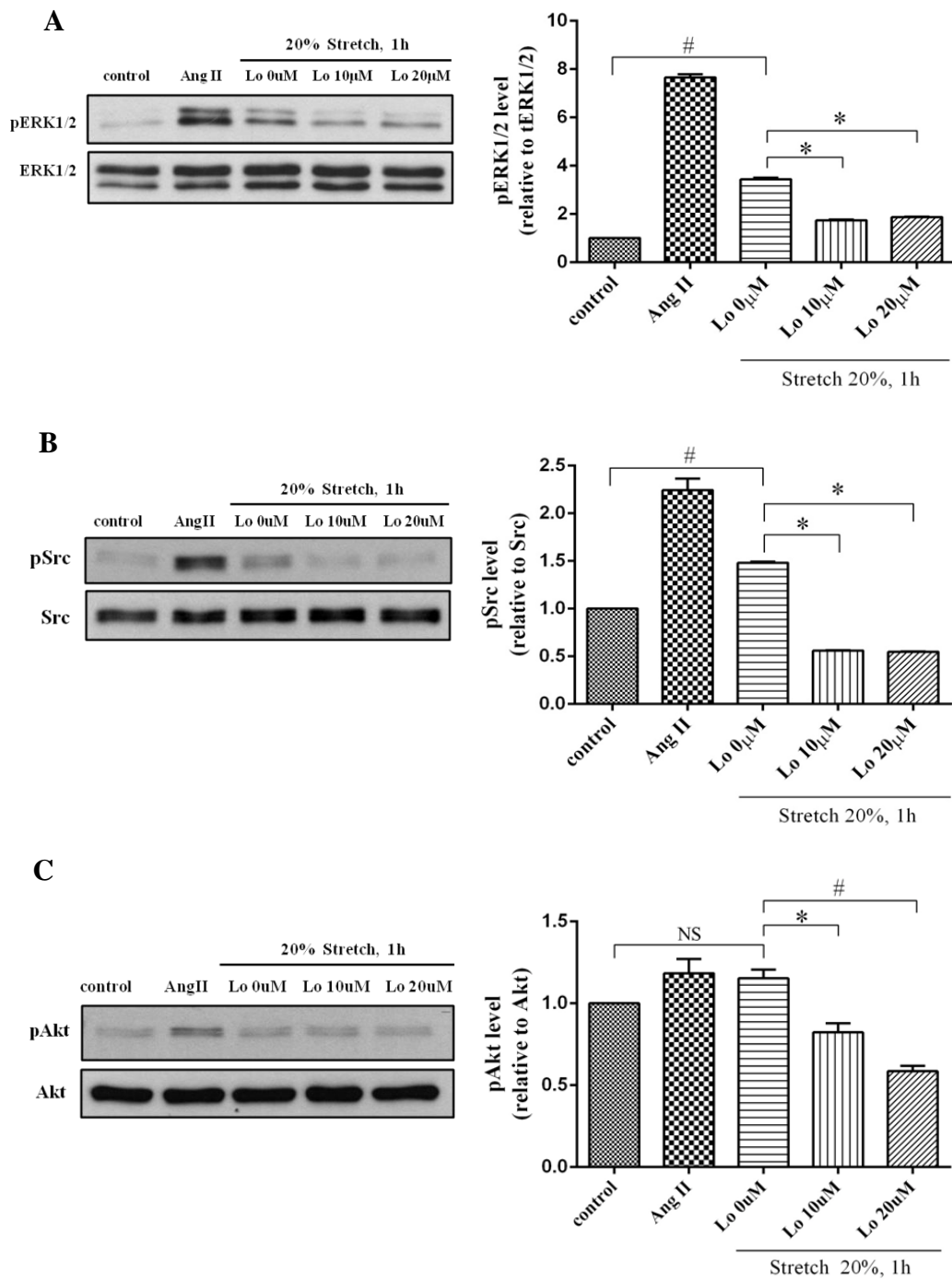


Figure 9. Effect of Losartan dose-dependent on mechanical stretch induced ERK1/2, Src and Akt activation.

Some cells were stimulated with Ang II 100nM for 10min and some cells were treated with Losartan (10 and 20 μ M) for 10min before stretch for 1h and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibodies to detect ERK1/2, Src and Akt phosphorylated. Values are mean \pm SD. # $P < 0.001$ compared to control. * $P < 0.005$ compare to Lo 0 μ M.

4.6. Effect of Losartan and AZD0530 on mechanical stretch induced ERK1/2, Src and Akt phosphorylation

To make sure of inhibitory effect of mechanical stretch on AT1 receptor induced ERK1/2, Src and Akt phosphorylation, we treated the cardiac fibroblasts with Losartan 20 μ M, AZD0530 20 μ M and Losartan plus AZD0530 20 μ M for 15min before took mechanical stretch 20% elongation for 1h. We found that Losartan and AZD0530 20 μ M compressed activation of ERK1/2 phosphorylation by stretch (Fig.11A). However, Losartan 20 μ M could little block the activation of Src phosphorylation by stretch, but the Src phosphorylation could significantly blocked by AZD0530 and Losartan plus AZD0530 20 μ M (Fig.11B). In contrast, both inhibitors could block the effect of mechanical stretch on Akt phosphorylation (Fig. 11C). From these results, we can assume that effect of acute mechanical stretch on AT1 receptor activated ERK1/2, Src, and Akt phosphorylation could be decrease by Losartan and AZD0530 20 μ M. So, Both AT1R and Src play role in stretch induced cardiac fibroblast dysfunction

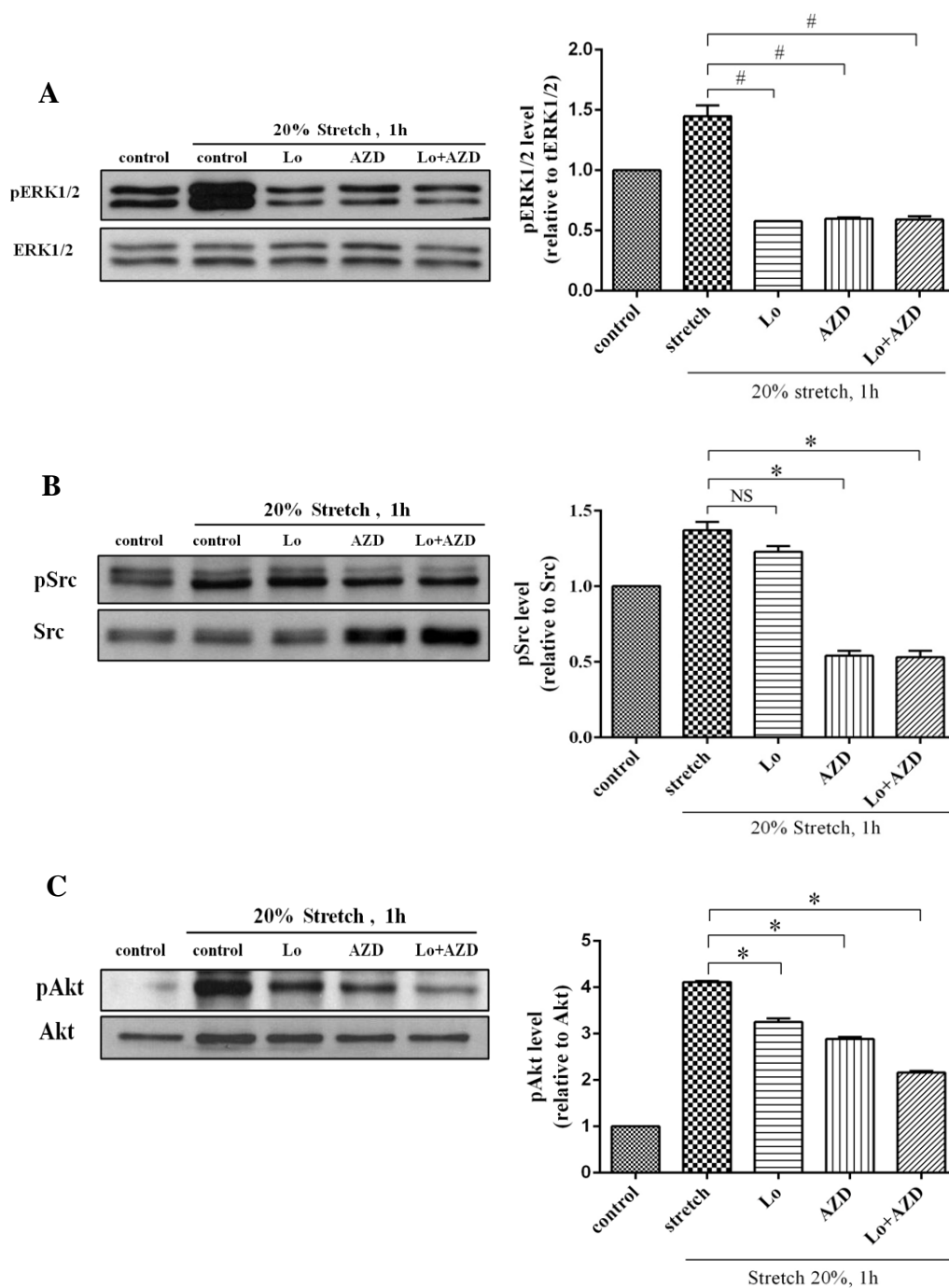


Figure 10. Effect of Losartan and AZD0530 on mechanical stretch induced ERK, Src and Akt activation.

Cells were treated with Losartan 20 μ M and AZD0530 (20 μ M) for 10min before stretch for 1h and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibodies to detect ERK1/2, Src and Akt phosphorylated. Values are mean \pm SD compared to stretch. # $P < 0.05$, * $P < 0.005$.

4.7. Effect of starvation time on ERK1/2, Src and Akt phosphorylation

To know the baseline of ERK1/2, Src and Akt phosphorylation, we investigated starvation time dependent on phosphorylation of the three kinases. We demonstrated that ERK1/2 and Akt phosphorylation were increased at 4hrs until 12hrs and decreased after 12hrs until 24hrs (Fig.12A, C). Src phosphorylation was decreased and kept the expression same until 24hrs (Fig. 10B). These result showed starvation time has effect on the expression of ERK1/2, Src and Akt phosphorylation.

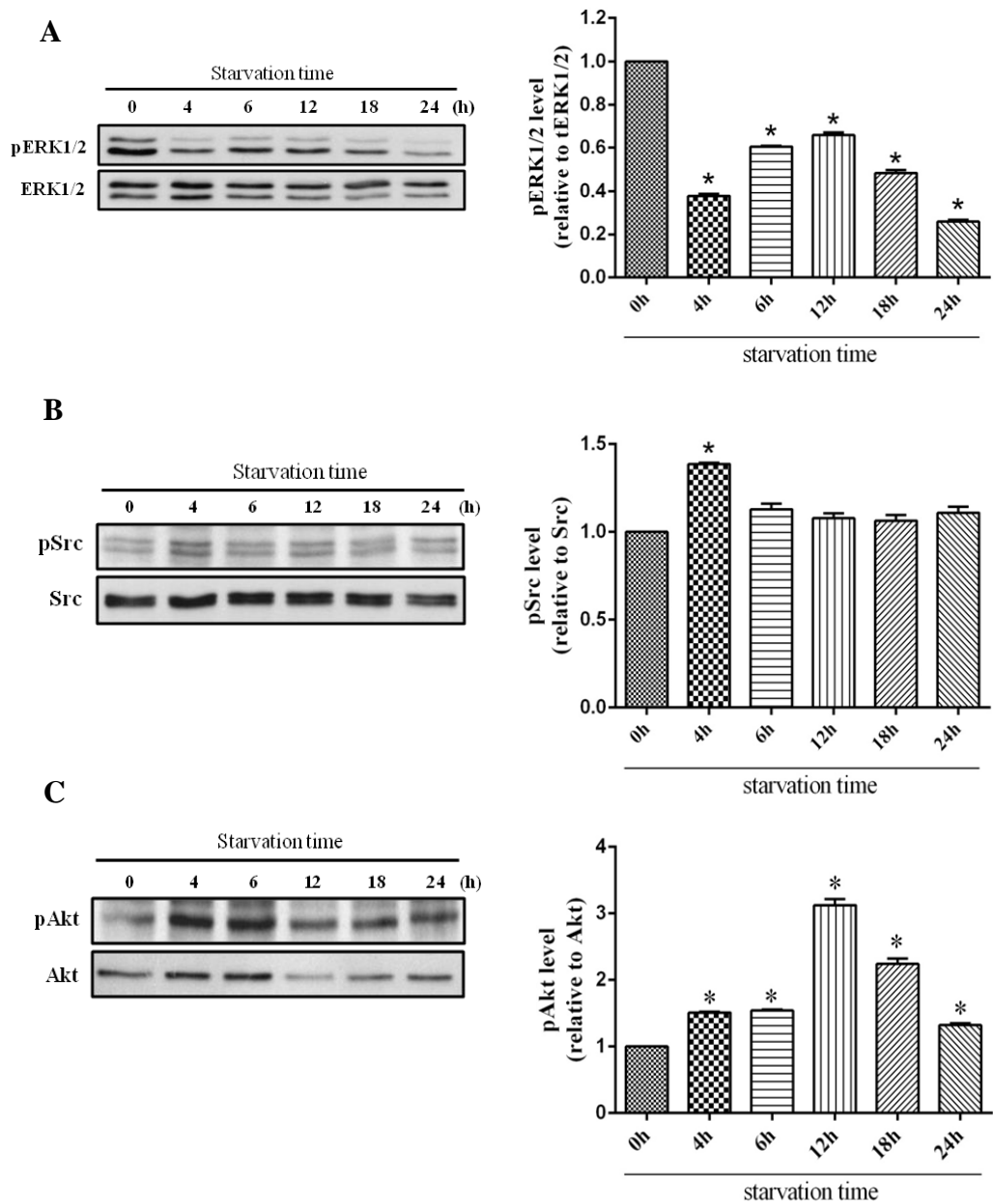


Figure 11. Effect of starvation time on ERK1/2, Src and Akt activation.

Cells were changed to serum starvation in different times and lysed. Proteins were separated by SDS-PAGE. Western analysis was performed by use of phospho-specific antibody to detect ERK1/2, Src and Akt. Values are mean \pm SD compared to stretch. * $P < 0.005$.

5. DISCUSSION

In this study, we demonstrated the signaling pathways that mediate ERK1/2, Src and Akt activation by Ang II and mechanical stretch in human cardiac fibroblast. Finding from the present study demonstrated that Ang II 100nM induced the activation of ERK1/2, Src and Akt phosphorylation in 10-15minutes. Mechanical stretch induced the activation of ERK1/2, Src and Akt phosphorylation at 1hour. Both Ang II and mechanical stretch induced phosphorylation of ERK1/2 was significantly blocked by Losartan 20 μ M in 10minutes, but it had no effect on Src phosphorylation. However, phosphorylation of Src was blocked by AZD0530 20 μ M in 10minutes. In contrast, AZD0530 was increased the phosphorylation of ERK1/2. Although the both inhibitors can inhibit ERK1/2 and Src phosphorylation, but it had no effect on Akt phosphorylation.

In human cardiac fibroblasts, Ang II stimulates cells proliferation and collagen synthesis (Chen *et al.*, 2004). Cardiac fibroblasts increased the production of ECM proteins when the heart is exposed to a different of injuries. The increasing number of cardiac fibroblasts and the content of proteins in ECM while cardiac remodeling is one of the major cause of cardiac dysfunction (Cheng *et al.*, 2003; Zou *et al.*, 1998). Ang II induced of both cardiomyocyte hypertrophy and fibroblast proliferation via AT1 receptor and increased activation of ERKs, JNK, and p38MAPK pathways (Cheng *et al.*, 2003; Wang *et al.*, 2013). Ang II activates ERKs through a pathway including the G β γ subunit of Gi protein, tyrosine kinases including Src family tyrosine kinases (Yamazaki, Komuro, and Yazaki, 1998). Src and Src-family protein tyrosine kinases are regulatory proteins that play major roles in cell differentiation, motility, proliferation, and survival

(Roskoski, 2005). Src was induced upregulation by Ang II (Yamazaki, Komuro, and Yazaki, 1998). In knockout mice, Src was recruited to the cell membrane and interact with AT1 receptor during stretching. The major role of Src in regulating AT1 receptor induced intracellular signaling transduction was stimulated by mechanical stretch (Wang *et al.*, 2014; Takeishi *et al.*, 2001).

Mechanical stretch activates AT1 receptor and integrins. The activation of these receptors can initiate several downstream signaling pathways, such as MAPK and Akt. In the heart, the Akt is implicated in the control of physiological cardiac hypertrophy, contractile function and Ca²⁺ handling (Dostal *et al.*, 2014). One of the principal targets of PI3K signaling is the serine/threonine kinase Akt, PI3K-Akt signaling pathway is essential in the induction of physiological cardiac hypertrophy (Rohini *et al.*, 2010). Mechanical stretch also activates AT1 receptor without the involvement of Ang II and caused an increase in the phosphorylation levels of ERKs through the Ang II independent pathway (Dostal *et al.*, 2014; Wang *et al.*, 2014; Yatabe *et al.*, 2009). Pretreatment of cardiomyocytes with AT1 receptor blockers such as candesartan can suppresses AT1 receptor activation by both Ang II and mechanical stretch-induced hypertrophic responses (Dostal *et al.*, 2014; Paravicini *et al.*, 2012). Moreover, stretch of cardiomyocytes caused activation of ERKs, indicating that ERKs and mechanical stress-induced hypertrophy may be linked. ERKs may partly participate in the mechanisms of mechanical stress-induced hypertrophy (Cheng *et al.*, 2003). Wang *et al* also reported that the Ang II-induced upregulation Src phosphorylation was inhibited by both candasartan and valsartan (AT1 receptor blockers) (Wang *et al.*, 2014), whereas our study indicated that Losartan could not block the activation of Src

phosphorylation. On the other hand, the report of Yamazaki *et al* demonstrated that inhibitors of tyrosine kinases abolish Ang II-induced ERKs activation in cardiac fibroblasts, while Rohini *et al* showed that inhibition the function of Src family tyrosine kinases, also has no effect on Ang II induced activation of ERKs in cardiac myocytes, which similar to the results in recent study (Rohini *et al.*, 2010; Yamazaki, Komuro, and Yazaki, 1998).

Taken together, the results suggest that Ang II and mechanical stretch effect on AT1 receptor and Src kinase induced cardiac fibroblast dysfunction such as fibrosis and hypertrophy. Ang II and mechanical stretch induced ERK1/2 phosphorylation was compressed by AT1 receptor blocker, Losartan, whereas Src phosphorylation increased by Ang II and mechanical stretch was inhibited by Src inhibitor, AZD0530. So, AT1 receptor and Src kinase play an important role involve in mechanical stretch induced cardiac fibroblast injury, which can be attenuated by Losartan and AZD0530.

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