Interaction of α-adrenergic stress and integrin-mediated signal transduction in hypertrophic cardiac myocytes

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Interaction of α-adrenergic stress and integrin-mediated signal transduction in hypertrophic cardiac myocytes

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Finally, I want to remember a God's word. "*In him we were also chosen, having been predestined according to the plan of him who works out everything.. Ephesians 1:11-3.*

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Interaction of α-adrenergic stress and integrin-mediated signal transduction in hypertrophic cardiac myocytes

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In cardiac myocytes, stimulation of alpha (1)-adrenoceptor (AR) leads to a hypertrophic phenotype. The G_h protein (transglutaminase II, TGII) is a tissue type transglutaminase and transmits the alpha (1B)-adrenoceptor signal with GTPase activity. Recent evidence suggests that integrin activation, in concert with G proteins activation, might be essential for cardiomyocyte growth. In this study, our objectives were to evaluate how G_h and integrin interact to promote cardiomyocyte hypertrophy and to identify G_h molecular determinants and downstream signals involved. First, we observed the selectivity of alpha-adrenoceptors in norepinephrine (NE)-induced cardiac hypertrophy by protein synthesis and estimation of constitutive gene, MLC-2 and MHC. NE-mediated hypertrophic responses activated ERK1, 2 and their upstream regulators MEK1,2, which were inhibited by siRNA for G_h. We also identified significant activation of β1-integrin in NE-induced cardiomyocyte hypertrophy. In addition, FAK and Shc, regulator of

FAK, were directly related with integrin and G_h protein in NE-induced cardiomyocyte hypertrophy. The activation of FAK and Shc were suppressed by siRNA for G_h. In conclusion, the hypertrophic response induced by norepinephrine in cardiomyocytes occurs through β1 Integrin/Shc/FAK/MEK1, 2/ ERKs signal pathways via α1-AR/G_h.

Key Words : cardiac myocyte: hypertrophy: integrin: α-adrenoeceptor

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I. INTRODUCTION

Human cardiac hypertrophy is a general pathological condition that often develops as a by-product of hypertension or valvular heart disease. Adult cardiomyocytes have no capacity to divide, but respond to stress and growth stimuli by enlarging.[1] Cardiac hypertrophy is a compensatory process that occurs in pathological conditions such as hypertension, myocardial infarction, and some genetic heart diseases, and is related to an increased risk of cardiac arrhythmias, diastolic dysfunction, congestive heart failure, and death[2, 3].

The relationship between increased adrenergic activity and cardiac hypertrophy has been documented for some time but the mechanisms involved have not been clearly understood until recently. The hypertrophic effect of α_1-adrenergic agonists has been observed in cultured cardiomyocytes from both neonates and adults. The direct parameters related to cardiac hypertrophy in cultured cardiocytes are

increased cell size and the protein content. Norepinephrine is a direct hypertrophic effector in cardiac myocytes without affecting afterload.[4, 5, 6] The mechanisms by which norepinephrine induces cardiomyocyte hypertrophy are as yet controversial. Norepinephrine-mediated adrenoceptor (AR) are divided into two major subtypes: α and β. The specific response mediated by these ARs has not been confirmed. The α_1-ARs, but not the α_2-ARs, exist in cardiomyocytes and α_1-ARs have been implicated in the pathogenesis of cardiac hypertrophy, in ischemia-induced cardiac arrhythmias, and ischemic preconditioning.[7] Norepinephrine, epinephrine and catecholamines activate α_1-ARs that are members of G-protein coupled receptor (GPCR) superfamily.[8] The specific G proteins that couple α_1-ARs to their intracellular effectors and the specificity of the various α_1-ARs subtypes for different G proteins remain to be clearly defined.

Whatever the etiology, the development of cardiac hypertrophy and progression towards heart failure include hypertrophy of cardiomyocytes, hyperplasia of fibroblasts, changes in the composition of interstitial tissue and ventricular remodeling.[9] Myocardial remodeling implies an alteration in the composition and distribution of the extracellular matrix (ECM), as well as changes in the spatial orientation of intracellular components and cells. Alterations in the density and distribution of the major constituents of the muscle cytoskeleton, i.e. intermediate filaments of desmin, microtubules have been associated with cardiac remodeling.[10, 11, 12, 13] Other cell types, mainly fibroblasts contribute to the increase

in the synthesis and secretion of proteins of the ECM, such as fibronectin (FN), laminin, collagen I and III, resulting in cardiac fibrosis.[14, 15] The accumulation of ECM components both in the myocardium and in the coronary vasculature contributes to cardiac failure. The ECM has historically been considered an inert fibrous network, but in fact, it exerts a specific regulatory influence on morphogenesis, cell function, proliferation, differentiation, and migration. According to in vitro analysis, cellular behavior varies according to cell interactions with the ECM that depend on trans-membrane proteins, such as integrins, and on a sarcolemmal cytoskeleton.[16, 17] When activated, integrins, a family of cell surface receptors, cluster in focal adhesion sites with cytoskeletal proteins associated to sarcolemma, such as vinculin, talin, paxillin and with signaling enzymes (protein-kinases and GTPases).[18] These complexes transmit external changes of the environment to the cells, via signaling pathways that include tyrosine phosphorylation of proteins, such as paxillin and activation of focal adhesion kinases such as FAK, Pyk2, and Src, and serine and threonine kinases; protein kinase C (PKC) and mitogen-activated protein kinase (MAPK).

The possible involvement of these signaling pathways in cardiac remodeling associated with hypertrophy and heart failure is strengthened by the fact that, in vitro, 1) activation of integrins induces the formation of focal adhesion plaques [19], 2) the integrin signal can overlap and even be synergic with those of growth factors [20], and angiotensin II [21, 22, 23], and 3) integrin signaling pathways may be directly

responsive to mechanical forces [24]. Insights into integrin mediated signaling pathways and regulation in cardiovascular system have been mostly restricted to in vitro studies of non-muscle, smooth muscle and endothelial cells. To our knowledge, little work has been done at the level of hypertrophic cardiomyocytes.

The integrins consist of two different chains, α (120-150 kDa) and β (110-190 kDa), linked by noncovalent bounds that are coded by different genes.[25] Integrins are associated in multiple ab dimers and differential splicing of cytoplasmic domains determine a large variety of combinations. The expression of α and β subunits is strictly regulated during development and is cell and ligand specific, several integrins can bind the same component of the ECM. The affinity of integrins to their ligand is low (Kd 10^{-6} M), but ligands have a high density (10^5-10^6 molecules per cell). In cardiomyocytes, β1 integrin is localized near the Z lines.[26] The beta 1D isoform is codistributed with vinculin at both costamere and intercalated disc levels.[27] Mechanical forces stabilize the cellular levels of β1 integrin and vinculin, possibly by regulating their association, with the formation and maintenance of focal adhesion and costameres.[28] Interaction of β1 integrin and the ECM is necessary for the differentiation and the maintenance of a specialized phenotype of cardiac muscle cells.[29] Both integrin ligation and signaling appear to be involved in the cardiac hypertrophic response pathway mediated by α-1 adrenergic agonist. Indeed, adenovirus-mediated overexpression of β1 integrin increases the hypertrophic response of neonatal rat ventricular myocytes to

catecholamines, and suppression of integrin signaling by over-expression of free β1 integrin cytoplasmic domain inhibits this response.[30] Alternative splicing of the β1 transcript results in different isoforms that modulate the integrin adhesive functions. An integrin β1 A isoform is widely expressed in non-muscle cell types and is present in fetal striated muscle cells including cardiomyocytes whereas integrin β1 D isoform, is specifically expressed in cardiac and skeletal muscle cells of adult mammals. In the neonatal cardiac myocytes, β1 integrin combines with the α1, α3, α5, α6 and α7 subunit to mediate attachment to laminin, collagen and fibronectin.[31] In adult, the integrin α7 B isoform is the major partner of β1 D isoform in the cardiomyocytes . On the other hand, the expression of the α1 chain increases together with that of collagen during cardiac hypertrophy.

Recent studies have demonstrated that integrin activation, in concert with G protein activation, might be essential for the growth of cardiomyocytes. For example, balloon-mediated ventricular stretch of isolated adult rat heart results in the rapid activation of FAK, promotes the association of Grb2 with FAK, and causes the activation of extra-cellular signal-regulated kinase 1/2 (ERK) . In addition, stretch of cultured rat neonatal cardiomyocytes results in the rapid activation of FAK and c-Src, the formation of a Grb2-FAK complex, and the activation of p38 MAPK [34]. Furthermore, expression of an integrin β_1 D inhibitor in cultured rat neonatal cardiomyocytes blocks phenylephrine-induced hypertrophy and atrial natriuretic factor (ANF) expression. [33] Finally, expression of a dominant

negative form of FAK, or expression of a mutant form of FAK that is unable to bind to Grb2, in cultured cardiomyocytes blocks phenylephrine-induced hypertrophy and ERK activation .[32, 33] In this study, we tested if G_h activation interacts intergrin activation to promote cardiomyocyte hypertrophic responses and attempted to identify G_h molecular determinants and potential downstream signals involved.

II. MATERIALS AND METHODS

1. Isolation of neonatal rat cardiomyocytes

Neonatal rat cardiomyocytes were isolated and purified using modifications of previously described methods. Briefly, hearts of 1-2 day-old Sprague Dawley rat pups were dissected, and the ventricles washed with Dulbecco's phosphate-buffered saline solution (PBS, pH 7.4, Gibco BRL) lacking Ca^{2+} and Mg^{2+}. Using micro-dissecting scissors, hearts were minced until the pieces were approximately 1 mm^3 and treated with 10ml of collagenase I (0.8 mg/ml, 262 units/mg, Gibco BRL) for 15 minutes at 37°C. The supernatant was then removed and the tissue was treated with fresh collagenase I solution for an additional 15 minutes. The cells in the supernatant were transferred to a tube containing cell culture medium (α-MEM containing 10% fetal bovine serum, Gibco BRL). The tubes were centrifuged at 1200 rpm for 4 minutes at room temperature, and the cell pellet was resuspended in

5 ml of cell culture medium. The above procedures were repeated 7-9 times until there little tissue was remained. Cell suspensions were collected and incubated in 100mm tissue culture dishes for 1-3 hours to reduce fibroblast contamination. The non-adherent cells were collected and seeded to achieve a final concentration of 5 x 10^5 cells/mL. After incubation for 4-6 hours, the cells were rinsed twice with cell culture medium and 0.1 mM BrdU was added. Cells were then cultured in a CO_2 incubator at 37°C.

2. Immunoblot analysis

Treated cells were washed once in PBS and lysed in a lysis buffer (Cell signaling, Beverly, MA, USA) containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 mg/ml leupeptin, and 1 mM PMSF. Protein concentrations were determined using the Bradford protein assay kit (BioRad, Hercules, CA, USA). Proteins were separated in a 12% SDS-polyacrylamide gel and transferred to PVDF membrane (Millipore Co, Bedford, MA, USA). After blocking the membrane with Tris-buffered saline-Tween 20 (TBS-T, 0.1% Tween 20) containing 5% non-fat dried milk for 1 hr at room temperature, membrane was washed twice with TBS-T for 10 min and incubated with primary antibodies for 1 hr at room temperature or for overnight at 4°C. The membrane was washed three

times with TBS-T for 10 min, and then incubated for 1 hr at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies. After extensive washing, bands were detected by enhanced chemoluminescence (ECL) reagent (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Band intensities were quantified using Photo-Image System (Molecular Dynamics, Uppsala, Sweden).

3. RNA interference

For function-blocking experiments, we used small interfering RNA molecules (siRNA) targeted at G_h and β1-integrin mRNA. A 21-nt sequence for siRNA was derived from the rat G_h (GenBank accession no. GI: 56083) and β1-integrin mRNA sequence (GenBank accession no. GI: 24511) and obtained from Ambion, Inc. (TX, USA): small interfering RNA against G_h (sense, 5'-GGGUUACCGGAAUAUCAUCTT-3'; antisense, 5'-GAUGAUAUUCCGGUAACCCTT-3') and β1-integrin (sense, 5'-CCUGGAAAAUAAUGUGUAUTT-3'; antisense, 5'-AUACACAUUAUUUUCCAGGTG-3'). Cardiomyocytes were transfected with si-G_h or β1-integrin duplexes by using siPORT NeoFX (Ambion Inc.). Briefly, RNA duplex (10nM of final concentration) was incubated in serum-free α-MEM containing 15 μl of siPORT NeoFX for 10 min. The complex was added to the empty 60 mm culture plate and then cardiomyocytes (1×10^5 cells per plate) was overlayed onto the culture plate wells containing transfection complexes.

Transfected cells were incubated in normal cell culture conditions until ready for assay.

4. Immunocytochemistry

Cells were grown on 4-well plastic dishes (SonicSeal Slide, Nalge Nunc, Rochester, NY, USA). Following incubation, The cells were washed twice with PBS and then fixed with 4% paraformaldehyde in 0.5 ml PBS for 30 min at room temperature. The cells were washed again with PBS and then permeabilized for 30 min in PBS containing 0.2% triton. The cells were then blocked in PBS containing 10% goat serum and then incubated for 1 hr with rabbit polyclonal HA antibody. The cells were rewashed three times for 10 min with PBS and incubated with FITC-conjugated goat anti-rabbit antibody as the secondary antibody for 1 hr. Photographs of cells were taken under fluorescence by immunofluorescence microscopy (Olympus, Melville, NY, USA). All images were made by using an excitation filter under reflected light fluorescence microscopy and transferred to a computer equipped with MetaMorph software ver. 4.6 (Universal Imaging Corp.).

6. Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis as performed by

student's t-test. Relationships were considered statistically significant when *p* value was less than 0.05.

III. RESULTS

1. Selectivity of adrenoceptors in protein synthesis and DNA

In cardiomyocytes, protein synthesis was widely used as an index for the hypertrophic phenotype. To confirm selectivity of adrenoceptors in neonatal cardiomyocytes, cells were pretreated for 30 min with α1 selective antagonist prazosin (100nM) and β1 selective antagonist propanol (2μM). After 48 hr of stimulation with norepinephrine, total protein content/DNA ratios were estimated. Norepinephrine significantly increased the protein/DNA ratio by about 130% compared to the control of no treatment. In addition, pretreatment of the α1-antagonist, prazocin suppressed the increase of the protein/DNA ratio but pretreatment of a β-antagonist did not (Figure 1). These data showed that the observed hypertrophy in neonatal cardiomyocytes was mediated mainly by α1-AR stimulation.

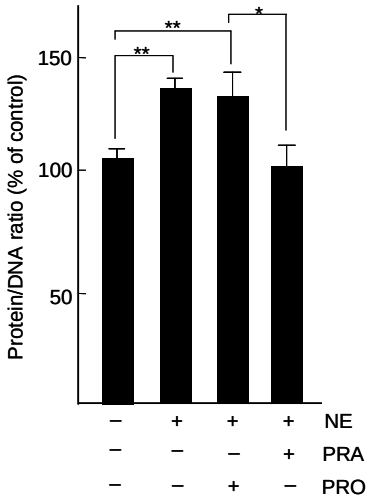

Figure 1. Norepinephrine-induced increase of protein synthesis in cardiomyocytes. After pretreatment with prazosin (PRA) (100 nM) or propranolol (PRO) (2 μM), cardiomyocytes were stimulated by norepinephrine (NE) (10^{-6} M) for 48hr. Total protein content was measured using a bicinchoninic acid (BCA) protein assay and evaluated as a ratio with DNA contents. P <0.05, when compared with the control value.

2. Identification of α1-adrenoceptor mediated hypertrophic response

To further confirm the selectivity of α1-adrenoceptor in norepinephrine-induced hypertrophy, changes of the expression of myosin heavy chain and the constitutive gene, myosin light chain-2v were detected by immumofluorescence images and RT-PCR, respectively. Norepinephrine is known to increase cell surface area and expression of genes encoded sarcomeric proteins and β-MHC, MLC-2v and α-skeletal actin, fetal gene are augmented in cardiac hypertrophy. When cardiac

hypertrophy occurs as a result of many stimuli including norepinephrine, several genes such as α-MHC, cardiac α-actin are transformed into β-MHC and α-skeletal actin.[34] We found that prolonged norepinephrine stimulation significantly increased cell surface area and expression of MLC-2v mRNA. In addition, norepinephrine-induced hypertrophy was reduced to basal control level by α1 selective antagonist prazosin (Figure 2). We interpret these results to mean that hypertrophic responses including protein synthesis and reorganization of actin cytoskeleton were induced by α1-adrenoceptor stimulation in neonatal cardiomyocytes.

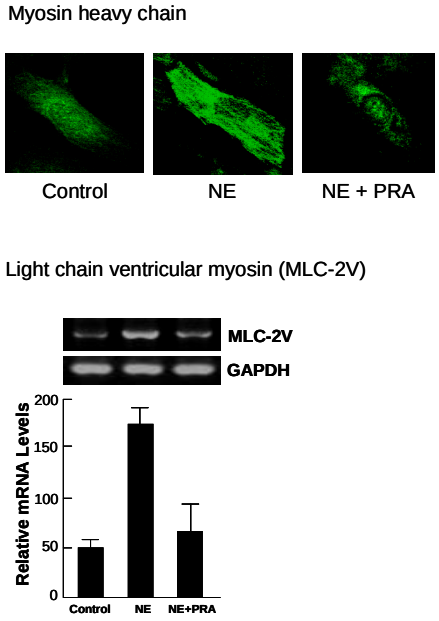

Figure 2. Identification of phenotypic response and mRNA expression of cardiomyocytes to norepinephrine treatment. Immunofluorescence images were obtained by confocal microscope after 48 hr treatment with 10 ($^{-6}$M) NE (×100). mRNA expression is represented by densitometrical analysis (ratio MLC-2v/GAPDH). P <0.05, when compared with the control value.

3. Effect of upstream regulators of ERKs activation in cardiomyocytes stimulated by norepinephrine

The activation of MAPKs is important as a mediator of cardiac hypertrophy but also provide a sensitive and quantitative marker in noncardiac cell lines.[35] Norepinephrine is able to stimulate ERK1,2 activity in cardiomyocytes.[36] In this study, the phosphorylation of ERKs (42 and 44 kDa) were upregulated following treatment with 10 ($^{-6}$M) norepinephrine for 10 min. The activation of ERKs was inhibited by preincubation with α1-AR selective antagonist prazosin (100 nM, 30 min) but not β1-AR selective antagonist propranolol. The inhibition pattern of MEK1, 2, upstream regulrator of ERKs was coincident with that of ERKs (Figure 3). These results show that intracellular signaling pathway induced norepinephrine is primarily processed by MEK1, 2/ERKs cascade through α1-AR in neonatal cardiomyocytes.

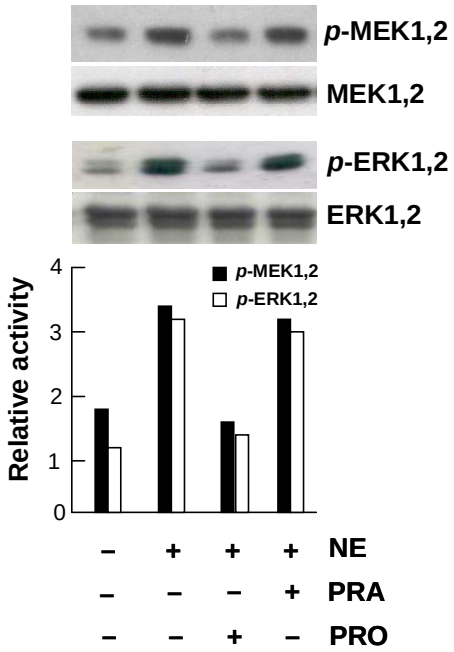

Figure 3. Activity of extra-cellular signal-regulated kinase (ERKs) and MEK1,2 upstream regulators of ERKs, in response to norepinephrine in neonatal rat cardiomyocytes. Activities of ERKs and MER1,2 were detected by immunoblot and their relative responses were based on a basal control level. $P < 0.05$, when compared with the control value.

4. Inhibitory effect of Gh by siRNA transfection in cardiac hypertrophy

Several reports have demonstrated the involvement of G_h, in addition to Gαq, in α1AR-induced hypertrophy. To further confirm this association, we estimated the activation level of ERKs after treatement with siRNA for G_h was treated. Western blot analysis showed that RNA interference inhibits the expression of G_h protein. The ERKs activation increased by norepinephrine was not decreased by propranolol but inhibition of G_h resulted in down-regulated ERK activation to the control level. The

result indicated that G_h partially mediated the α1-AR stimulation by norepinephrine.

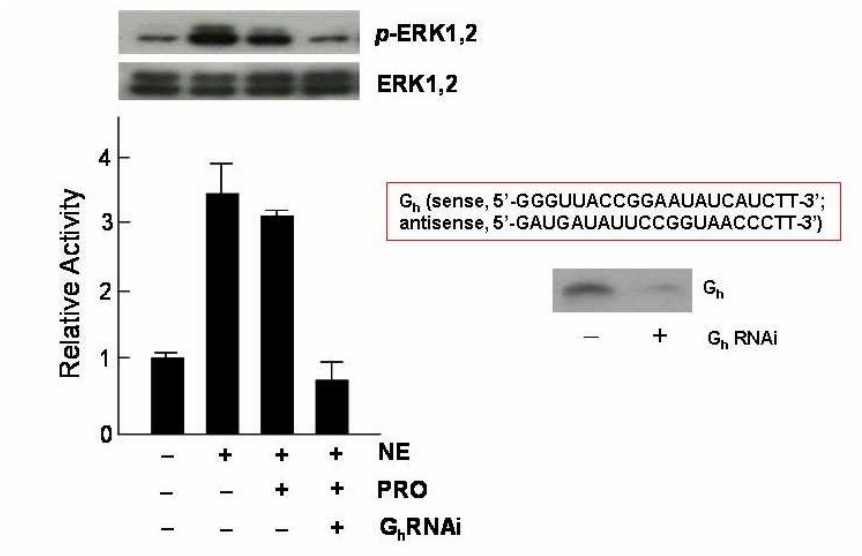

Figure 4. Inhibition of G_h expression by siRNA for G_h inhibited norepinephrine stimulated hypertrophy in cardiomyocytes. Neonatal cardiomyocytes were transfected with siRNA to a final concentration of 10nM or vehicle. After pretreatment with propranolol (2μM), cardiomyocytes was stimulated by 10^{-6}M norepinephrine for 10 min. Each signal was detected by immunoblot analysis using anti-ERK antibody and was quantified by densitometrical analysis. P <0.05, when compared with the control value.

5. Integrin-mediated signal transduction in hypertrophic cardiac myocytes

To determine whether β1-integrin is coupled to cardiomyocyte hypertrophy via norepinephrine, the expression level of β1-integrin was examined after pretreatment with α1/ β-antagonist and siRNA for G_h. Although β1-integrin expression increased about 3 fold after the stimulation with norepinephrine, expression decreased after prazosin pretreatment and G_h inhibition. The pattern of β1-integrins expression was similar to that of ERKs activation. The similarity between β1-integrin expression patterns and phosphorylation of ERKs demonstrates that β1-integrin relates α1-adrenoceptor mediated hypertrophic response in cardiomyocytes and β1-integrin might correlate with G_h pathway.

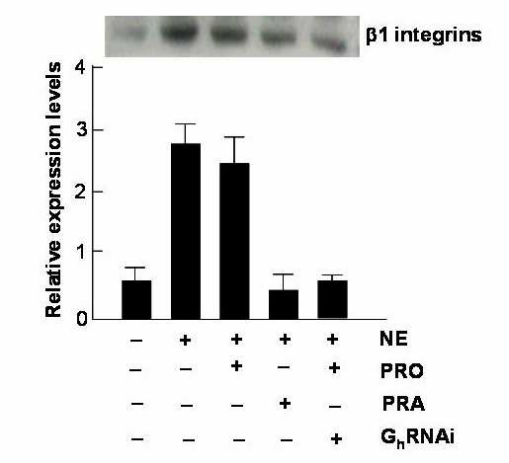

Figure 5. β1-integrins participate in the hypertrophic response of cardiomyocytes. β1-integrins were estimated by immunoblot assay and quantified by densitometrical analysis. P <0.05, when compared with the control value.

6. Effect of G_h and β1 integrin on hypertrophic response in NE-stimulated cardiomyocytes

Previous data have showen that G_h and β1 integrin are related to cardiac hypertrophy. We investigated other factors influencing the hypertrophic response in addition to phosphorylation level of ERKs. It has been documented that β1 integrin participate in the hyeprtrophic response by β1 integrin overexpression.[37] After G_h and β1 integrin was individually blocked by RNA interference, MLC-2v typically constitutive gene of cardiomycoytes, was examined by RT-PCR. Western blot analysis showed that the expression of β1 integrin was inhibited by RNA interference. The level of MLC-2v mRNA was increased with norepinephrine treatment and significantly reduced by inhibition of G_h and β1 integrin about to basal level (Figure 6).

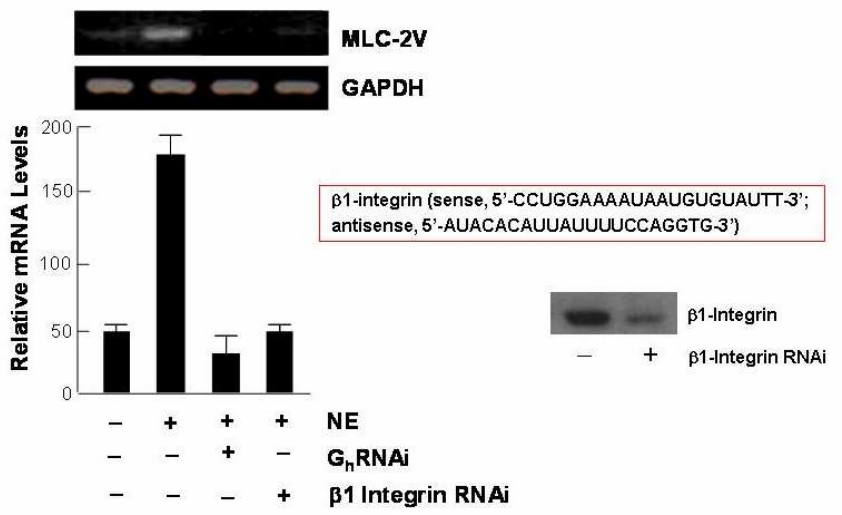

Figure 6. Effect of G_h and β1 integrin on hypertrophic response in norepinephrine-stimulated cardiomyocytes. Carciomyocytes was treated siRNA for 12 hr to block each gene , then incubated for 10min with 10μM norepinephrine, followed by 12 hr with media containing 0.1% FBS.

7. Regulation of Gh and β1 integrin for downstream factors in NE-stimulated cardiomyocytes

In general, the cytoplasmic protein tyrosine kinase focal adhesion kinase (FAK) is activated by many factors including growth factors or G-protein coupled receptor and plays an important role in mechanism of integrin-dependent adhesion on the ECM. To examine whether FAK might be associated with the hypertrophic signaling pathway through α1-AR, phosphorylation of FAK was measured after G_h

or β1-integrin expression was inhibited by siRNA treatment. Treatment of cardiomyocte with 10 μM NE for 10 min enhanced the phosphorylation of FAK but FAK activity was prevented in spite of norepinephrine stimulation by inhibition of each gene (Figure 7).

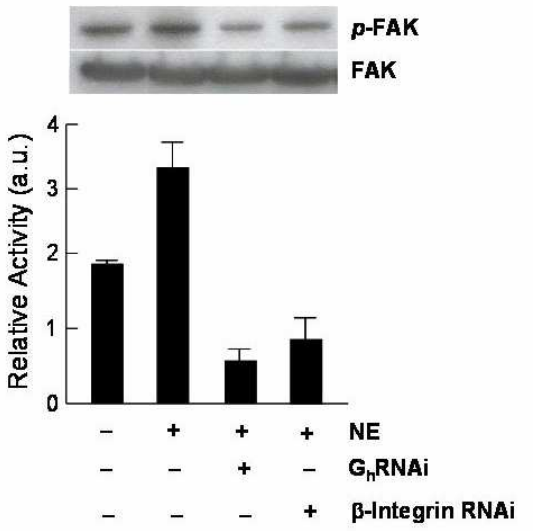

Figure 7. Effect of G_h and β1 integrin on FAK activation in norepinephrine-stimulated cardiomyocytes.

Shc, a regulator of FAK, was also examined by flow cytometry. Expression of Shc was increased to 2 fold relative to the basal level, and reduced by siRNA treatment for G_h and β1-integrin (Figure 8). These results suggest that both G_h and β1-integrin can affect hypertrophic responses via the Shc and FAK signaling pathway.

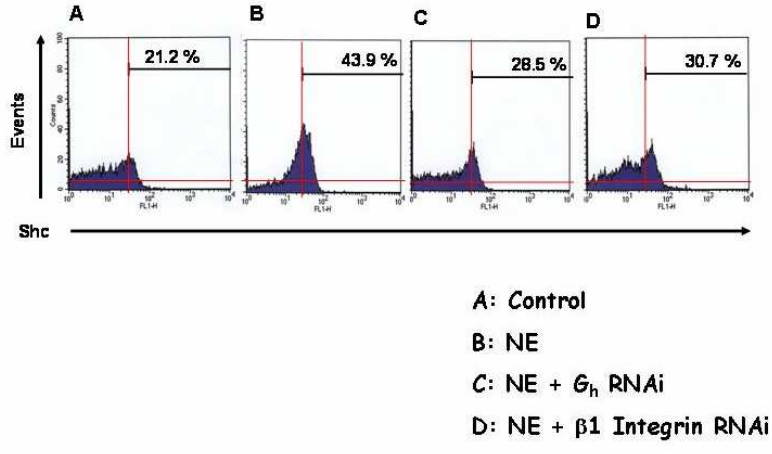

Figure 8. Effect on G_h and β1 integrin on Shc expression in NE-stimulated cardiomyocytes. Shc expression was analyzed by flow cytometry with anti-Shc integrin antibody.

IV. DISCUSSION

Cardiac hypertrophy is related to an increased risk of cardiac arrhythmias, diastolic dysfunction, congestive heart failure, and death[2,3] and is a compensatory process which occurs in pathological conditions, such as hypertension, myocardial infarction, and some genetic heart diseases.[3] Although many previous studies have underlined the relationship of Gαq and $G\alpha_{12/13}$ with hypertrophy in cardiomyocytes,[61] Some groups have been recently reported that cardiac hypertrophy by α1-AR stimulation was also related to the G_h pathway.[39,40,41,42]

The relationship between increased adrenergic activity and cardiac hypertrophy has been documented for quite a some time, but the mechanisms have not been clearly

understood until recent studies. The present study shows that norepinephrine induces cardiac hypertrophy through α1-AR stimulation and G_h is partially mediated hypertrophy through β1 integrin/MEK1,2/ERKs pathway. It is first demonstrated that G_h-coupled receptor was related to β1 integrin in cardiomyocytes except chondrocytes.

In neonatal cariomyocytes, α1-AR mediation plays an important role in hypertrophic response. In the current study, confocal microscopy was widely applied to prove that adrenergic stimulation caused significant increases in the cell volume of cultured cardiomyocytes. Figure 1 and 2 demonstrate that the selectivity of adrenoceptors in cardiac hypertrophy has an effect on protein synthesis and estimation of constitutive gene MLC-2 and MHC.

Cardiac hypertrophy altered the phosphorylation of both ERK 1, 2 and their upstream regulator MEK 1,2 which is important because the change of MAPK is an important result of hypertrophy (Figure 3). We demonstrated through RNA interference experiment that G_h might be related to cardiac hypertrophy (Figure 4). Tushi et al[62] and Lee et al[63] showed PLC might be a key component in initiation of the cardiomyocytes hypertrophic response. Protein synthesis was attenuated by PLC inhibitor, U73122 in a concentration dependent manner and DAG formation was decreased. In addition, PLC-δ1 mediates the signal pathway through G_h-coupled receptor signaling.[63]

Many stimuli are known to promote cardiac hypertrophy including endothelin-1

(ET-1), phenylepinephrine, and norepinephrine. Such agonists stimulate cellular signaling pathway via protein kinase cascade. Protein kinase cascade also activates the extra-cellular signal-regulated protein kinase 1, 2 (ERK 1, 2), subfamily of MAPKs.

Although integrins were initially regarded as necessary molecules for adhesive interactions between cells and the ECM, present studies have indicated that integrins might be bidirectional signaling molecules.[59] Glembotski et al. examined the regulatory role of β1 integrins in the hypertrophic response of adrenergically stimulated NRVMs because β1 integrins are abundant in the cardiac cell.[60] Figure 5 and Figure 6 firmly demonstrate that β1-integrin is related to cardiomyocyte hypertrophy. Ross et al showed that overexpression of β1 integrins in cardiomyocytes increased hypertrophic marker gene expression and protein synthesis but had no effect on cellular DNA synthesis. Likewise, inhibition of integrin signaling downregulated the adrenergically stimulated hypertrophic gene response.[59] Our results are in agreement with our these data.

β1 integrin induced focal adhesion formation at focal adhesion sites. Focal adhesion complexes including FAK, Src, cytoskeletal proteins and signal transduction molecules can directly regulate transcription and translation.[64,65,66] Figure 7 and Figure 8 show that such signaling molecules are directly related to both integrin and G_h protein.

V. CONCLUSIONS

In conclusion, we found that G_h partially mediated the α1-AR stimulation by norepinephrine. We linked β1 integrin signaling to the hypertrophic pathway in a cellular model using α1 adrenergic specific stimulation of neonatal cardiomyocytes. Finally, this study was the first to evaluate whether the hypertrophic response induced by norepinephrine in cardiomyocytes occurs through β1 Integrin/Shc/FAK/MEK1, 2/ERKs signal pathways via α1-AR/Gh.

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국문 요약 (In Korean)

Interaction of α-adrenergic stress and integrin-mediated signal transduction in hypertrophic cardiac myocytes

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심근세포에서 alpha(1)-adrenoceptor(AR)의 자극은 심근비대를 유발한다. G_h 단백질(transglutaminase II, TGII)는 tissue type transglutaminase이며 GTPase 활성을 통해 alpha(1B)-adrenoceptor 신호를 전달한다. 최근에, G 단백질뿐만 아니라 인테그린 활성화는 심근세포의 생장에 필수적일 수 있다. 여기서 우리 연구의 목적은 활성화된 G_h와 integrin이 상호 반응하여 심근세포의 비대 반응을 야기하는지를 검증하고 G_h 결정인자와 관련된 하위 신호전달 체계를 규명하는 것이다. 첫째, 우리는 MLC-2와 MHC 구조 유전자의 측정과 단백질 합성에 의해 norepinephrine(NE)에 의해 야기된 심장 비대증에서 α-adrenoceptor의 특이성을 관찰하였다. NE에 의한 심장비대 반응은 ERK1,2와 이들의 상위조절자인 MEK1,2를 활성화시키나 이들은 G_h에 대한 siRNA 처리에 의해 억제된다. 우리는 또한 NE에 의한 심장비대증에서 β1-integrin의 상당한 활성화를 관찰하였다. 게다가, NE에 의해 야기되는 심근세포의 비대에서 FAK과

이것의 조절자인 Shc는 직접적으로 integrin β1과 Gh 단백질과 연관되어 있다. FAK과 Shc의 활성화는 G_h에 대한 siRNA처리로 억제할 수 있다. 결론적으로, norepinephrine에 의한 심근세포의 비대는 α1-AR/ G_h를 거쳐서 β1 Integrin/Shc/FAK/MEK1,2/ERKs 신호 전달체계를 통해 일어난다.

핵심되는 말 : 심근세포: 비대: 인테그린: 알파 교감신경 수용체