Transglutaminase II (G_h) mediates MEK1,2/ERKs activation through the α_1-adrenergic receptor in norephinephrine-stimulated neonatal rat cardiomyocytes

Thesis by

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Directed by Professor Seung Yun Cho

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ABSTRACT

Transglutaminase II (G_h) mediates MEK1,2/ERKs activation through the α_1-adrenergic receptor in norephinephrine-stimulated neonatal rat cardiomyocytes

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Background In cardiac myocytes, stimulation of α_1-adrenoceptor (α_1-AR) leads to a hypertrophic phenotype. The G_h protein is tissue type transglutaminase and transmits the α_{1B}-adrenoceptor(AR) signal with GTPase activity. Little is known about G_h involvement in the activation of mitogen-activated protein (MAP) kinase cascade to develop the hypertrophy. The aim of this study was to elucidate whether G_h mediates norepinephrine-stimulated intracellular signal transductions leading to activation of extracellular signal-regulated kinases (ERKs) and neonatal rat cardiomyocyte hypertrophy,

Methods and Results To elucidate the molecular mechanism of α_1-adrenoceptor/G_h-mediated hypertrophic responses, we examined the effects of norephinephrine on the activation of ERKs and on the protein synthesis in neonatal rat cardiomyocytes. Norepinephrine-induced ERKs activation was inhibited by an α_1-adrenoceptor blocker (prazosin), but not by an β-adrenoceptor blocker (propranolol). Overexpression of the

G_h protein potentiated norepinephrine-induced ERKs activation, which was inhibited by α-adrenoceptor blocker (prazosin). Co-overexpression of G_h and calreticulin, the regulatory protein of G_h, abolished norepinephrine-induced ERKs activation.

Conclusion Norepinephrine induces hypertrophy in neonatal rat cardiomyocytes through α_1-AR stimulation and G_h is partly involved in norepinephrine-induced MEK1,2/ERKs activation. Activation of G_h-mediated MEK1,2/ ERKs is completely inhibited by calreticulin.

Key Words: Neonatal rat cardiomyocyte; hypertrophy; ERKs; G_h; calreticulin.

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I. INTRODUCTION

Norepinephrine is a direct hypertrophic effector on cardiac myocytes without afterload[1,2,3]. The mechanisms through which norepinephrine induces cardiomyocyte hypertrophy are as yet controversial. Two major subtypes of norepinephrine-mediated receptor are α and β-AR. The precise responses mediated by these ARs have not yet been confirmed. Among α-ARs, the α_1-ARs but not the α_2-ARs exists in cardiomyocytes and α_1-ARs have been implicated in the pathogenesis of cardiac hypertrophy, in ischemia-induced cardiac arrythmias, and ischemic preconditioning[4]. At the present time, α_1-ARs are divided into three subtypes, α_{1A}[5], α_{1B}[6], and α_{1D}[7,8,9,10], based on the differential affinity of receptors for the agonist oxymetazoline and the antagonists WB-4104 and phentolamine. It has been reported that α_1-ARs are involved in cell growth and hypertrophy. In neonatal cardiomyocytes, the hypertrophic response

is not mediated by β-ARs but is due to an exclusive α_1-AR mechanism[11]. Thus, activation of myocyte α_1-ARs increases protein synthesis on account of increased transcriptional and translational activity. In case of α_1-ARs stimulation, there is an initial rapid induction of protooncogenes within 15 to 30 minutes, leading to translation of immediate-early genes, *c-fos, c-jun, c-myc*, and *egr-1*. After α_1-ARs consistent stimulation for 12 to 24 hours, there is reactivation of a series of embryonic genes including atrial natriuretic factor, β-myosin heavy chain, and skeletal α-actin[12]. In vascular smooth muscle, α_1-AR activation also causes a rapid (within 6 hours) increase in the expression of the growth-related genes, platelet-derived growth factor–A chain, and ornithine decarboxylase, without an increase in DNA synthesis[13]. After 24 to 48 hours of α_1-AR consistent activation, the expression of contractile protein genes, including myosin light chain-2 and cardiac α-actin, is increased in cardiac myocytes and is associated with an increase in the number of contractile units[14]. This pattern of gene induction is specific for α_1-ARs activation and is distinct from that observed with β-AR stimulation or with thyroid hormone–induced hypertrophy[15]. The cellular pathways involved in these α_1-AR-mediated responses remain to be understood, but some evidences indicate the relationship between the α_1-AR subtype and G_q. This receptor activation affects the growth factor pathway involving the

protooncogene, ras, and its downstream kinases, raf-1, mitogen-activated protein kinase kinase, mitogen-activated protein kinase, and S6 kinase[16]. Activation of this cascade probably involves receptor-stimulated polyphosphoinositol turnover as well as activation of PKCβ[17]and a tyrosine kinase[18]. The nuclear pathways involved have not been completely elucidated, but a 9-bp "core" enhancer element corresponding to transcriptional enhancer factor-1 has recently been identified on the β-myosin heavy chain promoter. The activation of this enhancer element is required for β-myosin heavy chain synthesis by both α_1-AR and by the β isoform of PKC[19]. Induction of skeletal actin gene expression by α_1-AR stimulation also involves both transcriptional enhancer factor-1 and a CArG box[19]. The physiological role for individual AR subtypes appears to have been eloquently dissected using transgenic/knockout mice[20].

Norepinephrine, epinephrine and catecholamines activate α_1-ARs which are members of G-protein coupled receptor superfamily. The G proteins coupling α_1-ARs to their intracellular effectors and the selectivity of the various α_1-ARs subtypes for different G proteins remain to be clearly defined. α_1-ARs couple predominantly to pertussis toxin–insensitive G proteins of the $G_{q/11}$ family. Specifically, G_q, G_{11}, G_{14}, and G_{16} can mediate phosphoinositol turnover by the α_{1B}-ARs, but the α_{1D}-ARs couples only via G_q or G_{11}[21]. In studies of intact tissue[22] and of the various cloned α_1

subtypes expressed in eukaryotic expression systems[23], the individual α_1 subtypes can activate multiple effectors via coupling to both pertussis toxin–sensitive (G_i or G_o family) and –insensitive G proteins. Moreover, both α and $\beta\gamma$ subunits of the pertussis toxin–insensitive G proteins may mediate activation of certain receptor-coupled effectors, such as the β isoform of PLC (PLCβ)[24]. In addition to activation of PLCβ via $G_{q/11}$, there is recent evidence that the α_{1B}-ARs activates a 69-kD PLC via coupling to a high molecular mass (74-kD) class of pertussis toxin–insensitive G proteins, termed G_h, that are distinct from the heterotrimeric G proteins[25]. These G_h proteins are multifunctional proteins with both transglutaminase and receptor signaling functions and are expressed in various tissues, including brain, heart, and liver. Interestingly, binding of GTP with G_h inhibits its transglutaminase activity, whereas binding of Ca^{2+}, which is required for transglutaminase activity, prevents GTP binding. The selectivity of α_1-ARs coupling to PLC via G_q versus G_h remains to be defined, although there is recent evidence to suggest that the G_h system may mediate α_1-ARs activated inositol bi-phosphate rather than inositol tri-phosphate production in intact myocardium[26] and Ca^{2+} mobilization in rat embryo brain cells[27].

Although the role of G_h is still ambiguous under physiological and pathological condition, G_h, a high molecular mass (74 kDa) class of pertussis toxin-insensitive G

proteins, couples the α_{1B}-AR signal to PLC-δ1 through its GTPase function in vitro (Fig. 1)[28,29,30]. These G_h proteins that are distinct from heterotrimeric G proteins, are multifunctional proteins with both transglutaminase and GTPase. The regulator of G_h as transglutaminase and GTPase function is GTP. Recently, it has been shown that the calreticulin down-regulates both GTP binding and transglutaminase activities of G_h[31].

Many investigations have focused on the potential role of MAP kinases as effectors that regulate cardiac hypertrophy in response to signals arising from the cell surface[32,33]. MAP kinases are serine/threonine protein kinases and the importance of MAP kinases in cardiac hypertrophy has been addressed using pharmacological inhibitors of MAP kinase signaling in cultured cardiomyocytes[34,35]. Among the MAP kinases, ERKs has been focused on the essential regulators of a hypertrophic response although the role JNK and p38 were recently studied in regulating cardiac hypertrophy[36].

The goal of the present study was to determine whether the α_1-AR/G_h system affects the generation of hypertrophic signals with MAP kinase activation and calreticulin down-regulates the function of G_h. Here, we provide data showing that norepinephrine induces hypertrophy in neonatal cardiomyocytes through α-adrenoceptor stimulation and G_h is partly involved in norepinephrin-induced ERKs activation. Activation of G_h-mediated MEK1,2/ERKs is completely inhibited by calreticulin.

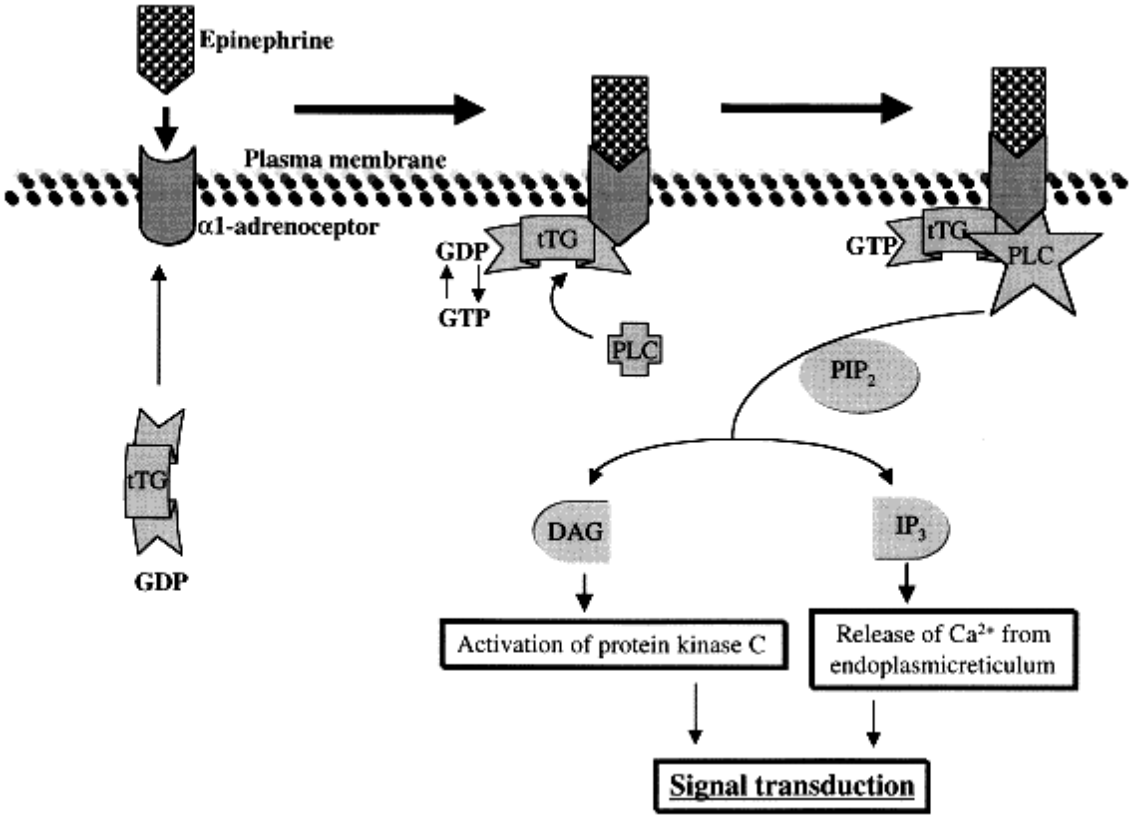

Fig. 1. G_h in α_1-adrenoceptor signaling pathway.
tTG; transglutaminase II, PLC; phospholipase C, DAG; diacylglycerol, PIP_2; phosphatidylinositol 4,5-biphosphate, IP_3; inositol 1,4,5-triphosphate, GDP; guanosine 5'-diphosphate; GTP; guanosine 5'-triphosphate

II. METHODS

1. Materials

Norepinephrine and prazosin were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Phospho-ERKs, calreticulin, and G_h polyconal antibodies were purchased from New England Biolabs (Beverly, MA,U.S.A.). Collagenase type I and elastase for cell preparations were obtained Sigma Chemical Co. and Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco Life technologies (Gaithersberg, MD, U.S.A.).

2. Isolation of neonatal rat cardiomyocytes.

Neonatal rat cardiomyocytes were isolated and purified by the modifications of previously described methods[37,38]. Briefly, hearts of 1-2 day-old Sprague Dawley rat pups were dissected, and the ventricles washed with Dulbecco's phosphate-buffered saline solution (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 left. 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 5×10^5 cells/mL. After incubation for 4-6 hours, the cells were rinsed twice with cell culture medium and 0.1 mM BrdU added. Cells were then cultured in a CO_2 incubator at 37°C. For the stimulation of norepinephrine (10^{-5} M), the confluent cells were rendered quiescent by culturing for 72 hr in 0.5% (v/v) FBS instead of 10%.

3. Transfection

Transfections of G_h and calreticulin genes cloned into the eukaryotic expression vector

pMT2' were performed using LIPOFECTAMIN PLUS^{TM} reagent (Gibco BRL). Breifly, neonatal rat cardiomyocytes cultured in a 60 mm culture plate ($5X10^5$ cells/plate) were washed twice with serum-free DMEM. LIPOFECTAMIN PLUS^{TM} reagent was diluted with serum-free DMEM and combined with 5μg of DNA for the each plate. The DNA and LIPOFECTAMIN PLUS^{TM} reagent was added into the each plate containing fresh medium on cells. After 12 h incubation in a CO_2 incubator at 37℃, the medium were exchanged with 10% FBS-DMEM. The cells were further incubated for 48 h at 37℃. For the stimulation of norepinephrine (10^{-6} M), the confluent cells were rendered quiescent by culturing for 24 hr in 0.5% (v/v) FBS.

4. Quantification of total protein and DNA from neonatal cardiomyocytes

Total protein content/DNA ratios were measured after solubilizing the cells in 1N NaOH at 60℃ for 30 minutes[2]. Total protein content was determined with BCA protein reagent (Pierce Chemicals, Rockford, IL, U.S.A.) with a bovine albumin standard according to the manufacturer's direction. For the quantitative measurement of DNA, cells were lysed by adding SDS and proteinase K, and extraction of DNA was performed with phenol. The absorbance of the purified DNA was measured at 260 nm.

5. Immunoblot analysis

Proteins were separated by SDS-PAGE using 10-12% polyacrylamide gel and then electrotransferred to methanol-treated polyvinylidene difluoride membranes. The blotted membranes were washed twice with water and blocked by incubation with 5% nonfat dried milk in PBS buffer (8.0 g NaCl, 0.2 g KCl, 1.5 g NaH_2PO_4, 0.2 g K_2HPO_4 per liter). After a 1 hour incubation at room temperature, the membranes were probed overnight at 4℃ with polyclonal antibodies against calreticulin and G_h followed by goat anti-rabbit IgG-peroxidase. The blots were detected using enhanced chemiluminescence kits (ECL, Amersham Pharmacia Biotech.). For the phosphorylation analysis of ERKs, the membranes were probed with antiphospho-ERKs antibody.

6. Statistical analysis

The data are expressed as mean ±SE. p values < 0.05 were considered to be statistically significant (analysis of variance and Student's t test)

III. RESULTS

1. Selectivity of adrenoceptors in norepinephrine-stimulated ERKs activation

In the mechanisms of cardiac hypertrophy, the activation of ERKs plays an important role in gene regulation[39,40] and is a sensitive and quantitative marker for hypertrophic responses of cardiac myocytes [41,42]. Although α_1-AR as well as β-AR agonists stimulate the protein synthesis and results in hypertrophy, the selective pathways through which norepinephrine induces cardiomyocyte hypertrophy are not defined yet. We first examined which AR was stimulated by norepinephrine in neonatal rat cardiomyocytes (Fig. 2). The phosphorylation activities of ERKs (42 and 44 kDa) and MEK1,2 were increased by the treatment with 10^{-6} M norepinephrine for 10 minutes. Activations of ERKs and MEK1,2 were abolished by preincubation with a α_1-AR-selective antagonist prazosin (100 nM, 30 min) but not β-AR-selective antagonist propanolol (2 μM, 30 min) indicating that activation of ERKs was mainly mediated by α_1-AR stimulation in neonatal rat cardiomyocytes. To further confirm the selectivity of ARs in norepinephrine-induced ERKs activation, 10-fold different concentrations of each inhibitor were treated at the above conditions. The activation

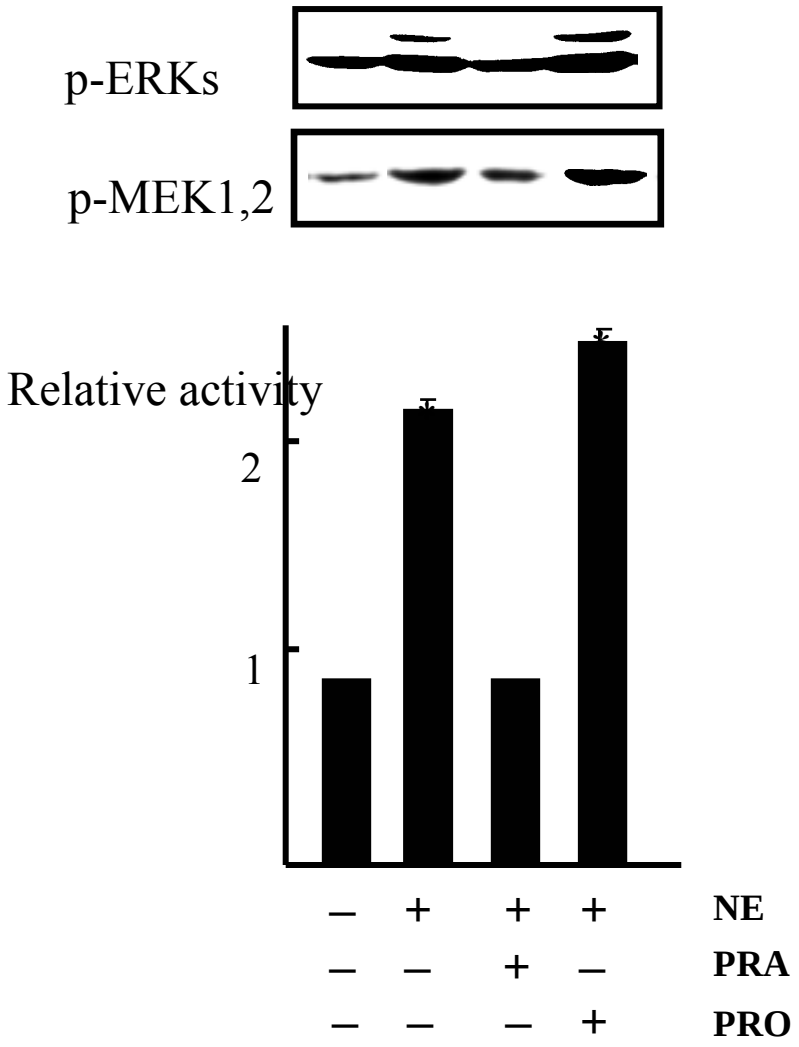

Fig.2. ERKs activation by norepinephrine through α_1-adrenoceptor. After pretreatment with prazosin(PRA) (100 nM) or propranolol (PRO) (2 μM), cardiomyocytes were stimulated by norepinephrine(NE) (10^{-6} M) for 10 minutes. Each column represents the mean ±SE(n=6). *$p<0.05$, when compared with the control value.

of ERKs was mediated through α_1-AR with a clear dose dependency of norepinephrine (Fig. 3). These results were in good agreement with the α_1-AR-stimulated hypertrophy in adult rat ventricular myocytes and the norepinephrine-induced hypertrophy in cultured cardiomyocytes of neonatal rats through α_1-AR stimulation[42]. Next, the activation of norepinephrine-induced ERKs was examined in different time. As was shown in Fig. 4, the maximal activity of ERKs by 10^{-6} M norepinephrine was detected at about 10 minutes and the activity reached to basal levels within 60 minutes after stimulation of norepinephrine, indicating consistent results with the previous report[43].

2. Increase of protein synthesis in in norepinephrine-stimulated neonatal rat cardiomyocytes.

To further confirm whether there are discrepancies between ERKs activation and hypertrophic responses, total protein content/DNA ratios were measured in cardiomyocytes after stimulation with norepinephrine (10^{-6} M) and prazosin (100 nM) and/or propanolol (2 μM). After 48 hours of stimulation, norepinephrine significantly increased the protein/DNA ratio to 127%, based on the no treatment. Whereas propanolol, a β-AR blocker, increased the protein/DNA ratio to 121%, prazosin, a α_1-

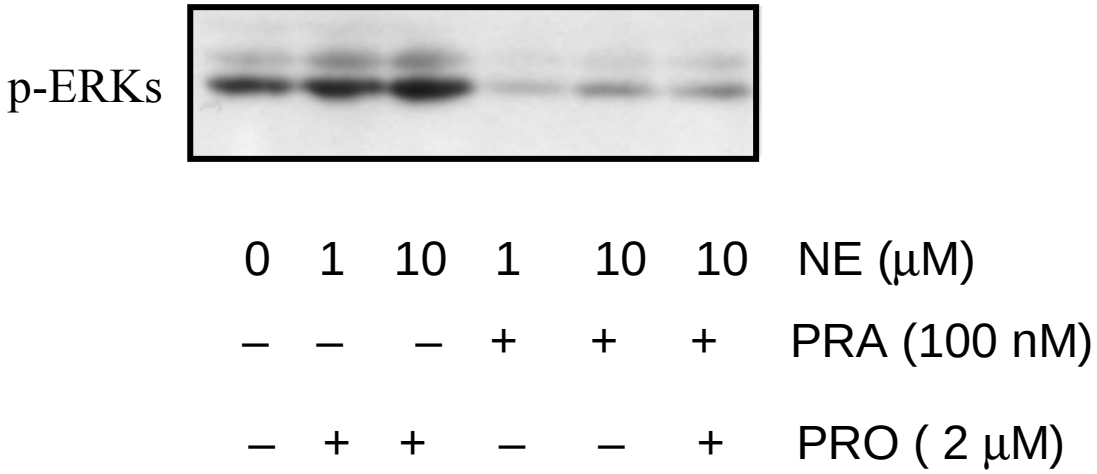

Fig. 3. Selectivity of adrenoceptors in norepinephrine-induced ERKs activation. After pretreatment with prazosin(PRA) (100 nM) or propranolol(PRO) (2 μM), cardiomyocytes were stimulated by norepinephrine (10^{-6} or 10^{-5} M) for 10 minutes.

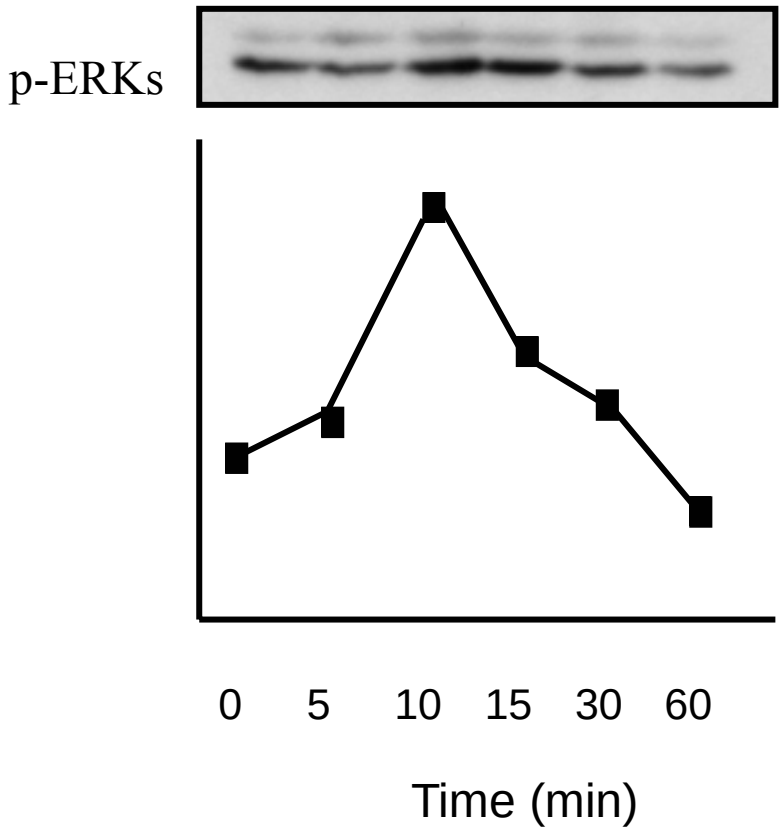

Fig. 4. Time course of ERKs activation induced by norepinephrine(NE). After pretreatment with prazosin(PRA) (100 nM) or propranolol(PRO) (2 μM), cardiomyocytes were stimulated by norepinephrine (10^{-6} M) for indicated times.

AR blocker did not increased the protein/DNA ratio (Fig 5). These data suggested that ERKs activation by norepinephrine played an essential role in cellular proliferation and differentiation in neonatal rat cardiomyocytes.

3. Effect of overexpression of G_h protein on the ERKs activation

We next examined the possibility that G_h protein mediates the activation of ERKs in norepinephrine-induced α_1-AR stimulation because α_{1B}-AR was coupled with G_h in human heart[30]. To characterize the mechanisms of ERKs via a pertussis toxin-insensitive G protein, G_h in neonatal rat cardiomyocytes, we employed a transiently transfected model system. The full-lengh G_h cDNA cloned into pMT2' was transfected into neonatal cardiomyocytes and the expressed protein was recognized by the guinea pig TGase II antibody and was of the expected sizes with ~80kDa. Overexpression of G_h resulted in a consistent 5-fold stimulation of ERKs phosphorylation and propanolol had no effect on the ERKs activation augmented by G_h overexpression. The ERKs activation increased by G_h overexpression was inhibited by prazosin but was not leaded to the control level, indicating that G_h partly mediated the α_1-AR stimulation by norepinephrine (Fig. 6). Although the selectivity of α_1-AR signal mediation via G_q

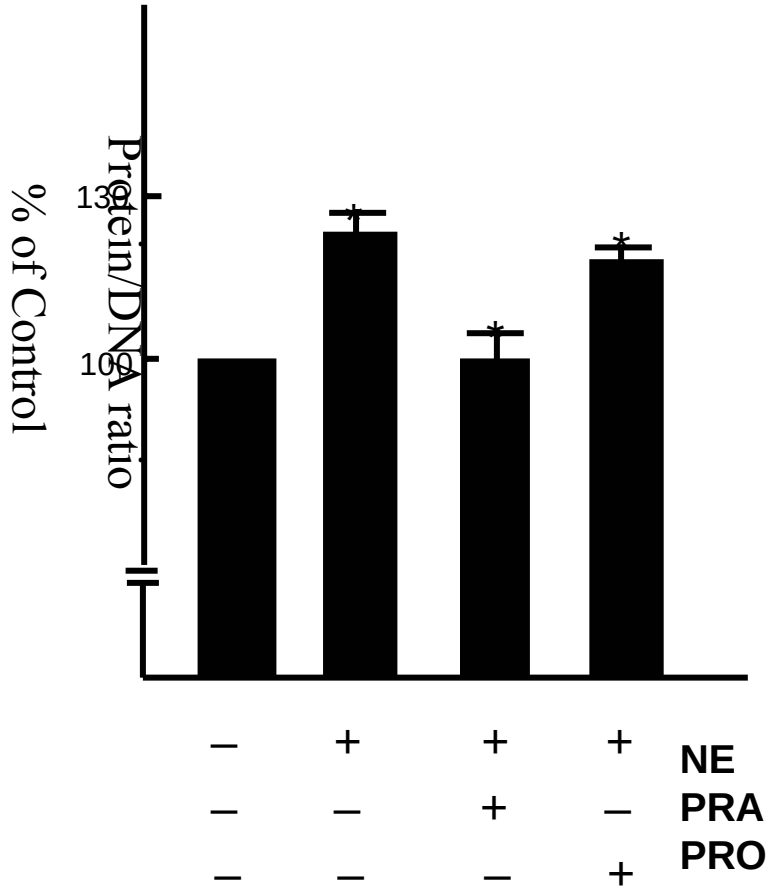

Fig. 5. Effect of norepinepnrine on protein systhesis in neonatal cardiomyocytes. Quiescent neonatal cardiomyocytes were treated with norepinephrine (10^{-6} M) for 48 hours. Total protein contents were measured with the BCA protein assay reagent and corrected by DNA contents. Each column represents the mean $\pm$ SE (n=6). *$p < 0.05$, when compaired with the control value.

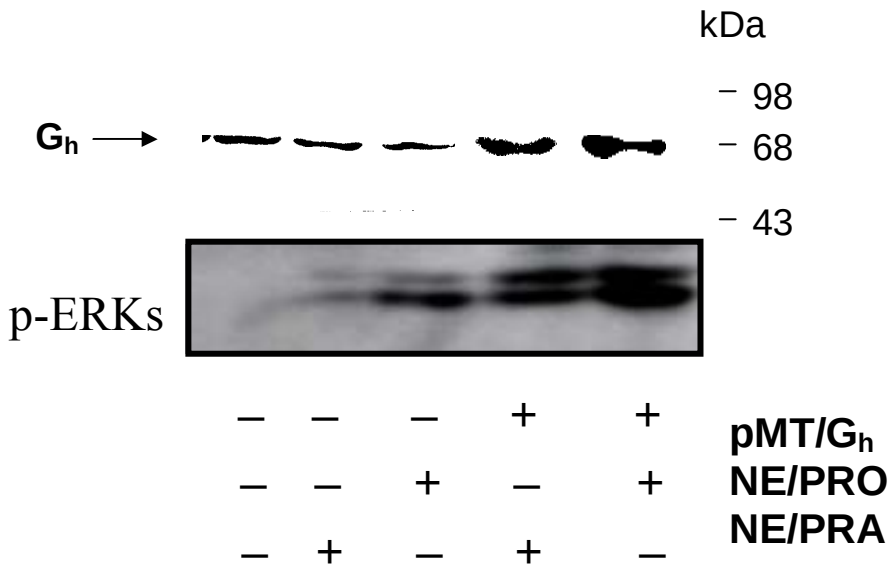

Fig. 6. Effect of overexpression of G_h on the ERKs activation. Neonatal rat cardiomyocytes were transiently transfected with plasmid DNA encoding G_h (5 μg/ well) or empty vector. ERKs activation in these cells was determined after 24 h of serum starvation. After pretreatment with prazosin(PRA) (100 nM) or propranolol(PRO) (2 μM), cardiomyocytes were stimulated by norepinephrine (NE) (10^{-6} M) for 10 minutes.

versus G_h remains to be defined[45], our results suggest that the α_1-AR/G_h systems play a role of the ERKs activation in norepinephrine-induced neonatal rat cardiomyocytes. To further confirm the ERKs activation increased by G_h, the different dose of G_h gene was transfected into neonatal rat cardiomyocytes. The norepinephrine-induced ERKs activation showed the dose dependency of the expressed G_h protein, suggesting that G_h overexpression leaded to the ERKs activation (data not shown). Although the activation of MAP kinases through α_1-AR was mediated by many different G-protein subunits, such as G_q, G_i and $G_{\beta\gamma}$[46], these mechanisms was independent from each signaling pathways.

4. Inhibitory effect of calreticulin on the MEK1,2/ERKs activation by the overexpression of G_h

G_h proteins that are distinct from heterotrimeric G proteins, are multifunctional proteins with both transglutaminase and GTPase. The regulators of G_h as transglutaminase and GTPase function are GTP and Ca^{2+}; binding of GTP by G_h inhibits its transglutaminase activity, whereas binding of Ca^{2+}, which is required for transglutaminase activity, prevents GTP binding. Recently, it was reported that the calreticulin down-regulates

both GTP binding and transglutaminase activities of TGII[31]. To address the possibility of down-regulation of calreticulin in G_h-mediated ERKs activation, both G_h and calreticulin was overexpressed in neonatal rat cardiomyocytes. As Fig. 7, overexpression of calreticulin decreased the MEK1,2/ERKs activation by G_h supporting the notion that calreticulin down-regulates both GTP binding and transglutaminase activities of TGII.

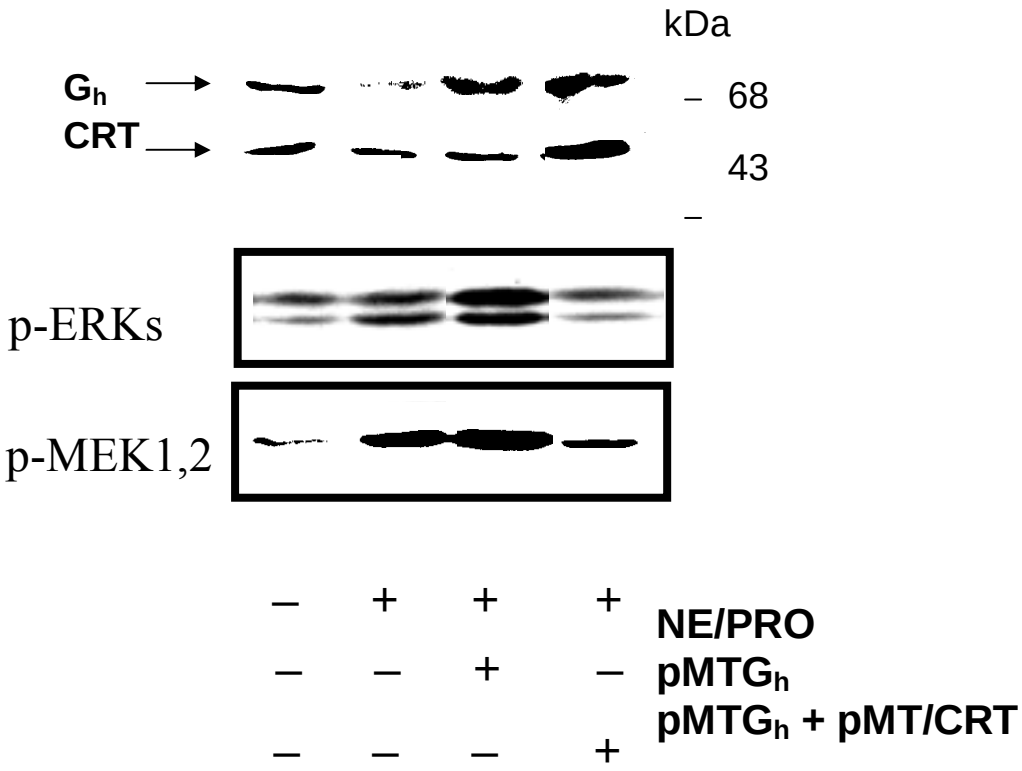

Fig. 7. Inhibitory effect of calreticulin (CRT) on the MEK1,2/ERKs activation induced by the overexpression of G_h. Neonatal rat cardiomyocytes were cotransfected with plasmid DNA encoding G_h (5 μg/well) plus plasmid DNA encoding CRT (5 μg/well). Cells were serum-starved for 24 h and stimulated for 10 min and ERKs phosphorylation was determined.

VI. DISCUSSION

Cardiac hypertrophy is a compensatory process that occurs in pathological conditions, such as hypertension, myocardial infacrtion, and some genetic heart disease[47]. Even though the relationship between increased adrenergic activity and cardiac hypertrophy has been documented previously, the precise mechanisms remain to be clearly defined. Up to now, it is generally accepted that both α_1- and β-adrenergic activations are related to cardiac hypertrophy.

The present study shows that norepinephrine induces hypertrophy in neonatal rat cardiomyocytes through α_1-AR stimulation and G_h is partly involved in norepinephrine-induced ERKs activation. It is first time to demonstrate that G_h-coupled receptors link to MAP kinase cascade, as G_i-, G_s-, and G_q-coupled receptors link to ras-MAP kinase cascade[48,49]. Activation of G_h-mediated ERKs was completely inhibited by calreticulin. Even though α_1-ARs predominantly interact with G_q leading to activation of phospholipase C, hydrolysis of phosphoinositides, activation of protein kinase C and mobilization of intracellular Ca^{2+}, the selectivity of the various α_1-AR subtypes for different G proteins were not clearly understood until recent studies. It has been shown that norepinephrine strongly induces cardiac hypertrophy[3]. Most experiments

identifying the effects of α_1-adrenergic stimulation on cardiac hypertrophy were done in cultured cardiomyocytes from both neonates and adults. In cultured neonatal cardiomyocytes, the direct parameters related to cardiac hypertrophy are increased cell size and the protein content. In Fig. 2 and Fig. 5, the relationship between the direct hypertrophic parameters and ERKs activation was confirmed in quantitative changes.

MAP kinase activation in cardiac myocyte was important in mediation of the hypertrophic response to adrenergic receptors by different signaling pathways[36]. Both α- and β-adrenergic agoinsts can activate the MEK/MAP kinase in the heart by different signaling pathways. It shows some different mechanisms, comparing that endothelin utilize both PKC-dependent and PKC-independent components in the signaling pathways leading to MAP kinase activation[50].

In neonatal cardiomyocytes, hypertrophic response is mediated by α_1-ARs but not β-ARs[11]. Thus, activation of α_1-ARs increases protein synthesis as a result of increased transcriptional and translational activity. Although α_1-ARs couple predominantly to $G_{q/11}$ family, $\alpha_{1B/\Delta}$–ARs activates G_h proteins, those are multifunctional proteins with transglutaminase and receptor signaling function[51]. Thus, we investigated whether G_h was involved in norepinephrine-stimulated ERKs activation. In Fig. 6, the ERKs activation was significantly increased by overexpression of G_h in neonatal

cardiomyocytes, suggesting that norepinephrine-stimulated hypertrophy is associated with enhanced G_h signaling. In addition to activation of G_q[42], G_h is another molecule in the initiation of myocardial hypertrophy.

Interestingly, calreticulin down-regulates both GTP binding and transglutaminase activities of G_h[54]. calreticulin is a highly versatile lectin-like chaperon, and it participates during the synthesis of a variety of molecules, ion channels, surface receptors, integrins and transporters[53]. The protein also affects intracellular Ca^{2+} homeostasis by modulation of endoplasmic reticulum Ca^{2+} storage and transport. We further investigated whether calreticulin inhibited the ERKs activation via α_1-ARs/G_h pathways. In Fig. 7, co-overexpression of calreticulin and G_h completely inhibited the MEK1,2/ERKs activation augmented by G_h-overexpression.

In conclusion, norepinephrine induced hypertrophy in neonatal cardiomyocytes through MEK1,2/ERKs signal pathways via α_1-AR/G_h. Activation of G_h-mediated ERKs was completely inhibited by calreticulin.

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국문요약

신생 쥐의 심근세포의 norephinephrine 으로 자극된 α_1-교감신경 수용체에서의 Transglutminase II (G_h)를 통한 MEK1,2/ERKs 활성화의 매개

배경: 심근세포에서 α_1- 교감신경 수용체는 심근세포의 비대에 관여한다. G_h (TG II)는 조직형 transglutaminase 이고 GTPase 활성이 있을때 교감신경 수용체의 신호를 전달한다. G_h에 의한 심근세포의 비대과정에서 MAP kinase cascade 의 활성여부에 관해서는 거의 알려져 있지 않다. 본 연구는 norepinephrine의 자극에 의한 세포내 신호 전달과 ERKs의 활성화의 관계와 신생 쥐의 심근 비대증을 일으키는 norepinephrine자극에 의한 세포내 신호 전달을 G_h가 중재하는지를 조사하는데 목적이 있다.

방법 및 결과: α_1-교감신경 수용체 /G_h에 의해 중재된 심근세포 비대증의 분자적 기전을 설명하기 위해서, norepinephrine에 의한 ERKs 의 활성화와 신생 쥐의 심근세포에서의 단백질 합성의 효과를 관찰했다. Norepinephrine에 의한 ERKs 의 활성은 α_1- 교감신경 수용체 차단제(prazosin) 에 의해 억제되었지만 β-교감신경 수용체 차단제(propranolol)에 의해서는 억제되지 않았다. G_h 단백질의 과잉 발현은 norepinephrine 에 의한 ERKs 의 활성을 자극하였고 α_1-교감신경 수용체 차단제(Prazosin)에 의해 억제되었다. G_h 와 CRT(G_h단백질

의 조절자) 의 공조-과잉발현은 norepinephrine 에 의한 ERKs 의 활성을 억제하였다.

결론: Norepinephrine은 α_1-교감신경 수용체 자극을 통해 신생 쥐의 심근세포에서 비대증을 유발했고 G_h 는 norepinephrine 에 의한 MEK1,2/ERKs 활성을 부분적으로 유발하였다. G_h 매개성 MEK1,2/ERKs 의 활성은 calreticulin에 의해 완전히 억제되었다.