

Propofol Depresses Angiotensin II–Induced Cardiomyocyte Hypertrophy *In Vitro*

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Cardiomyocyte hypertrophy is formed in response to pressure or volume overload, injury, or neurohormonal activation. The most important vascular hormone that contributes to the development of hypertrophy is angiotensin II (Ang II). Accumulating studies have suggested that reactive oxygen species (ROS) may play an important role in cardiac hypertrophy. Propofol is a general anesthetic that possesses antioxidant action. We therefore examined whether propofol inhibited Ang II–induced cardiomyocyte hypertrophy. Our results showed that both ROS formation and hypertrophic responses induced by Ang II in cardiomyocytes were partially blocked by propofol. Further studies showed that propofol inhibited the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and mitogen-activated protein kinase/ERK kinase 1/2 (MEK1/2) induced by Ang II *via* a decrease in ROS production. In addition, propofol also markedly attenuated Ang II–stimulated nuclear factor- κ B (NF- κ B) activation *via* a decrease in ROS production. In conclusion, propofol prevents cardiomyocyte hypertrophy by interfering with the generation of ROS and involves the inhibition of the MEK/ERK signaling transduction pathway and NF- κ B activation. *Exp Biol Med* 233:200–208, 2008

Key words: propofol; angiotensin II; cardiomyocyte hypertrophy; reactive oxygen species; extracellular signal-regulated kinase; nuclear factor- κ B

Introduction

Cardiomyocyte hypertrophy increases the risk for cardiac ischemia, left ventricular dysfunction, and sudden cardiac death; this represents a very strong predictor of cardiovascular mortality and death of all causes and is recognized as a risk factor for the development of congestive heart failure (1). One of the most important factors contributing to the development of cardiomyocyte hypertrophy is angiotensin II (Ang II), which increases protein synthesis and cell diameter in cultured neonatal rat myocytes through the type 1 Ang II receptor (2). These processes directly lead to cardiomyocyte hypertrophy.

As a multifunctional agonist for cardiomyocyte hypertrophy, Ang II stimulates protein phosphorylation, gene expression, and cell growth (3). Many of the signaling events stimulated by Ang II are mediated by members of the mitogen-activated protein kinase (MAPK) family, including the extracellular signal-regulated kinase (ERK), p38, and the c-Jun NH₂-terminal kinase (JNK) (4). Among the MAPKs, ERK has been focused on as the essential regulator of a hypertrophic response, although JNK and p38 were recently studied in regulating cardiac hypertrophy (5–7). The activation of the ERK1/2 is triggered by MAPK/ERK kinase-1/2 (MEK1/2) *via* phosphorylation of serine/threonine residues (8).

Reactive oxygen species (ROS) have emerged as important triggers of the hypertrophic responses, both *in vivo* and *in vitro*, whether in response to stretch (9) or to other hypertrophic stimuli, such as Ang II (10–12). Production of ROS triggers activation of ERK1/2 (13, 14), which is one of the first indicators of hypertrophy. Also, nuclear factor- κ B (NF- κ B) is known to be redox-sensitive, which translocates to the nucleus after activated by Ang II and plays a leading role in hypertrophic responses of cardiomyocytes (15–18).

Many reports have revealed the stimulation of the renin-angiotensin system in critically ill patients (19, 20), which are congruent with other studies in animals (21, 22).

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Propofol (2,6-diisopropylphenol) is a potent intravenous anesthetic agent that is widely used for continuous sedation (for a duration that ranges from less than 24 hrs to 24 days) in critically ill patients (23, 24). Accordingly, it is important to determine the effects of propofol on the cardiovascular system. Propofol is known to act as an antioxidant, reacting with free radicals to prevent oxidative cell damage in several types of preparations (25–27), and to protect myocardial contractile performance during ischemia (28). However, no study has addressed the effects of propofol on cardiomyocyte hypertrophy. The aims of this study were, therefore, to investigate the antihypertrophic effect of propofol on Ang II-induced cardiomyocyte hypertrophy *in vitro* and to identify the potential mechanisms that may be responsible for the putative effect of propofol.

Materials and Methods

Materials. This study complied with the U.S. National Institutes of Health Guidelines for the Care and Use of Experimental Animals and was approved by the Animal Research Committee of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). One-day-old Wistar rats were obtained from the Experimental Animal Center of Tongji Medical College, Grade II, Certificate 19-050.

Pure propofol was purchased from Sigma-Aldrich (St. Louis, MO) and was diluted in 0.1% dimethylsulfoxide (DMSO), which was used instead of the commercially available 10% intralipid emulsion. Ang II was obtained from Sigma-Aldrich. U0126 (a MEK1/2 inhibitor) was obtained from Alexis Biochemicals (Lausen, Switzerland). Antibodies for ERK, phosphorylated ERK, MEK, phosphorylated MEK, NF- κ B p65, and histone were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Texas Red-X phalloidin was obtained from Molecular Probes Inc. (Eugene, OR). All other chemicals used were of the highest grade available commercially.

Neonatal Cardiomyocyte Cultures. Primary cultures of 1-day-old neonatal Wistar rat myocytes were prepared as previously described (29). Minced ventricular myocardium was placed into D-Hanks' salt solution with pH 7.4. The cells were dissociated by a trypsin (0.125%) digestion in D-Hanks' salt solution. After each of five successive 8-min incubations, the dissociated cells were mixed with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and were centrifuged and pooled. The dissociated cells were enriched for cardiomyocytes by the technique of differential adhesion for 90 mins and were plated at a concentration of 10^6 cells/well. Cultures were incubated in a humidified environment of 5%CO₂–95%O₂ at 37°C. Bromodeoxyuridine (0.1 mM) was added into the medium to inhibit proliferation of nonmyocytes. This procedure yielded cultures with 90%–95% myocytes, as assessed by microscopic observations of cellular contractions. After a 2-night incubation in DMEM

containing 10% FBS, the attached cells were rinsed and were maintained in DMEM containing 0.1% FBS. After 48 hrs of serum starvation, cardiomyocytes were pretreated with propofol or with other agents for 30 mins and subsequently were stimulated with 0.1 μ M Ang II for the indicated times.

Evaluation of Cytotoxicity. Equal numbers of cardiomyocytes were plated on a 96-well microplate (5×10^4 cells/well). Increasing concentrations of propofol (3, 10, or 30 μ M) were added to cultures after medium renewal. After 24 hrs of incubation, cytotoxicity was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The assay is based on the transformation of the tetrazolium salt MTT by active mitochondria to an insoluble formazan salt. MTT was added to each well under sterile conditions (with a final concentration of 0.5 mg/ml), and the plates were incubated for 4 hrs at 37°C. Untransformed MTT was removed by aspiration, and formazan crystals were dissolved in DMSO (150 μ l/well). Formazan was quantified at 540 nm with a Bio-Rad automated Enzyme Immunoassay Analyzer (Bio-Rad, Hercules, CA).

Measurement of ROS Production. The determination of intracellular ROS production was based on the oxidation of 2',7'-dichlorodihydrofluorescein (DCFH) to a fluorescent 2',7'-dichlorofluorescein (DCF). Briefly, cardiomyocytes were loaded with 20 μ M DCFH (Molecular Probes) for 30 mins at 37°C in the dark.

Cells loaded with DCFH were treated with 0.1 μ M Ang II in the presence or absence propofol for 30 mins. Subsequently, the cells were washed twice with phosphate-buffered saline (PBS), were detached by trypsin, and then were measured for DCF fluorescence intensity by fluorospectrophotometer analysis at an excitation wavelength of 488 nm and at an emission wavelength of 535 nm. The cell number in each sample was counted and was utilized to normalize the fluorescence intensity of DCF.

Protein Synthesis Measurement ([³H]Leucine Incorporation). Protein synthesis was determined by assessing the incorporation of labeled leucine from the extracellular medium into total trichloroacetic acid (TCA)-precipitable cell proteins (30). Cardiomyocytes cultured for 48 hrs in DMEM with 10% FBS were starved in 0.1% FBS-DMEM for 48 hrs. Then, cells were treated with 0.1 μ M Ang II alone or with propofol (3, 10, or 30 μ M) for 24 hrs. [³H]leucine (1 μ Ci/ml) was added 2 hrs before harvest. At the end of the labeling incubation, plates were placed on ice, were quickly washed twice with ice-cold PBS, were incubated for 30 mins with 5% TCA, and were washed again. Precipitates were solubilized for 30 mins in 0.5 M sodium hydroxide and were neutralized. Radioactivity was measured by a liquid scintillation counter.

Northern Blot Analysis. Total mRNA extraction and Northern blot analysis were performed as previously described (31). Briefly, after 24 hrs of incubation with various agents, the cultured myocytes were submitted to

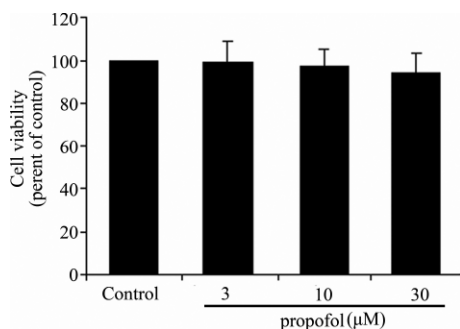


Figure 1. Effects of propofol on the viability of cardiomyocytes. Myocytes were cultured in microplate and were incubated with increasing concentration of propofol. After 24 hrs, cell viability was determined with the MTT assay. Data reported as means $\pm$ SD. The experiments were repeated five times with reproducible results.

RNA extraction. Total RNA was extracted from cultured cells with TRIZOL Reagent (Sigma Chemical Co., St. Louis, MO). The cDNA probes used were as follows: rat atrial natriuretic peptide (*ANP*) cDNA—an 850-bp fragment, and rat GAPDH—a 1300-bp fragment. The cDNA probes were labeled with [32 P]dCTP (3 mCi/mmol; New England Nuclear, Waltham, MA) by random primer extension. Autoradiography was performed on a Kodak XAR-5 film (Emeryville, CA) with an intensifying screen at -80°C . Autoradiograms were quantified by an image analyzer (LEICA, DC200; Vertrieb, Germany). Results were normalized to GAPDH gene expression.

Immunofluorescence. After being treated with 0.1 μM Ang II alone or with propofol (30 μM) for 24 hrs, cardiomyocytes were fixed in 4% paraformaldehyde and were permeabilized with 0.25% Triton X-100. Cells were incubated for 1 hr in blocking serum and for 2 hrs at room temperature in the presence of Texas Red-X phalloidin (1:500). Immunostained cardiomyocytes were viewed by fluorescence microscopy. Quantitation of cell surface area was performed on actin-stained cardiomyocytes. At least 100 random cardiomyocytes from five independent experiments were measured by planimetry.

Nuclear Protein Extracts. Following appropriate experimental treatment, cultured cardiomyocytes were rinsed with PBS at 0°C and were scraped into the same buffer. All steps were performed at 0° – 4°C . Nuclear protein extracts from cardiomyocytes were prepared according to the protocol of the Nuclear Extract Kit (Active Motif, Carlsbad, CA). The protein concentration of the cell extract was determined by a Bradford-based assay, and the extracts were stored at -80°C until use.

Western Blot Analysis. After various treatments, myocytes were harvested and were lysed for 20 mins in 200 μl lysis buffer (10 mM Tris-HCl, 0.5% NP40, 150 mM NaCl, 1 mM Na_3VO_4 , 10 mM NaF, 1 mM phenylmethyl sulfonylfluoride (PMSF), 1 mM EDTA, 10 $\mu\text{g}/\text{ml}$ aprotinin, 10 $\mu\text{g}/\text{ml}$ leupeptin, and 1 $\mu\text{g}/\text{ml}$ pepstatin). Equal protein loading in each lane, resolved by SDS-PAGE, was blotted on nitrocellulose membrane. Membranes were blocked in

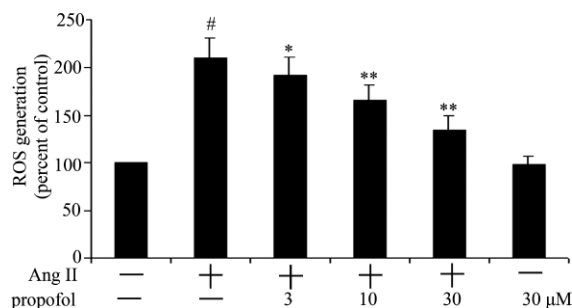


Figure 2. Inhibitory effects of propofol on Ang II-induced production of ROS. Myocytes were preincubated with propofol (3, 10, or 30 μM) for 30 mins and were stimulated with Ang II (0.1 μM) for 30 mins. DCF fluorescence was measured by fluorospectrophotometer analysis. Data reported as means $\pm$ SD. [#] $P < 0.01$ vs. control group; ^{*} $P < 0.05$ and ^{**} $P < 0.01$ vs. Ang II group. The experiments were repeated five times with reproducible results.

5% nonfat milk powder in Tris-buffered saline (TBS)/0.1% Tween-20 for 1 hr at room temperature and then were incubated with specific antibodies in 5% bovine serum albumin (BSA) in TBS for another hour. Membranes were incubated with peroxidase-conjugated second antibody in blocking buffer for 1 hr. The labeled proteins were detected with enhanced chemiluminescence.

Electrophoretic Mobility Shift Assays (EMSA).

Binding reactions were performed with 10 μg of nuclear protein in 10 mM Tris, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol, 0.1% Triton X-100, 1 mg poly(dIdC), 5 μg BSA, and 10,000 cpm of [32 P]labeled oligonucleotide. The specific NF- κB consensus oligonucleotides were 5'-AGTTGAGGG-GACTTTCCTCCAGGC-3' (Promega, Madison, WI), which contains a high-affinity NF- κB -binding site. The unlabeled oligonucleotides were used for competition. DNA complexes were separated on a 6% nondenaturing polyacrylamide gel in Tris-HCl (6.7 mM), EDTA (1 mM), and ammonium acetate (3.3 mM), and then autoradiography was performed.

Statistical Analysis. Results were expressed as means $\pm$ standard deviation (SD). Statistical significance was determined using one-way analysis of variance. The differences were considered statistically significant at a value of $P < 0.05$.

Results

Effects of Propofol on Cardiomyocytes Viability. Exposure of cultured cardiomyocytes to propofol (3, 10, or 30 μM) for 24 hrs did not induce any significant effect on cardiomyocytes viability (Fig. 1). Additionally, myocyte monolayers continued to contract synchronously in the presence of propofol.

Propofol Inhibits Ang II-Induced Intracellular ROS. A previous study showed that Ang II increased intracellular ROS formation (13), which played a key role in cardiomyocyte hypertrophy. Therefore, we examined the effect of propofol (3, 10, or 30 μM) on Ang II-increased intracellular ROS generation. Propofol, in a concentration-dependent manner, suppressed ROS formation in cardio-

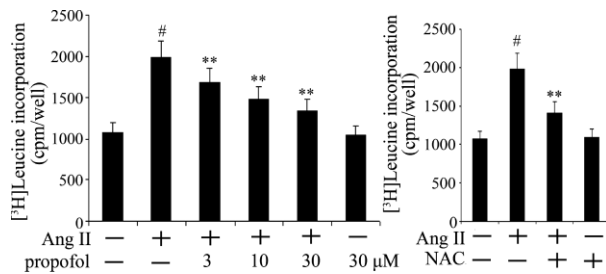


Figure 3. Inhibitory effects of propofol on Ang II-induced increase in [^3H]leucine incorporation. Myocytes were preincubated with either propofol (3, 10, or 30 μM) or NAC (10 mM) for 30 mins and were stimulated with Ang II (0.1 μM) for 24 hrs. Protein synthesis rate was measured as [^3H]leucine incorporation. Data reported as means $\pm$ SD. [#] $P < 0.01$ vs. control group; ^{**} $P < 0.01$ vs. Ang II group. The experiments were repeated five times with reproducible results.

myocytes as measured after Ang II treatment for 30 mins (Fig. 2). Propofol was solubilized in 0.1% DMSO. No significant difference was observed between control and vehicle (DMSO)-treated groups on the protein synthesis in cardiomyocytes (data not shown). These data, therefore, clearly demonstrated that propofol could decrease ROS production.

Effects of Propofol on Ang II-Induced Cardiomyocyte Hypertrophy. Cardiomyocyte hypertrophy is characterized by increased cell surface area, protein synthesis, and induction of fetal-type genes (e.g., *ANP*). Therefore, we examined the effects of propofol on these parameters. Cardiomyocytes were pretreated with propofol (3, 10, or 30 μM) for 30 mins before stimulation with 0.1 μM Ang II. After 24 hrs, hypertrophy was determined. The increase in [^3H]leucine incorporation detected after 24 hrs of culture in the presence of Ang II was markedly inhibited in cells preincubated with propofol (Fig. 3). No significant difference was observed between control and vehicle

(DMSO)-treated groups on the [^3H]leucine incorporation in cardiomyocytes (data not shown). Moreover, propofol, in a concentration-dependent manner, significantly depressed the expression of *ANP* mRNA induced by Ang II (Fig. 4). Further, Ang II induced a marked increase in cell surface area, which was partially inhibited by propofol (Fig. 5). To determine whether inhibition of Ang II-induced cardiomyocyte hypertrophy by propofol is *via* a decrease in ROS production, we employed N-acetyl-L-cysteine (NAC), a known antioxidant. The results showed that NAC (10 mM) significantly reduced protein synthesis and *ANP* mRNA expression induced by Ang II in cardiomyocytes (Figs. 3 and 4). These findings implied that propofol inhibited Ang II-induced hypertrophic responses *via* attenuation of ROS in cardiomyocytes.

Effects of Propofol on ERK Phosphorylation in Cardiomyocytes. We further investigated whether propofol inhibited the ERK pathway in Ang II-treated cardiomyocytes. To investigate the effects of propofol on ERK activation, myocytes were pretreated with propofol (3, 10, or 30 μM) for 30 mins followed by treatment with 0.1 μM Ang II for 30 mins. The activation of ERK was examined by Western blot with the phosphospecific antibody. The data suggested that propofol markedly inhibited the activations of ERK1/2 induced by Ang II in a concentration-dependent manner (Fig. 6). The observed inhibitory effects of propofol on Ang II-induced ERK1/2 activation were not caused by the decreases in total ERK1/2 protein levels. Moreover, cardiomyocytes treated with NAC (10 mM) showed significantly decreased ERK1/2 phosphorylation stimulated by Ang II (Fig. 6). These findings suggested that the inhibitory effects of propofol on Ang II-activated ERK1/2 might be *via* a decrease in ROS production.

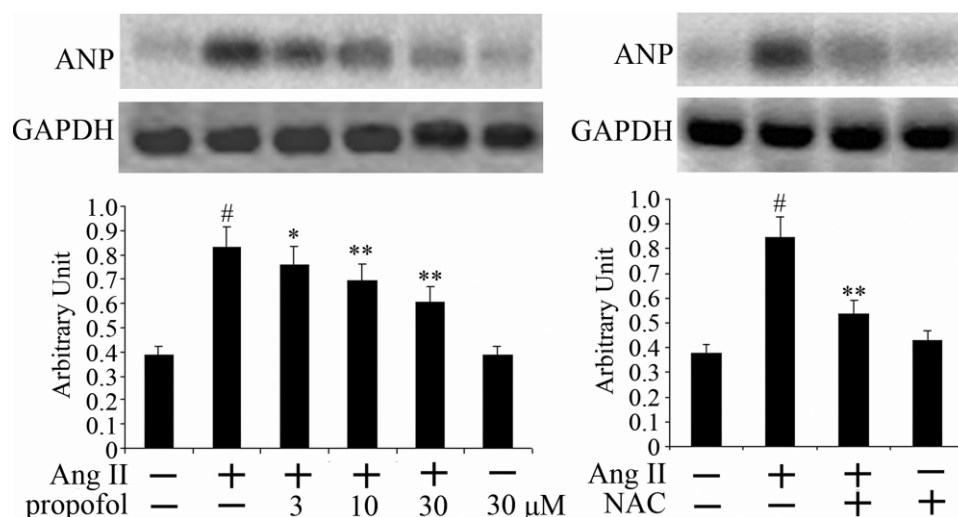


Figure 4. Inhibitory effects of propofol on the Ang II-induced increase in *ANP* expression. Myocytes were preincubated with either propofol (3, 10, or 30 μM) or NAC (10 mM) for 30 mins and were stimulated with Ang II (0.1 μM) for 24 hrs. *ANP* mRNA expression was measured by Northern blot. Data reported as means $\pm$ SD. [#] $P < 0.01$ vs. control group; ^{*} $P < 0.05$ and ^{**} $P < 0.01$ vs. Ang II group. The experiments were repeated five times with reproducible results.

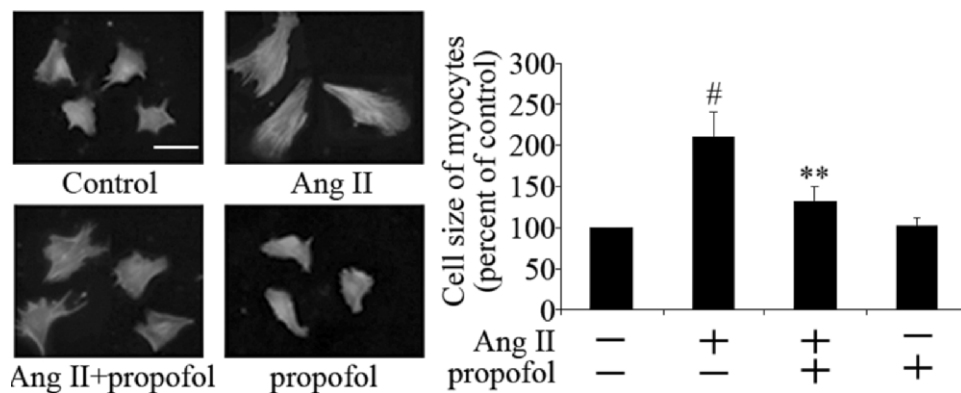


Figure 5. Inhibitory effects of propofol on Ang II-induced increase in the cell surface area. Myocytes were preincubated with propofol (30 μ M) or NAC (10 mM) for 30 mins and were stimulated with Ang II (0.1 μ M) for 24 hrs. Immunofluorescence was performed with Texas Red-X phalloidin. Scale bar: 50 μ m. The cell surface area of cardiomyocytes was measured by directly tracing actin-stained cardiomyocytes. Data reported as means $\pm$ SD. [#] $P < 0.01$ vs. control group; ^{**} $P < 0.01$ vs. Ang II group. The experiments were repeated five times with reproducible results.

Effects of Propofol on MEK Phosphorylation in Cardiomyocytes. MEK1/2 phosphorylates and activates ERK1/2 in response to mitogenic stimuli (32). To elucidate the mechanism by which propofol inhibited ERK activation, we examined the effect of propofol on MEK activation in cardiomyocytes. The protein content of p-MEK1/2 was also examined by Western blot analysis. As shown in Figure 7, treatment of cardiomyocytes with 0.1 μ M Ang II for 30 mins markedly increased the phosphorylation of MEK1/2. However, cardiomyocytes pretreated with propofol (3, 10, or 30 μ M) showed significantly decreased Ang II-induced MEK1/2 phosphorylation. The observed inhibitory effects of propofol on Ang II-induced MEK1/2 activation were not caused by the decreases in total MEK1/2 protein levels. Moreover, cardiomyocytes treated with NAC (10 mM) showed significantly decreased MEK1/2 phosphorylation induced by Ang II. These findings suggested that propofol

inhibited the Ang II-activated MEK1/2 *via* a decrease in ROS production in cardiomyocytes (Fig. 7). To further prove the roles of MEK1/2 on the Ang II-induced cardiac hypertrophy, cardiomyocytes were pretreated with 20 μ M U0126 for 30 mins followed by stimulation with Ang II for 24 hrs. U0126 showed strong decreases on the Ang II-induced [³H]leucine incorporation and expression of *ANP* mRNA (Fig. 8).

Effects of Propofol on NF- κ B Activation. Activation of NF- κ B involves its translocation to the nucleus, where it binds to DNA and regulates the transcription of a wide variety of genes. Cardiomyocytes were treated with Ang II for the indicated times or were preincubated for 30 mins with different concentrations of propofol and then were treated with Ang II (0.1 μ M) for 30 mins. Nuclear extracts were prepared and were assayed for NF- κ B activation by EMSA. As shown in Figure 9, Ang II induced

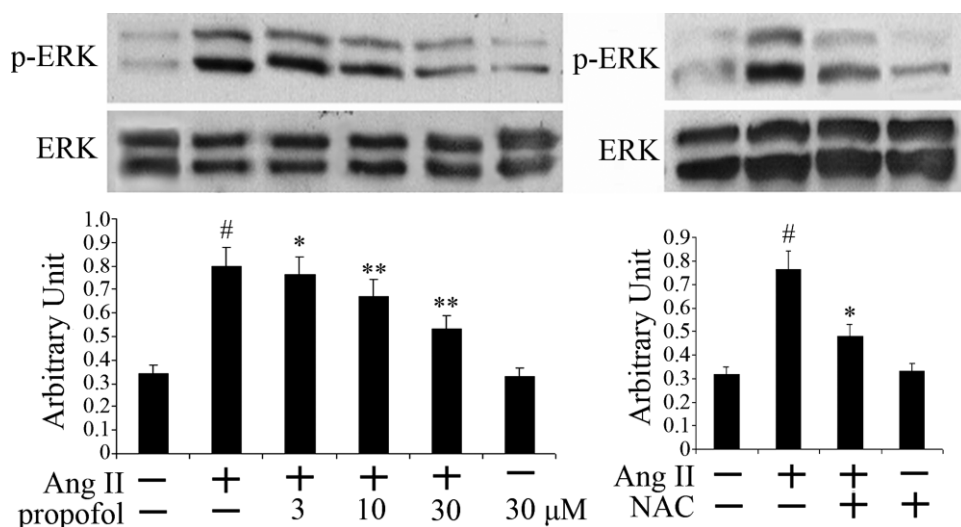


Figure 6. Inhibitory effects of propofol on Ang II-induced ERK activation. Myocytes were preincubated with either propofol (3, 10, or 30 μ M) or NAC (10 mM) for 30 mins and were stimulated with Ang II (0.1 μ M) for 30 mins. ERK activation was examined by Western blot with the phosphospecific antibody. Data reported as means $\pm$ SD. [#] $P < 0.01$ vs. control group; ^{*} $P < 0.05$ and ^{**} $P < 0.01$ vs. Ang II group. The experiments were repeated five times with reproducible results.

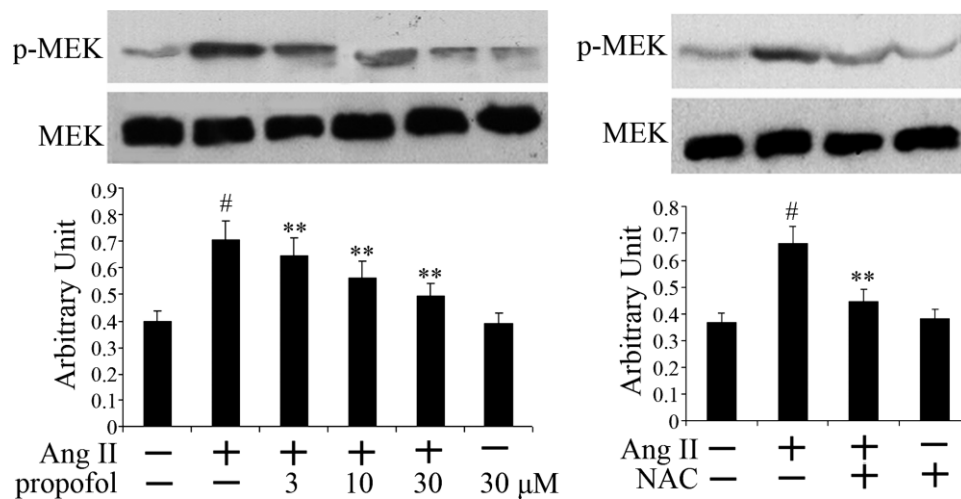


Figure 7. Inhibitory effects of propofol on Ang II-induced MEK activation. Myocytes were preincubated with either propofol (3, 10, or 30 μ M) or NAC (10 mM) for 30 mins and were stimulated with Ang II (0.1 μ M) for 30 mins. MEK activation was examined by Western blot with the phosphospecific antibody. Data reported as means $\pm$ SD. [#] $P < 0.01$ vs. control group; ^{**} $P < 0.01$ vs. Ang II group. The experiments were repeated five times with reproducible results.

NF- κ B activation and propofol inhibited Ang II-mediated NF- κ B activation in a concentration-dependent manner. Moreover, nuclear extracts treated with NAC (10 mM) showed significantly decreased NF- κ B activity mediated by Ang II. Further characterization of NF- κ B activation was performed by Western blot by using a p65-specific antibody. Figure 10 shows the Western blot profile of nuclear fractions from various treated cells. Histone monoclonal antibody was used as an internal protein loading control. Ang II increased the content of NF- κ B p65 in nuclear extracts, which was inhibited by propofol in a concentration-dependent manner. Moreover, nuclear ex-

tracts treated with NAC (10 mM) showed significantly decreased amount of NF- κ B p65 stimulated by Ang II (Fig. 10). These findings suggest that propofol inhibited NF- κ B activation induced by Ang II, and the effects of propofol might be *via* a decrease in ROS production in cardiomyocytes.

Discussion

The key finding that emerges from this study is the display of antihypertrophic actions of propofol in cardiomyocytes. Moreover, the antihypertrophic actions of propofol are mediated, at least in part, *via* reduction of

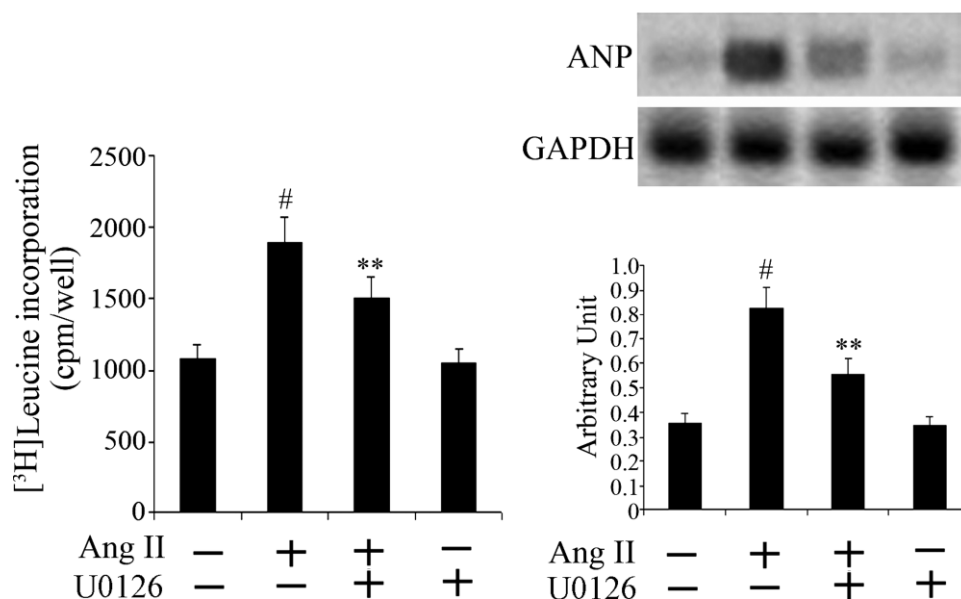


Figure 8. Inhibitory effects of U0126 on Ang II-induced cardiomyocyte hypertrophy. Myocytes were preincubated with U0126 (20 μ M) for 30 mins and were stimulated with Ang II (0.1 μ M) for 24 hrs. Protein synthesis rate was measured as [³H]leucine incorporation and ANP mRNA expression was measured by Northern blot. Data reported as means $\pm$ SD. [#] $P < 0.01$ vs. control group; ^{**} $P < 0.01$ vs. Ang II group. The experiments were repeated five times with reproducible results.

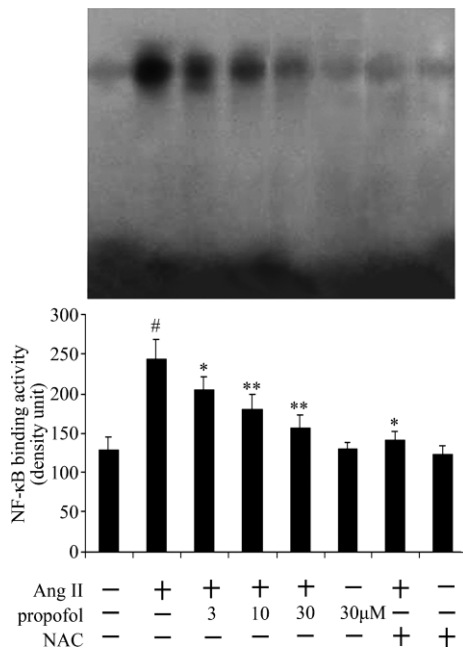


Figure 9. Inhibitory effects of propofol on Ang II-induced NF-κB binding activity. Myocytes were preincubated with either propofol (3, 10, or 30 μ M) or NAC (10 mM) for 30 mins and were stimulated with Ang II (0.1 μ M) for 30 mins; then, nuclear extracts were prepared and were assayed for NF-κB activation by EMSA. Data reported as means $\pm$ SD. [#] $P < 0.01$ vs. control group; ^{*} $P < 0.05$ and ^{**} $P < 0.01$ vs. Ang II group.

superoxide levels in neonatal cardiomyocytes. Further, both MEK and ERK phosphorylation and NF-κB activation in Ang II-stimulated cells are inhibited by propofol.

Cardiomyocyte hypertrophy refers to the increase of a cell's volume and myocomma and to the changes of types of contracting proteins at the same time. In its early stage, it is a compensatory response, but, if prolonged, the heart may undergo a transition to heart failure. Therefore, it is

important to prevent the process of cardiomyocyte hypertrophy induced by extracellular signals for any proposed therapy to regulate the myocardial hypertrophic responses (33, 34). Ang II, as an important neuroendocrine factor, can not only regulate the physiologic functions of the cardiovascular system, but also make a vital role in physiopathologic processes, such as myocardial hypertrophy or heart failure (35); in addition, multiple intracellular pathways in Ang II signaling have been reported. Adult rat ventricular myocytes are strongly physically connected by intercalated discs and an extracellular matrix and therefore are more difficult to isolate. Thus, adult mouse cardiac myocytes are particularly difficult to maintain in culture, although success has been reported. Even if successfully cultured, these cells begin to dedifferentiate over time and lose important aspects of the adult cardiomyocyte phenotype. Therefore, it is not optimal for biochemical or molecular biological approaches. For these reasons, neonatal rat ventricular myocytes were chosen for models in present study.

In the present work, accompanied by changes in [³H]leucine incorporation and fetal gene (*ANP*) expression, Ang II enhanced the cell surface area of cardiomyocytes, a direct indication of cellular hypertrophy, which is consistent with previous results (36). Moreover, the hypertrophic effects of Ang II were also accompanied by the increased generation of ROS in cardiomyocytes. All these above actions of Ang II were inhibited by either propofol or by the antioxidant NAC. Taken together, Ang II induced cardiomyocytes hypertrophy in part *via* the generation of ROS, in line with previous studies (11, 13, 37).

ROS, including superoxide anion, hydrogen peroxide, and hydroxyl radical (38), have been proposed to activate crucial signaling cascades, have mitogenic effects, and play an important role in cardiomyocyte hypertrophy mediated by Ang II (10, 11, 39). Upon Ang II stimulation, ROS are generated *via* membrane-bound NAD(P)H oxidase (11, 12),

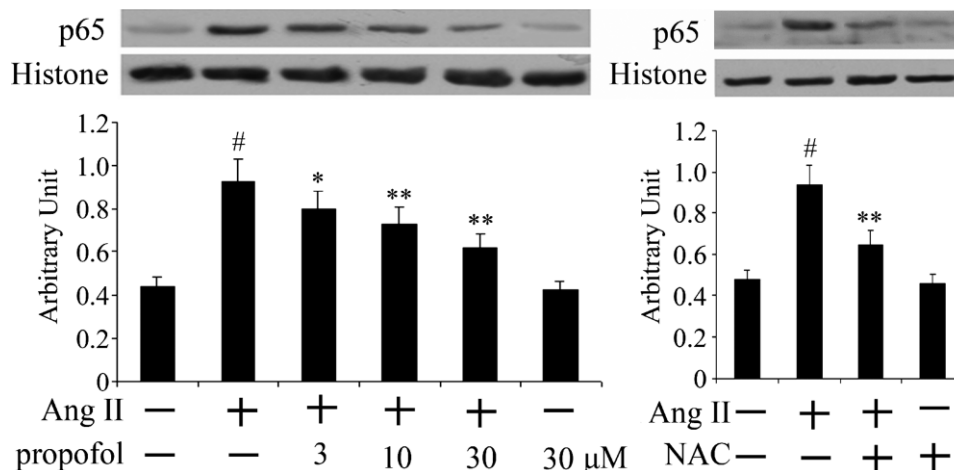


Figure 10. Inhibitory effects of propofol on Ang II-induced NF-κB p65 translocation. Myocytes were preincubated with either propofol (3, 10, or 30 μ M) or NAC (10 mM) for 30 mins and were stimulated with Ang II (0.1 μ M) for 30 mins. The translocation of NF-κB p65 into the nucleus was examined by Western blot with p65 antibody. Data reported as means $\pm$ SD. [#] $P < 0.01$ vs. control group; ^{*} $P < 0.05$ and ^{**} $P < 0.01$ vs. Ang II group. The experiments were repeated five times with reproducible results.

might alter the activities of MAPK pathways, and modulate the subsequent development of cardiomyocyte hypertrophy (13, 40). Additionally, suppression of ROS formation inhibits Ang II-induced hypertrophy (40, 41).

Propofol, known as a general anesthetic, is chemically similar to α -tocopherol (42) and exhibits potent scavenging action against superoxide anion and hydrogen peroxide. It has been reported that propofol, at clinically relevant concentrations, can protect cardiomyocyte function during ischemia by counteract the effects of increased production of free radical compounds (28). In the present study, propofol inhibited cardiomyocyte hypertrophy and the phosphorylation of ERK1/2 induced by Ang II. One possible explanation for the effects of propofol in cardiomyocytes thus may be its ability to decrease ROS production. However, further experiments will be necessary to identify the detailed mechanisms by which propofol exerts its antigrowth effects in cardiomyocytes.

MEK1/2, an immediate upstream regulator of ERK, phosphorylates and activates ERK1/2 but not JNK or p38 in response to Ang II stimulation (32). Transgenic overexpression of MEK1/2 results in considerable cardiac hypertrophy (43). As an inhibitor of MEK, U0126 has marked antihypertrophic effects on cardiomyocyte hypertrophy and downregulates some hypertrophic gene expressions (44). U0126 also abolishes prostaglandin E_2 -induced cardiac hypertrophy in a dose-dependent manner (45). Furthermore, U0126 inhibits endothelin-1 and phenylephrine-induced protein synthesis and increased cell surface area, sarcomeric reorganization, and expression of the β -myosin heavy chain in myocytes (46). Our results indicate that U0126 inhibits Ang II-induced protein synthesis and expression of *ANP* mRNA. The inhibition pattern of MEK activation by propofol was consistent with that of ERK1/2 activation, which indicates that the MEK/ERK pathway may be involved in the antihypertrophic effect of propofol and that propofol inhibited the Ang II-activated MEK1/2 *via* a decrease in ROS production in cardiomyocytes.

Transcriptional regulation is critical for molecular signaling in a cellular response that involves interactions between the proteins of the general transcriptional apparatus and proteins that bind gene-specific enhancer elements. Oxidative stress has long been known to induce nuclear transcriptional factors, such as NF- κ B, and has been thought to be crucial for NF- κ B activation in cells. Ang II has been implicated in the activation of NF- κ B in cardiac myocytes (19), and the vasoactive peptides induce hypertrophy, at least in part, *via* ROS-dependent activation of NF- κ B (18). It is of note that the activation of NF- κ B is a downstream target of the phopho-ERK. We therefore examined the effects of propofol on the activation of NF- κ B. Our results indicate that either propofol or NAC inhibits Ang II-induced NF- κ B activation. These results suggest that a decrease of Ang II-induced ROS production by propofol leads to inhibition of NF- κ B. Nevertheless, we can not rule out the possibilities that some other mechanisms are also

involved in the growth-inhibitory effects of propofol in cardiomyocytes. The molecular mechanism underlying antihypertrophic effect of propofol remains to be further explored.

Taken together, the present study delivers important new insights to the molecular mechanisms of actions of propofol on cardiomyocytes. Our results indicated that propofol markedly depresses Ang II-induced cardiomyocyte hypertrophy *in vitro*. The inhibition of MEK/ERK pathway may be involved in the antihypertrophic effect. Moreover, propofol could modulate the redox-sensitive steps involved in the Ang II signaling pathway. Although the precise mechanism by which propofol inhibits the development of cardiac hypertrophy remains to be further clarified, understanding the pharmacologic actions of propofol on cardiac cells may contribute to the choice of anesthetic in anesthesia and sedation for the people who suffer from cardiovascular diseases.

1. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 322:1561–1566, 1990.
2. Baker KM, Aceto JF. Angiotensin II stimulation of protein synthesis and cell growth in chick heart cells. *Am J Physiol* 259:610–618, 1990.
3. Kagiya S, Eguchi S, Inagami T, Zhang YC, Phillips MI. Angiotensin II-induced cardiac hypertrophy and hypertension are attenuated by epidermal growth factor receptor antisense. *Circulation* 106:909–912, 2002.
4. Thorburn J, Carlson M, Mansour SJ, Chien KR, Ahn NG, Thorburn A. Inhibition of a signaling pathway in cardiac muscle cells by active mitogen-activated protein kinase. *Mol Biol Cell* 6:1479–1490, 1995.
5. Sugden PH. Signalling pathways in cardiac myocyte hypertrophy. *Ann Med* 33:611–622, 2001.
6. Maller JL. Signal transduction: fishing at the cell surface. *Science* 300:594–595, 2003.
7. Ptashne M, Gann A. Signal transduction: imposing specificity on kinases. *Science* 299:1025–1027, 2003.
8. Bueno OF, Molkentin JD. Involvement of extracellular signal-regulated kinases 1/2 in cardiac hypertrophy and cell death. *Circ Res* 91:776–781, 2002.
9. Pimentel DR, Amin JK, Xiao L, Miller T, Viereck J, Oliver-Krasinski J, Baliga R, Wang J, Siwik DA, Singh K, Pagano P, Colucci WS, Sawyer DB. Reactive oxygen species mediate amplitude-dependent hypertrophic and apoptotic responses to mechanical stretch in cardiac myocytes. *Circ Res* 89:453–460, 2001.
10. Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyazaki M, Ohe T, Namba M. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor- α and angiotensin II. *Circulation* 98:794–799, 1998.
11. Bendall JK, Cave AC, Heymes C, Gall N, Shah AM. Pivotal role of a gp91(phox)-containing NADPH oxidase in angiotensin II-induced cardiac hypertrophy in mice. *Circulation* 105:293–296, 2002.
12. Hingtgen SD, Tian X, Yang J, Dunlay SM, Peek AS, Wu Y, Sharma RV, Engelhardt JF, Davisson RL. Nox2-containing NADPH oxidase and Akt activation play a key role in angiotensin II-induced cardiomyocyte hypertrophy. *Physiol Genomics* 26:180–191, 2006.
13. Shih NL, Cheng TH, Loh SH, Cheng PY, Wang DL, Chen YS, Liu SH, Liew CC, Chen JJ. Reactive oxygen species modulate angiotensin II-induced beta-myosin heavy chain gene expression *via* Ras/Raf/extra-

- cellular signal-regulated kinase pathway in neonatal rat cardiomyocytes. *Biochem Biophys Res Commun* 283:143–148, 2001.
14. Liu JC, Chana P, Chen JJ, Lee HM, Lee WS, Shih NL, Chen YL, Hong HJ, Cheng TH. Inhibitory effect of trilinolein on norepinephrine-induced β -myosin heavy-chain promoter activity, reactive oxygen species generation, and extracellular signal-regulated kinase phosphorylation in neonatal rat cardiomyocytes. *J Biomed Sci* 11:11–18, 2004.
 15. Rouet-Benzineb P, Gontero B, Dreyfus P, Lafuma C. Angiotensin II induces nuclear factor-kappa B activation in cultured neonatal rat cardiomyocytes through protein kinase C signaling pathway. *J Mol Cell Cardiol* 32:1767–1778, 2000.
 16. Purcell NH, Tang G, Yu C, Mercurio F, DiDonato JA, Lin A. Activation of NF- κ B is required for hypertrophic growth of primary rat neonatal ventricular cardiomyocytes. *Proc Natl Acad Sci* 98:6668–6673, 2001.
 17. Hirotani S, Otsu K, Nishida K, Higuchi Y, Morita T, Nakayama H, Yamaguchi O, Mano T, Matsumura Y, Ueno H, Tada M, Hori M. Involvement of nuclear factor- κ B and apoptosis signal-regulating kinase 1 in G-protein- coupled receptor agonist-induced cardiomyocyte hypertrophy. *Circulation* 105:509–515, 2002.
 18. Freund C, Schmidt-Ullrich R, Baurand A, Dunger S, Schneider W, Loser P, El-Jamali A, Dietz R, Scheidereit C, Bergmann MW. Requirement of nuclear factor-kappaB in angiotensin II- and isoproterenol-induced cardiac hypertrophy *in vivo*. *Circulation* 111:2319–2325, 2005.
 19. Annat G, Viale JP, Bui Xuan B, Hadj Aissa O, Benzoni D, Vincent M, Gharib C, Motin J. Effect of PEEP ventilation on renal function, plasma renin, aldosterone, neurophysins and urinary ADH, and prostaglandins. *Anesthesiology* 58:136–141, 1983.
 20. Mannix ET, Farber MO, Aronoff GR, Brier ME, Weinberger MH, Palange P, Manfredi F. Hemodynamic, renal, and hormonal responses to lower body positive pressure in human subjects. *J Lab Clin Med* 128:585–593, 1996.
 21. Bond GC, Lightfoot B. Role of renal nerves in mediating the increased renin secretion during continuous positive pressure ventilation. *Proc Soc Exp Biol Med* 173:104–108, 1983.
 22. Kaczmarczyk G, Vogel S, Krebs M, Bünger H, Scholz A. Vasopressin and renin-angiotensin maintain arterial pressure during PEEP in nonexpanded, conscious dogs. *Am J Physiol* 271:1396–1402, 1996.
 23. Sanchez-Izquierdo-Riera JA, Caballero-Cubedo RE, Perez-Vela JL, Ambros-Checa A, Cantalapiedra-Santiago JA, Alted-Lopez E. Propofol versus midazolam: safety and efficacy for sedating the severe trauma patient. *Anesth Analg* 86:1219–1224, 1998.
 24. Carrasco G, Molina R, Costa J, Soler JM, Cabre L. Propofol vs midazolam in short-, medium-, and long-term sedation of critically ill patients: a cost-benefit analysis. *Chest* 103:557–564, 1993.
 25. Balyanskikova IV, Vistintine DJ, Gunnerson HB, Paisansatan C, Baughman VL, Minshall RD, Danilov SM. Propofol attenuates lung endothelial injury induced by ischemia-reperfusion and oxidative stress. *Anesth Analg* 100:929–936, 2005.
 26. Peng Z, Luo M, Ye S, Critchley LA, Joynt GM, Ho AM, Yao S. Antioxidative and anti-endotoxin effects of propofol on endothelial cells. *Chin Med J* 116:731–735, 2004.
 27. Lee H, Jang YH, Lee SR. Protective effect of propofol against kainic acid-induced lipid peroxidation in mouse brain homogenates: comparison with trolox and melatonin. *J Neurosurg Anesthesiol* 17:144–148, 2005.
 28. McDermott BJ, McWilliams S, Smyth K, Kelso EJ, Spiers JP, Zhao Y, Bell D, Mirakhur RK. Protection of cardiomyocyte function by propofol during simulated ischemia is associated with a direct action to reduce pro-oxidant activity. *J Mol Cell Cardiol* 42:600–608, 2007.
 29. Hannan RD, Luyken J, Rothblum LI. Regulation of ribosomal DNA transcription during contraction-induced hypertrophy of neonatal cardiomyocytes. *J Biol Chem* 271:3213–3220, 1996.
 30. Yamazaki T, Komuro I, Kudoh S, Zou Y, Shiojima I, Mizuno T, Takano H, Hiroi Y, Ueki K, Tobe K, Kadowaki T, Nagai R, Yazaki Y. Angiotensin II partly mediates mechanical stress-induced cardiac hypertrophy. *Circ Res* 77:258–265, 1995.
 31. Wollert KC, Studer R, von Bulow B, Drexler H. Survival after myocardial infarction in the rat. Role of tissue angiotensin-converting enzyme inhibition. *Circulation* 90:2457–2467, 1994.
 32. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, Ye Q, Lobo JM, She Y, Osman I, Golub TR, Sebolt-Leopold J, Sellers WR, Rosen N. BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 439:358–362, 2005.
 33. Frey N, Katus HA, Olson EN, Hill JA. Hypertrophy of the heart: a new therapeutic target? *Circulation* 109:1580–1589, 2004.
 34. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling-concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf on an international forum on cardiac remodelling. *J Am Coll Cardiol* 35:569–582, 2000.
 35. Sven W, Georg N. The role of the AT1 receptor in the cardiovascular continuum. *Eur Heart J Suppl* 6:Suppl H (h3–h9), 2004.
 36. Hayashi D, Kudoh S, Shiojima I, Zou Y, Harada K, Shimoyama M, Imai Y, Monzen K, Yamazaki T, Yazaki Y, Nagai R, Komuro I. Atrial natriuretic peptide inhibits cardiomyocyte hypertrophy through mitogen-activated protein kinase phosphatase-1. *Biochem Biophys Res Commun* 322:310–319, 2004.
 37. Wu R, Laplante MA, de Champlain J. Cyclooxygenase-2 inhibitors attenuate angiotensin II-induced oxidative stress, hypertension, and cardiac hypertrophy in rats. *Hypertension* 45:1139–1144, 2005.
 38. Li Y, Trush MA. Diphenyleneiodonium, an NAD(P)H oxidase inhibitor, also potently inhibits mitochondrial reactive oxygen species production. *Biochem Biophys Res Commun* 253:295–299, 1998.
 39. Sugden PH, Clerk A. Cellular mechanisms of cardiac hypertrophy. *J Mol Med* 76:725–746, 1998.
 40. Tanaka K, Honda M, Takabatake T. Redox regulation of MAPK pathways and cardiac hypertrophy in adult rat cardiac myocyte. *J Am Coll Cardiol* 37:676–685, 2001.
 41. Laskowski A, Woodman OL, Cao AH, Drummond GR, Marshall T, Kaye DM, Ritchie RH. Antioxidant actions contribute to the antihypertrophic effects of atrial natriuretic peptide in neonatal rat cardiomyocytes. *Cardiovasc Res* 72:112–123, 2006.
 42. Murphy PG, Davies MJ, Columb MO, Stratford N. Effect of propofol and thiopentone on free radical mediated oxidative stress of the erythrocyte. *Br J Anaesth* 76: 536–543, 1996.
 43. Bueno OF, De Windt LJ, Tymitz KM, Witt SA, Kimball TR, Klevitsky R, Hewett TE, Jones SP, Lefer DJ, Peng CF, Kitsis RN, Molkentin JD. The MEK1-ERK1/2 signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. *EMBO J* 19:6341–6350, 2000.
 44. Kennedy RA, Kemp TJ, Sugden PH, Clerk A. Using U0126 to dissect the role of the extracellular signal-regulated kinase 1/2 (ERK1/2) cascade in the regulation of gene expression by endothelin-1 in cardiac myocytes. *J Mol Cell Cardiol* 41:236–247, 2006.
 45. Frias MA, Rebsamen MC, Gerber-Wicht C, Lang U. Prostaglandin E2 activates Stat3 in neonatal rat ventricular cardiomyocytes: a role in cardiac hypertrophy. *Cardiovasc Res* 73:57–65, 2007.
 46. Yue TL, Gu JL, Wang C, Reith AD, Lee JC, Mirabile RC, Kreutz R, Wang Y, Maleeff B, Parsons AA, Ohlstein EH. Extracellular signal-regulated kinase plays an essential role in hypertrophic agonists, endothelin-1 and phenylephrine-induced cardiomyocyte hypertrophy. *J Biol Chem* 275:37895–37901, 2000.