K_{ATP} channels mediate the antihypertrophic effects afforded by κ-opioid receptor stimulation in neonatal rat ventricular myocytes

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Received March 12, 2012; Accepted May 8, 2012

DOI: 10.3892/etm.2012.578

Abstract. Recent evidence suggests that κ-opioid receptor (OR) agonists and K_{ATP} channel activation exert antihypertrophic effects on cardiac myocytes. We studied the role of K_{ATP} channels in the antihypertrophic effects of ORs in primary cultures of neonatal rat ventricular myocytes exposed for 48 h to the α_1 adrenoceptor agonist phenylephrine and the relative contributions of mitochondrial K_{ATP} (mitoK_{ATP}) and sarcolemmal K_{ATP} (sarcK_{ATP}). Furthermore, we elucidated the pathway between ORs and K_{ATP} channels and their impact on intracellular Ca^{2+} ($[Ca^{2+}]_i$) transients. Hypertrophy of cardiomyocytes was characterized by increases in i) total protein content; ii) cell size and iii) [^{3}H]leucine incorporation. Phenylephrine (10 μM) increased the three parameters. Trans-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamid methanesulfonate salt (U50,488H), a selective κ-opioid receptor agonist, prevented phenylephrine-induced hypertrophy and $[Ca^{2+}]_i$ transients. The effect of U50,488H was abolished by nor-binaltorphimine, a selective κ-OR antagonist, indicating that the effect was κ-OR-mediated. The protein kinase C inhibitor chelerythrine and the K_{ATP} channel inhibitors glibenclamide (50 μM), a nonselective K_{ATP} antagonist, and 5-hydroxydecanoic acid (100 μM), a mitochondrial selective K_{ATP} antagonist, reversed the antihypertrophic effect of U50,488H, and there was no significant difference between the two K_{ATP} channel blockers. Moreover, we also determined the expression of the Kir6.2 subunits of the K_{ATP} channel, which increased in response to U50,488H in the presence of phenylephrine, but was suppressed by chelerythrine, glibenclamide and 5-hydroxydecanoic acid. U50,488H also attenuated the elevation of $[Ca^{2+}]_i$. This study suggests that K_{ATP}, and particularly the mitochondrial $K_{ATP,}$ mediates the antihypertrophic effects of κ-opioid receptor stimulation via the PKC signaling pathway.

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Key words: U50,488H, κ-opioid receptor, cardiac hypertrophy, ATP-sensitive potassium channel, protein kinase C, intracellular Ca^{2+}

Introduction

Cardiac hypertrophy has historically been considered to be an adaptive response; however, prolonged hypertrophy is associated with increased risk of sudden death or progression to heart failure (1). Recent studies have also demonstrated an antihypertrophic effect of κ-opioid receptor activation in cardiac myocytes. For example, U50,488H, a selective κ-opioid receptor agonist, inhibits the effects of norepinephrine, an α-adrenoceptor agonist, on the electrically induced intracellular Ca^{2+} transient in cardiac myocytes (2). We also demonstrated that U50,488H inhibits the Ca^{2+} transient and cardiac hypertrophy induced by isoprenaline, a β-adrenoceptor agonist (3). Emerging evidence indicates that K_{ATP} activation reduces the remodeling process and inhibits cardiac hypertrophy. For example, the K_{ATP} opener nicorandil has been shown to reduce myocardial remodeling in rats (4), whereas the putative mitoK_{ATP} opener diazoxide inhibited phenylephrine (PE)-induced cardiac hypertrophy in rat neonatal cardiomyocytes (5). Thus, these studies indicate a direct antihypertrophic effect of K_{ATP} activation in the heart. Cardiac K_{ATP} channels are composed of SUR2A and Kir6.2 subunits (6). There are two types of K_{ATP} channels, namely the mitochondrial K_{ATP} channel (mitoK_{ATP}) and the sarcolemmal K_{ATP} channel (sarcK_{ATP}). Previous studies have found that the opening of mitoK_{ATP} also plays an important role in cardiac protection, such as in ischemic preconditioning (7). The mitoK_{ATP} channel has been established to play a critical role in various types of preconditioning, whereas that of the sarcK_{ATP} channel is controversial (8).

Although the mechanism of the antihypertrophic effect of κ-opioid receptors is uncertain, we can refer to the relationship of K_{ATP} channels and κ-opioid receptor in ischemic preconditioning (IP). Previous studies have shown that the opening of K_{ATP} channels and activation of κ-opioid receptor exert cardioprotective effects against ischemic and reperfusion (I/R) injury. In IP, U50,488H reduced the infarct size induced by I/R in the rat and intracellular Ca^{2+} ($[Ca^{2+}]_i$). The infarct-reducing effect of U50,488H was reversed by blockade of the K_{ATP}

channel, which abolished the protective effect of preconditioning with U50,488H (9). It has also been shown that activation of PKC prevented the $[Ca^{2+}]_i$ overload and conferred cardioprotection against hypoxic insults, and blockade of the mitoK_{ATP} channel attenuated the effects of PKC activation (10). κ-opioid receptor signaling was impaired in cardiac hypertrophy due to a defect in the coupling of PKC signaling with its effector (11). δ_1-opioid receptor mediates a potent cardioprotective effect via protein kinase C and the mitochondrial K_{ATP} channel (12) and Wang *et al* (13) demonstrated that the mitochondrial K_{ATP} channel is dependent on PKC for protection against calcium and ischemia-induced injury.

In view of this body of evidence and the finding that K_{ATP} opener and κ-opioid receptor agonist attenuate hypertrophy, we hypothesized that the direct antihypertrophic effects of κ-opioid receptor stimulation may involve K_{ATP} activation and likely occur via the PKC pathway. Accordingly, the present study was designed to determine whether K_{ATP} channels mediate the antihypertrophic effect of κ-opioid receptors in neonatal rat ventricular myocytes and, if so, to assess and identify the nature of K_{ATP} involvement in mediating the antihypertrophic effect of κ-opioid receptor activation.

Materials and methods

Chemicals. Trans-(±)-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamid methanesulfonate salt (U50,488H, U50) was used as a selective κ-opioid receptor agonist (14,15), and nor-binaltorphimine (NBI) was used as an antagonist (16-18). Phenylephrine (PE), an α-adrenoceptor agonist, was used to induce hypertrophy. 5-Hydroxydecanoic acid (5-HD) was used as a specific blocker of the mitochondrial ATP-sensitive potassium channel. Glibenclamide was used as a nonselective K_{ATP} channel blocker. Chelerythrine was used as the protein kinase C inhibitor. The concentrations of U50,488H (19-21), PE, 5-HD, glibenclamide (22) and chelerythrine (12) were based on previous studies. All drugs were initially dissolved in distilled water and subsequently diluted in culture medium, except for glibenclamide and Fura-2/AM, which were dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO was <0.1%, which itself had no effect.

U50,488H, NBI, 5-HD, glibenclamide, PE, chelerythrine, Fura-2/AM, trypsin and DMEM were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Calf serum was obtained from Si Ji Qing Chemical Co., Hangzhou, China.

Culture of neonatal rat ventricular myocytes. In the experiment 65 neonatal rats were used, and the protocols were approved by the Committee of Liaoning Medical College for the Use of Experimental Animals for Research and Teaching. Sprague-Dawley rats, 2-3 days old, were sacrificed, and the heart was removed immediately. The ventricles were separated from the atrium, trisected, and digested with trypsin (Sigma) in 0.8 mg/ml for 20 min at 37˚C. Ventricular myocytes were cultured as described previously (21). The supernatant was removed following centrifugation and the pellet was re-suspended in fetal bovine serum. The above steps were repeated 4-6 times until the ventricle was completely digested. The cell suspension was diluted to $1x10^6$/ml and placed in 24-well tissue culture plates in humidified 5% CO_2/95% air at 37˚C for 48 h. The culture medium comprised 15% heat-inactivated fetal bovine serum, 84% Dulbecco's modified Eagle's medium (DMEM) and 1% penicillin-streptomycin, conditions shown to enhance the growth of cultured ventricular myocytes. Bromodeoxyuridine (0.1 mM) was added to prevent non-myocyte proliferation without toxicity to myocytes (23). In experiments involving treatment with PE, U50, NBI, 5-HD, glibenclamide or chelerythrine, a low-serum (0.4%) DMEM was used. Myocardial cells become 'quiescent' in low-serum medium and grow without multiplication and/or proliferation (24).

Determination of cellular protein content. Cells were cultured for 72 h with various treatments (72 h was chosen as preliminary studies showed that the maximum effects were obtained at that time). Dishes were washed rapidly three times with Hank's solution, the cells were dissolved in 1% sodium dodecylsulphate (SDS), and the protein content was measured using the method described by Lowry *et al* (25).

Estimation of cell volume. The volume of ventricular myocytes was calculated from measurement of cell diameter (26). The medium was aspirated and cells were washed rapidly three times with D-Hank's solution. Cells were then treated with 0.3 ml of 0.1% trypsin per well at 37˚C for 10 min and the process was terminated with 10% fetal bovine serum (0.2 ml/well). Digested cells were collected and measured using an inverted microscope. For measurements, four or five fields were randomly selected from 16 or 20 fields and photographed at high power (magnification, x400), and 80 individual cell areas were calculated using CIAS Daheng computer photograph analysis system (China Da Heng Co., Beijing, P.R. China).

Incorporation of [^{3}H]leucine. [^{3}H]leucine uptake was used as an index of protein synthesis. The medium from myocardial cells grown in 24-well plates was aspirated and replaced with a medium contaning 1 Ci [^{3}H]leucine. Drugs were added and incubation was continued for 48 h. The medium was then aspirated and cells were washed rapidly three times with cold Hank's solution. They were then lysed by addition of l ml per well 1% SDS. Lysates were collected and precipitated by the addition of 1 ml 5% trichloroacetic acid and then applied to fiberglass GF/C filters. After washing three times with 5 ml Hank's solution, filters were dried and transferred to vials containing 4 ml scintillation fluid and the radioactivity was determined using liquid scintillation counting (27). The radioactivity, which represented the [^{3}H]leucine incorporated into newly synthesized protein, was expressed as cpm per well.

Loading of cells with Fura-2/AM. Myocytes were cultured in wells, each with a coverslip. The coverslips with myocytes were incubated with Fura-2/AM (4 μM) in medium for 25 min. The unincorporated dye was removed by washing twice with fresh medium. To allow the Fura-2/AM in the cytosol to de-esterify, the loaded cells were maintained at room temperature (24-26˚C) for 60 min prior to the measurement of $[Ca^{2+}]_i$.

Measurement of cytosolic calcium transient. A spectrofluorometric method was used to measure the cytosolic

Ca^{2+} transient, using Fura-2/AM as the Ca^{2+} indicator. After loading with Fura-2/AM, the coverslips with myocytes were transferred to a superfusion chamber on the stage of an inverted microscope, which was coupled to a TILL imaging system (Munich, Germany), and superfused with Hank's buffer. The emitted light was filtered at 510 nm. Fluorescence signals at 340 nm (F340) and 380 nm (F380) were recorded on a personal computer for data processing and analysis. Maximal fluorescence for each coverslip was obtained after addition of the Ca^{2+} ionophore ionomycin (20 μM). Ethylene glycol tetraacetic acid (EGTA) was added to a final concentration of 20 mM for the Ca^{2+}-free condition. Cytosolic $[Ca^{2+}]$ was calculated by the following formula: $[Ca^{2+}]_i = Kd \times (Sf_2/Sb_2) \times (R_{340/380} - R_{min})/(R_{max} - R_{340/380})$ (28). Kd is the dissociation constant of Fura-2/AM for Ca^{2+} and was assumed to be 225 nM at 37˚C. $R_{340/380}$ is the ratio of corrected fluorescence signals. R_{max} is the ratio obtained following ionomycin treatment. R_{min} is the ratio of the corrected signals obtained after EGTA treatment. Sf_2 and Sb_2 represent the emission intensities at 380 nm excitation at saturation and under Ca^{2+}-free conditions, respectively.

Western blotting. Cells were washed once with ice-cold PBS containing 100 μM sodium orthovanadate and solubilized in the lysis buffer (50 mM Tris-HCl, 137 mM NaCl, 10% glycerol, 100 μM sodium orthovanadate, 1 mM phenylmethylsulfonylfluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1% Nonident P-40; pH 7.4). After centrifugation at 12,000 x g for 20 min, the supernatant was removed. Cells were dissolved in buffer containing 65 mM Tris-HCl (pH 6.8), 3% SDS, 10% glycerol, and 6 mol urea. After measurement of protein concentration (BCA kit, Pierce, Rockford, IL, USA), β-mercaptoethanol and bromophenol blue were added to the buffer for electrophoresis. Protein (60 μg) thus obtained (for Kir6.2) was separated on 10% SDS-PAGE and transblotted to polyvinylidene difluoride membranes (BioRad, Hercules, CA, USA). The blots were incubated at 4˚C overnight with antibodies and the resulting bands were detected using enhanced chemiluminescence. Antibodies to Kir6.2 at Thr-276 (1:1000 dilution; Santa Cruz) were used to detect the activated form of the kinase. Intensities in the resulting bands were quantified using CAMIAS008 image analysis system.

Statistical analysis. All data are expressed as the mean ± SEM. For the effects of drugs at various concentrations, analysis of variance (one-way ANOVA) was used to compare the control and treatment groups. The post-LSD test was used to evaluate differences between two groups. $P<0.05$ was considered to indicate statistical significance.

Results

Effects of U50,488H, glibenclamide, 5-HD or chelerythrine on PE-induced enhancement of spontaneous $[Ca^{2+}]_i$ transients. PE (10 μM) significantly increased the resting $[Ca^{2+}]_i$ (Fig. 1D) and reduced the peak amplitude (Fig. 1B) of spontaneous $[Ca^{2+}]_i$ transients (Fig. 1A). Both were abolished by 1 μM U50,488H, which had no effect on normal cells. The effect of U50,488H was abolished by 1 μM NBI, 50 μM glibenclamide, 100 μM 5-HD and 2 μM chelerythrine, each of which alone had no effect. None of the treatments had any effect on the increased frequency of spontaneous $[Ca^{2+}]_i$ transients (Fig. 1C).

Effects of U50,488H, glibenclamide, 5-HD or chelerythrine on PE-induced enhancement of total protein content, cell size and [^{3}H]leucine incorporation. PE (10 μM) significantly increased the total protein content (Fig. 2A), cell size (Fig. 2B) and [^{3}H]leucine incorporation (Fig. 2C) in myocytes. These effects were abolished by 1 μM U50,488H, which itself had no effect. The inhibitory effects of U50,488H were abolished by 1 μM NBI, 100 μM 5-HD, 50 μM glibenclamide and 2 μM chelerythrine, each of which alone had no effect.

Effects of U50,488H, glibenclamide, 5-HD or chelerythrine on Kir6.2 expression. U50,488H increased the expression of Kir6.2 in myocytes exposed to PE, which itself had no effect (Fig. 3). Glibenclamide (50 μM), 5-HD (100 μM), NBI (1 μM) or chelerythrine (2 μM) abolished the effects of U50,488H.

Discussion

The present study demonstrated that administration of U50,488H attenuated the increase in total protein content, cell size and [^{3}H]leucine incorporation induced by PE in rat neonatal cardiomyocytes and that the effects were abolished by nor-binaltorphimine. Having elucidated the identity of the antihypertrophic effect of κ-opioid receptor, the goal of the study focused on the signaling pathway involved. The initial hypothesis was that the pathway was probably quite similar to that involved in the κ-OR-mediated cardioprotective effect observed in previous studies (9). The involvement of a K_{ATP} channel in the adenosine receptor-mediated antihypertrophic effect has also been well characterized (22). A previous study revealed that an opioid agonist potentiated the opening of cardiac K_{ATP} channels produced by a K_{ATP} channel opener to produce an additive cardioprotective effect (29). To examine whether the same mechanisms are at work in the present study, K_{ATP} channel blockers were administered individually during treatment with U50. Thus, our study demonstrated for the first time that the antihypertrophic effect of κ-OR activation in rat neonatal cardiomyocytes, at least with respect to PE-induced hypertrophy, is dependent on K_{ATP} activation. This hypothesis is based on the finding that the role of K_{ATP} in mediating the antihypertrophic effect of κ-OR activation was clearly indicated by the ability of pharmacological inhibitors of the channels to abrogate the effect of U50. To determine the relative contributions of mitochondrial K_{ATP} ($mitoK_{ATP}$) and sarcolemmal K_{ATP} ($sarcK_{ATP}$) in the effect of U50, we administered the nonspecific K_{ATP} blocker glibenclamide or the $mitoK_{ATP}$ specific blocker 5-HD, both of which reversed the effect of U50. Surprisingly, the reversing effect of the two blockers in response to U50 was equivalent. This indicates that $mitoK_{ATP}$ plays a critical role. These data show that the K_{ATP} channel, and most likely the mitochondrial channel, is a downstream effector of the κ-opioid receptor.

The relative contributions of sarcolemmal and mitochondrial K_{ATP} channel opening were revealed. The consequences of sarc/mito K_{ATP} channel opening affect various measures of antihypertrophy. $SarcK_{ATP}$ channel increases potassium efflux

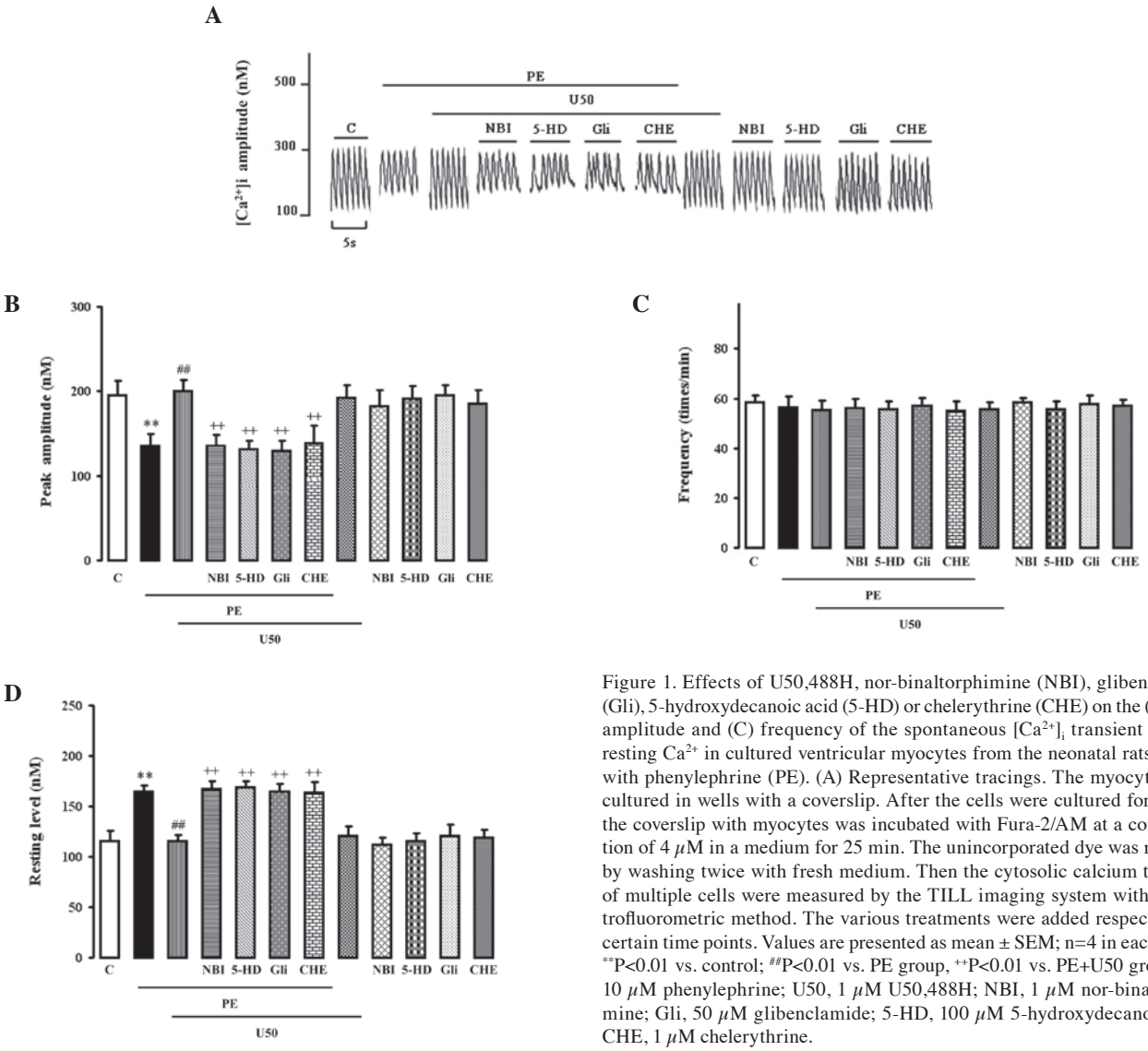

Figure 1. Effects of U50,488H, nor-binaltorphimine (NBI), glibenclamide (Gli), 5-hydroxydecanoic acid (5-HD) or chelerythrine (CHE) on the (B) peak amplitude and (C) frequency of the spontaneous $[Ca^{2+}]_i$ transient and (D) resting Ca^{2+} in cultured ventricular myocytes from the neonatal rats treated with phenylephrine (PE). (A) Representative tracings. The myocytes were cultured in wells with a coverslip. After the cells were cultured for 2 days, the coverslip with myocytes was incubated with Fura-2/AM at a concentration of 4 μM in a medium for 25 min. The unincorporated dye was removed by washing twice with fresh medium. Then the cytosolic calcium transient of multiple cells were measured by the TILL imaging system with a spectrofluorometric method. The various treatments were added respectively at certain time points. Values are presented as mean ± SEM; n=4 in each group. $^{**}P<0.01$ vs. control; $^{\#\#}P<0.01$ vs. PE group, $^{++}P<0.01$ vs. PE+U50 group. PE, 10 μM phenylephrine; U50, 1 μM U50,488H; NBI, 1 μM nor-binaltorphimine; Gli, 50 μM glibenclamide; 5-HD, 100 μM 5-hydroxydecanoic acid; CHE, 1 μM chelerythrine.

from the cell, hastening repolarization and shortening the potential duration of the action. Mitochondrial K_{ATP} channel activation is associated with numerous effects, including membrane depolarization and changes in Ca^{2+} homeostasis (30). A brief depolarization of the mitochondrial membrane may exert antihypertrophy by preventing Ca^{2+} entry into the matrix. Therefore, it is likely that κ-OR activation opens mitochondrial K_{ATP} and results in the decrease in the mitochondrial membrane potential, thus reducing the driving force of Ca^{2+} influx and attenuating the mitochondrial Ca^{2+} overload induced by PE. Thus, a reduction in 'mitochondrial remodeling' may constitute a significant contributor to the antihypertrophic effect of κ-OR. The study has also provided the first evidence that the effect of the K_{ATP} channels is accompanied by prevention/attenuation of the changes in $[Ca^{2+}]_i$ homeostasis, namely $[Ca^{2+}]_i$ overload, indicating that the prevention/attenuation of the changes in $[Ca^{2+}]_i$ homeostasis may contribute, at least partly, to the roles of the K_{ATP} channels by attenuation of the $[Ca^{2+}]_i$ overload in response to PE-induced hypertrophy. PE significantly increased the resting $[Ca^{2+}]_i$ and reduced the peak amplitude and spontaneous $[Ca^{2+}]_i$ transients. Both were abolished by U50,488H, which had no effect on normal cells. The effect of U50,488H was abolished by 1 μM nor-binaltorphimine, glibenclamide, 5-HD and chelerythrine, each of which alone had no effect.

Although our data support the hypothesis that κ-OR activation inhibits PE-induced cardiac hypertrophy through the opening of K_{ATP}, and particularly mito$K_{ATP,}$ channels, via attenuation of the $[Ca^{2+}]_i$ overload in neonatal cardiomyocytes, the signaling pathway between them remains uncertain. An additional set of experiments was performed to determine whether PKC was involved in the signal transduction pathway. A study by Seymour *et al* (31) showed that opioid receptors result in activation of PKC, which then functions to open the K_{ATP} channel to further enhance a cardioprotective signal, and Wang *et al* (13) found that the mitochondrial K_{ATP} channel is dependent on PKC for protection against calcium and ischemic-induced injury. Opioid agonists act through Gi protein-coupled opioid receptors, leading to the translocation and activation of protein kinase C. Active PKC then initiates cardioprotection through multiple kinase pathways, which phosphorylate undetermined effectors (32,33). Mitochondrial K_{ATP} channels opened by opioid-agonist stimulation also play a critical role in PKC-mediated cardioprotection (34,35). Therefore, we used

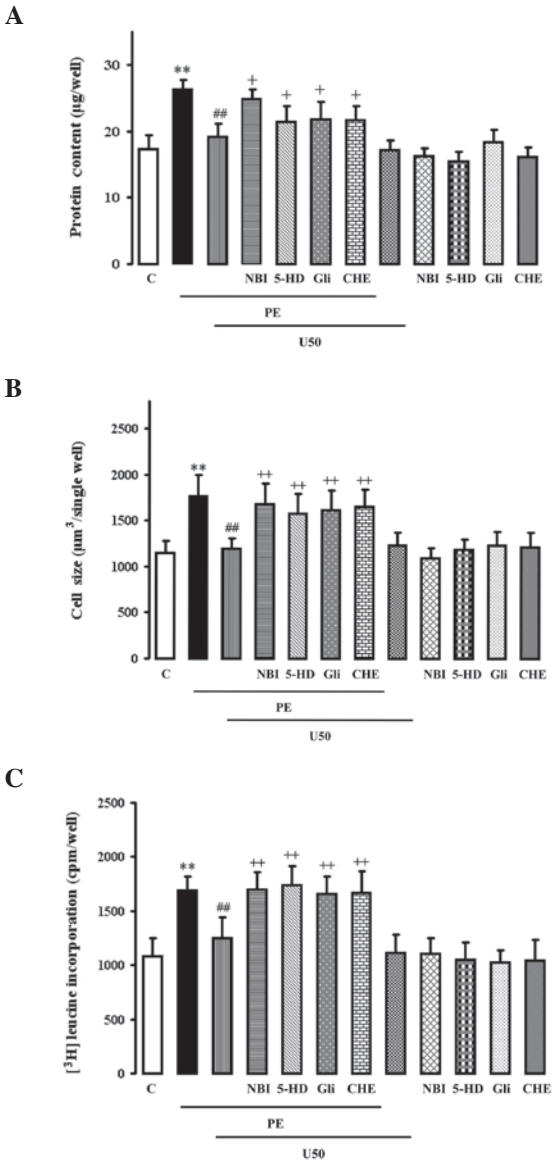

Figure 2. Effects of U50,488H, nor-binaltorphimine (NBI), glibenclamide (Gli), 5-hydroxydecanoic acid (5-HD) or chelerythrine (CHE) on (A) protein content, (B) cell size and (C) [^{3}H]leucine uptake in cultured ventricular myocytes from the neonatal rats treated with phenylephrine (PE). Methods and times of cell culture are as described in Materials and methods. After the cells were cultured for 2-3 days, the medium was changed to DMEM supplemented with 0.4% calf serum. The various treatments were added to the medium as described in Materials and methods and cultured for 48 h. Values are presented as mean ± SEM; n=6. **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. PE group; $^{+}$P<0.05, $^{++}$P<0.01 vs. PE+U50 group. PE, 10 μM phenylephrine; U50, 1 μM U50,488H; NBI, 1 μM nor-binaltorphimine; Gli, 50 μM glibenclamide; 5-HD, 100 μM 5-hydroxydecanoic acid; CHE, 1 μM chelerythrine.

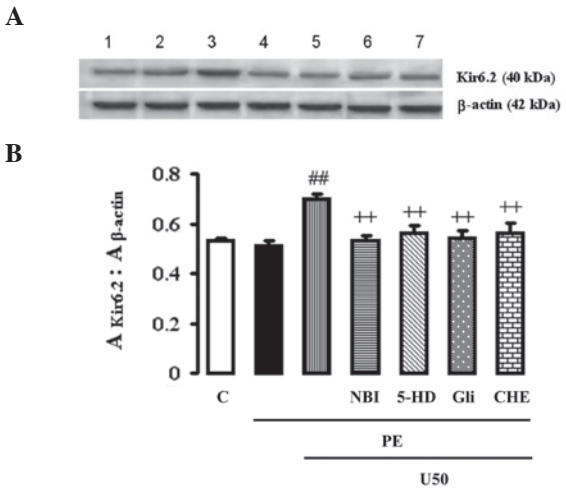

Figure 3. Effects of U50,488H, nor-binaltorphimine (NBI), glibenclamide (Gli), 5-hydroxydecanoic acid (5-HD) and chelerythrine (CHE) on Kir6.2 expression in cultured myocardial cells from neonatal rats treated with phenylephrine (PE). Apart from the cells (1x10^6 cells per flask), the method and time of cell culture were the same as in Fig. 2. The different treatments were added to the medium at the same time and cultured for 48 h. (A) Representative autoradiograms of Kir6.2 and β-actin. Lane 1, normal control; lane 2, PE 10 μM; lane 3, PE 10 μM + U50 1 μM; lane 4, PE 10 μM + NBI 1 μM + U50 1 μM; lane 5, PE 10 μM + 5-HD 100 μM + U50 1 μM; lane 6, PE 10 μM + Gli 50 μM + U50 1 μM; lane 7, PE 10 μM + CHE 1 μM + U50 1 μM. (B) Relative levels of Kir6.2 expressed as the absorbance ratio of each group:control (%). Values are presented as the mean ± SEM., n=4 in each group. $^{\#\#}$P<0.01 vs. PE group. $^{++}$P<0.01 vs. PE + U50 group. PE, 10 μM phenylephrine; U50, 1 μM U50,488H; NBI, 1 μM nor-binaltorphimine; Gli, 50 μM glibenclamide; 5-HD, 100 μM 5-hydroxydecanoic acid; CHE, 1 μM chelerythrine.

chelerythrine, a PKC inhibitor, to determine whether blocking PKC has any effect upon the observed opioid receptor-mediated antihypertrophic effect in myocytes. The data clearly show an inhibition of the effect of U50, and indicate that PKC is involved in the pathway. Moreover, to determine whether K_{ATP} channels act downstream of PKC we assessed the expression of Kir6.2, a subunit of the K_{ATP} channel, in the presence of chelerythrine. The data indicated that U50 increased the expression of Kir6.2 in the presence of PE, which suggests that U50 activated the opening of the K_{ATP} channel. However, when chelerythrine was administered prior to U50, the expression of Kir6.2 decreased compared with that of the PE+U50 group, which showed that the PKC inhibitor blocked the activation of the K_{ATP} channel. This reveals that PKC acts upstream of the K_{ATP} channel. Activation of G-protein-coupled receptors may stimulate PKC to enhance K_{ATP} channel activity.

In conclusion, our study shows an important role for K_{ATP} in mediating the antihypertrophic effects of κ-OR activation. Based on our results, we propose that the antihypertrophic effect of κ-OR activation is dependent on K_{ATP} channel activity, particularly mitoK_{ATP} activity, via attenuation of $[Ca^{2+}]_i$ overload. Although PKC was associated with the antihypertrophic effects of κ-OR receptor activation, the precise role of this pathway and the role of PKC subtypes require further study. Furthermore, evaluation of SUR2A and Kir6.2 mRNAs is clearly warranted.

Acknowledgements

We thank Professor I.C. Bruce for the advice, particularly regarding the language editing of the manuscript, and Z.M. Qi, X.L. Xu and Z.H. Zong for their expert technical assistance. This work was supported by the National Natural Science Foundation (30973898/C190702).

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