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PRECLINICAL RESEARCH

Effect of ATP-Sensitive Potassium Channel Agonists on Ventricular Remodeling in Healed Rat Infarcts

Tsung-Ming Lee, MD, FESC,* Mei-Shu Lin, PHD,† Nen-Chung Chang, MD, PHD, FACC‡
Tainan and Taipei, Taiwan

Objectives The purpose of this study was to determine whether ATP-sensitive potassium (K_{ATP}) channel agonists exert a beneficial effect on the structural, functional, and molecular features of the remodeling heart in infarcted rats.

Background Myocardial K_{ATP} channels have been implicated in the ventricular remodeling after myocardial infarction by inhibition of 70-kDa S6 (p70S6) kinase.

Methods Male Wistar rats after induction of myocardial infarction were randomized to either vehicle, agonists of K_{ATP} channels nicorandil and pinacidil, an antagonist of K_{ATP} channels glibenclamide, or a combination of nicorandil and glibenclamide or pinacidil and glibenclamide for 4 weeks. To verify the role of p70S6 kinase in ventricular remodeling, rapamycin was also assessed.

Results Significant ventricular hypertrophy was detected by increased myocyte size at the border zone isolated by enzymatic dissociation after infarction. Increased synthesis of p70S6 kinase messenger ribonucleic acid after infarction in vehicle-treated rats was confirmed by reverse transcription-polymerase chain reaction, consistent with the results of immunohistochemistry and Western blot for phosphorylated p70S6 kinase. Rats in the nicorandil- and pinacidil-treated groups significantly attenuated cardiomyocyte hypertrophy and phosphorylated p70S6 kinase expression with similar potency, as compared with vehicle. The beneficial effects of nicorandil and pinacidil were abolished by administering either glibenclamide or 5-hydroxydecanoate. Addition of rapamycin attenuated ventricular remodeling and did not have additional beneficial effects compared with those seen in rats treated with either nicorandil or pinacidil alone.

Conclusions Activation of K_{ATP} channels by either nicorandil or pinacidil can attenuate ventricular remodeling, probably through a p70S6 kinase-dependent pathway after infarction. (J Am Coll Cardiol 2008;51:1309–18) © 2008 by the American College of Cardiology Foundation

Cardiac remodeling is an unfavorable evolution associated with myocardial hypertrophy after myocardial infarction (MI) (1). Cardiac remodeling is a complex process involving numerous signaling pathways. Pharmacological therapies, such as angiotensin-converting enzyme inhibitors and beta-blockers through different molecular targets, have been used to improve cardiac remodeling (2). Previous studies have shown that left ventricular (LV) remodeling is improved after nicorandil treatment (3), implying myocardial ATP-sensitive potassium (K_{ATP}) channels may play a role in ventricular remodeling after infarction. We have shown a direct relation between the activation of myocardial K_{ATP} channels and ventricular remodeling by administering K_{ATP} channel antagonists in hyperlipidemic rabbits (4). Cabo and Boyden (5) have shown a marked reduction of the current density of potassium current at the border zone cells compared with that in the remote sites. Activation of K_{ATP} channels has been shown to attenuate cardiac hypertrophy by inhibition of 70-kDa S6 (p70S6) kinase (6), a key trigger of protein synthesis for hypertrophic changes. The activity of p70S6 kinase is controlled by multiple phosphorylation. Phosphorylation of Thr389 most closely correlates with p70S6 kinase activity in vivo (7). Although we have demonstrated a cardioprotection against infarct of any size and myocardial ischemia by activation of K_{ATP} channels in animals (8) and in humans (9) in acute settings, the observed beneficial effects do not provide information on whether similar effects would be present on ventricular remodeling with long-term administration of K_{ATP} channel agonists.

Cardiac myocytes contain 2 distinct K_{ATP} channels with 1 subtype located in the sarcolemma and the other in the

From the *Cardiology Section, Department of Medicine, Taipei Medical University and Chi-Mei Medical Center, Tainan, Taiwan; †Department of Pharmacy, National Taiwan University and Hospital, Taipei, Taiwan; and the ‡Cardiology Section, Department of Medicine, Taipei Medical University and Hospital, Taipei, Taiwan. This work was supported by a grant from Chi-Mei Medical Center (CMFHT 9501, CMFHR9502, CMFHR9506, and CM-TMU9501) and a grant from National Science Council, Republic of China (NSC 95-2314-B-384-009).

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Abbreviations and Acronyms

GAPDH = glyceraldehyde-3-phosphate-dehydrogenase
HD = hydroxydecanoate
K_{ATP} = ATP-sensitive potassium
LV = left ventricle/ventricular
LVEDD = left ventricular end-diastolic diameter dimension
LVESD = left ventricular end-systolic diameter dimension
MI = myocardial infarction
p70S6 = 70-kDa S6
RT-PCR = reverse transcription-polymerase chain reaction

inner membrane of the mitochondria (10). Mitochondrial K_{ATP} channels share some pharmacological properties with sarcolemmal K_{ATP} channels, while possessing a distinct pharmacologic response. K_{ATP} channels are activated by numerous compounds with distinct chemical structures (11). These agents, collectively termed as K_{ATP} channel agonists, cause various biological effects through modulation of the sarcolemmal and mitochondrial K_{ATP} channels (11). Pinacidil has been shown to activate both sarcolemmal and mitochondrial K_{ATP} channels, whereas nicorandil activates only mitochondrial K_{ATP} channels (11). The purpose of this study was: 1) to investigate how 2 K_{ATP} channel agonists, nicorandil and pinacidil, affect ventricular remodeling; 2) to assess the role of p70S6 kinase systems; and 3) to determine which subtype of K_{ATP} channels plays a role in ventricular remodeling in a rat MI model by the use of 5-hydroxydecanoate (5-HD), a specific mitochondrial K_{ATP} channel blocker.

Methods

Animals. Male Wistar rats (250 to 300 g) were subjected to ligation of the anterior descending artery, resulting in infarction of the LV free wall. Rats were randomly assigned into 9 groups so as to have approximately the same number of survivors in each group: 1) vehicle group; 2) nicorandil (0.1 mg/kg/day, Chugai Pharmaceutical Co., Tokyo, Japan), a specific mitochondrial K_{ATP} channel agonist; 3) pinacidil (0.1 mg/kg/day, Sigma Chemical Co., St. Louis, Missouri), a nonspecific K_{ATP} channel agonist; 4) glibenclamide (1.4 mg/kg/day), a nonspecific K_{ATP} channel blocker; 5) a combination of nicorandil and glibenclamide; 6) a combination of pinacidil and glibenclamide; 7) rapamycin (0.5 mg/kg/day), a p70S6 kinase inhibitor; 8) a combination of nicorandil and rapamycin; and 9) a combination of pinacidil and rapamycin. The doses of nicorandil (12), pinacidil (13), and glibenclamide (14) used in this study have been shown to specifically modulate K_{ATP} channels without the interference of hemodynamics. To verify the role of p70S6 kinase in K_{ATP} channel inhibition-related ventricular hypertrophy, we further assessed rapamycin in the role of ventricular remodeling. The oral dose of rapamycin was according to previous studies (6), showing effective attenuation of cardiac remodeling induced by long-term inhibition of nitric oxide synthesis. The drugs were given orally by gastric gavage once a day. The drugs were started 24 h after MI, during which drugs can maximize benefits at this timing window (15) and minimize the possibility of a direct effect on infarct size. The study duration was designed to be 4 weeks because the majority of the myocardial remodeling process in the rat (70% to 80%) is complete within 3 weeks (16). To prevent hypoglycemic attacks during the administration of glibenclamide, 2.5% (weight/volume) sucrose in filtered tap water was supplied and glucose examinations were performed once per week by the 1-touch method. Sham operation served as controls to exclude the possibility of drugs themselves directly to alter cardiomyocyte hypertrophy.

Although results of the above study showed that K_{ATP} channel antagonist-induced ventricular remodeling was altered at 4 weeks after infarction (see Results section), glibenclamide was used as an antagonist of K_{ATP} channels, which can block both mitochondrial and sarcolemmal K_{ATP} channels and have multiple actions independent of K_{ATP} channels. To further confirm the role of K_{ATP} channel agonist-induced ventricular remodeling and assess which subtype of K_{ATP} channels plays a role in attenuating p70S6 kinase expression, we used 5-HD, a specific mitochondrial K_{ATP} channel blocker, in an in vitro model. Four weeks after induction of MI by coronary ligation, infarcted rat hearts were isolated and subjected to no treatment (vehicle), nicorandil (50 μM), pinacidil (50 μM), 5-HD (100 μM), nicorandil + 5-HD, or pinacidil + 5-HD. Each heart was perfused with a noncirculating modified Tyrode's solution containing (in mM): NaCl 117.0, $NaHCO_3$ 23.0, KCl 4.6, NaH_2PO_4 0.8, $MgCl_2$ 1.0, $CaCl_2$ 2.0, and glucose 5.5, equilibrated at 37°C and oxygenated with a 95% O_2 to 5% CO_2 gas mixture. The perfusion medium was maintained at a constant temperature of 37°C with a constant flow at 4 ml/min as previously described (17). Drugs were infused for 30 min. The doses of nicorandil (18), pinacidil (19), and 5-HD (20) used in this study have been shown to modulate K_{ATP} channels in an isolated heart. At the end of the study, all hearts (n = 10 in each group) were used for performing Western blot from samples at the border zone. The animal experiment was approved and conducted in accordance with local institutional guidelines for the care and use of laboratory animals in the Chi-Mei Medical Center.

Experimental MI. After anesthesia with ketamine (90 mg/kg) intraperitoneally, rats were intubated and the anterior descending artery was ligated using a 6-0 silk as previously described (17). Sham rats underwent the same procedure except the suture was passed under the coronary artery and then removed. Mortality in the animals with MI was ~50% within the first 24 h. None of the sham-operated animals died.

Echocardiogram. At 28 days after operation, rats were lightly anesthetized with intraperitoneal injection of ketamine HCl (25 mg/kg). Echocardiographic measurements were done with an HP Sonos 5500 system with a 15-6L (6 to 15 MHz, SONOS 5500, Agilent Technologies, Palo Alto, California) probe. M-mode tracing of the LV was

obtained from the parasternal long-axis view to measure left ventricular end-diastolic diameter dimension (LVEDD) and left ventricular end-systolic diameter dimension (LVESD), and fractional shortening (%) was calculated. After this, the hearts quickly underwent hemodynamic measurement after systemic heparinization.

Hemodynamics and infarct size measurements. Using a 2-F micromanometer-tipped catheter (Model SPR-407, Millar Instruments, Houston, Texas) inserted through the right carotid artery as in our previous description (17), we measured the maximal rate of LV pressure rise (+dP/dt) and decrease (−dP/dt). Next, the heart was rapidly excised and divided into right and left atria, right ventricles, LV, and the scarred area. Each tissue was then weighed individually. Infarct size (%) was expressed as the ratio of the sum of external and internal perimeters of LV as described by Pfeffer and Braunwald (16). Samples of the LV from the border zone (0 to 2 mm outside the infarct) were cut transmurally, frozen rapidly in liquid nitrogen, and stored at −80°C until use. It has been shown that hypertrophy of the residual myocardium progresses after MI if infarct size is larger than 30% of the LV (16). Thus, with respect to clinical importance, only rats with infarction larger than 30% of the LV were selected for analysis.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis of p70S6 kinase. To further confirm the downstream activation of K_{ATP} channels, messenger ribonucleic acid (mRNA) levels of p70S6 kinase were measured by real-time quantitative RT-PCR from samples obtained from the border zone with the TaqMan system (Prism 7700 Sequence Detection System, Applied Biosystems, Courtaboeuf, France) as in our previous description (17). For p70S6 kinase the primers were sense, 5′-GAAGATTTATTGGTAGCCCACGAA-3′; antisense, 5′-GCACCTCGTCCCCAGAAA-3′. For glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), the primers were 5-CTTCACCACCATGGAGAAGGC-3′ and 5′-GGCATGGACTGTGGTCATGAG-3′. For quantification, p70S6 kinase expression was normalized to the expressed housekeeping gene GAPDH. Reaction conditions were programmed on a computer linked to the detector for 40 cycles of the amplification step.

Western blot analysis of p70S6 kinase. Samples from the border zone were homogenized, and the supernatant protein concentration was determined with the BCA protein assay reagent kit (Pierce, Rockford, Illinois); 20 μg protein was separated by 8% SDS-PAGE and electrotransferred onto a nitrocellulose membrane. After incubation with anti–phospho-p70S6 kinase antibodies (Thr389, #9205L, Cell Signalling, Hitchin, Kent, United Kingdom) and anti–total-p70S6 kinase antibodies (#9202, Cell Signalling, Hitchin), antigen-antibody complexes were detected with 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium chloride (Sigma). Films were volume-integrated within the linear range of the exposure using a scanning densitometer. Experiments were replicated 3 times and results expressed as the mean value.

Immunohistochemical analysis of p70S6 kinase. To confirm the downstream pathways of K_{ATP} channel, immunohistochemical staining of phospho-p70S6 kinase was performed on LV muscle from the border zone. Cryosections were performed at a thickness of 5 μm and incubated with a rabbit polyclonal anti–phospho-p70S6 kinase antibody (Santa Cruz Biotechnology, Santa Cruz, California) at dilution 1:10. Immunostaining with p70S6 kinase antibodies was performed using a standard immunoperoxidase technique (N-Histofine Simple Stain Rat MAX PO kit, Nichirei Co., Tokyo, Japan). The antibody used had been tested for specificity in the rat. Isotype-identical directly conjugated antibodies served as a negative control.

Cell isolation. Because cardiac hypertrophy is a combination of reactive fibrosis and myocyte hypertrophy, we measured cardiomyocyte sizes at the border zone besides using myocardial weight to avoid the interference of nonmyocytes on post-infarction hypertrophy. Myocytes were enzymatically isolated with collagenase (type II, Sigma) and protease (type XIV, Sigma) according to previously described techniques (17). Random high-power fields of the rod-like relaxed myocytes with clear striations were selected to eliminate selection bias. In the sham-operated group, cell width and length were measured from the LV free wall for comparisons. At least 100 cells from each section were selected for measurement of cell length, width, and area, and the mean value was used as the individual value for each section.

Morphometric determination of cardiac fibrosis. Heart sections from the remote regions were stained with Sirius red stain to distinguish areas of connective tissue as previously described (21). The percentage of red staining, indicative of fibrosis, was measured in 10 fields randomly selected on each section (Image Pro Plus, Media Cybernetics, Silver Spring, Maryland). The value was expressed as the ratio of Sirius red-stained fibrosis area to total infarct area. All sections were evaluated under blind conditions without prior knowledge as to which section belonged to which rat.

Laboratory measurements. To determine the confounding roles of glucose and insulin in ventricular remodeling, blood samples from the aorta were assayed at the end of the study. Plasma insulin was measured by ultrasensitive rat enzyme immunoassay (Mercodia, Uppsala, Sweden).

Statistical analysis. Results were presented as mean ± standard deviation. Statistical analysis was performed using the SPSS statistical package (version 10.0, SPSS Inc., Chicago, Illinois). A 1-way analysis of variance was used to search for possible effects of nicorandil, pinacidil, and glibenclamide on the measurements of hemodynamics and myocyte sizes. In case of a significant effect, the measurements between the groups were compared, with a value of $p < 0.007$ (Bonferroni's correction) as significant. Probability values were 2-tailed, and a value of $p < 0.05$ was considered to be statistically significant.

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Results

Differences in mortality between vehicle (n = 3, 25%) and treated groups (n = 20, 20%) were not found throughout the study. No sham-operated rats had evidence of cardiac damage. Blood pressure, heart rate, infarct size, and glucose levels did not significantly differ among the infarcted groups (Table 1). Insulin concentrations were significantly increased in rats that were administered glibenclamide.

Morphometric studies. In the sham-operated rats, treatment with either nicorandil, pinacidil, or glibenclamide had little effect on cardiac gross morphology (data not shown), whereas there were significant effects on the cardiac morphology after MI (Table 1). Four weeks after MI, the infarcted area of the LV was very thin and was totally replaced by fully differentiated scar tissue. The vehicle-, glibenclamide-, and a combination of nicorandil and glibenclamide- or pinacidil- and glibenclamide-treated groups had an increase in right ventricular weight/body weight ratio, and lung weight/body weight ratio compared with the sham-operated rats.

To characterize the cardiac hypertrophy on a cellular level, we isolated cardiomyocytes from different treated groups (Table 2). The cell areas isolated from the border zone in the vehicle significantly increased by 63% compared with those from the same area of sham-operated hearts (4,664 ± 187 μm^2 in the vehicle vs. 2,863 ± 92 μm^2, $p < 0.0001$). Nicorandil and pinacidil reduced cell areas 20% and 19% compared with vehicle (both $p < 0.0001$, respectively). Conversely, the rats to which glibenclamide was administered developed cardiomyocyte hypertrophy compared with the nicorandil- and pinacidil-treated groups alone. Besides, treatment of rapamycin significantly attenuated cardiomyocyte hypertrophy and blunted the increase in cell area by about 15% compared with those treated with vehicle at the border zone, a figure similar to that in either the nicorandil- or pinacidil-treated group. However, addition of rapamycin did not further attenuate ventricular hypertrophy in either the nicorandil- or pinacidil-treated group.

Fibrosis of the LV from the remote zone was examined in tissue sections after Sirius red staining as shown in Figure 1. Infarcted rats treated with vehicle had significantly larger areas of intense focal fibrosis compared with sham-operated rats (0.09 ± 0.04% vs. 0.02 ± 0.02%, $p < 0.0001$). Compared with vehicle, treatment with either nicorandil or pinacidil attenuated fibrosis. Collagen formation in the LV was significantly increased in rats treated with a combination of K_{ATP} channel agonists and glibenclamide compared with K_{ATP} channel agonists-treated rats alone.

Echocardiographic data. Compared with sham-operated hearts, MI hearts showed structural changes such as increased LV diastolic and systolic diameters (Figs. 2 and 3), consistent with LV remodeling. Both LVEDD and LVESD in rats with MI were significantly reduced by nicorandil or pinacidil treatment ($p < 0.0001$). Left ven-

Table 1 Cardiac Morphometry, Hemodynamics, and Levels of Glucose and Insulin at End of Study

Parameters	Sham	Infarction Treated With								
		Vehicle	Nic	Pin	Glib	Nic + Glib	Pin + Glib	Rap	Rap + Nic	Rap + Pin
Number of rats	10	9	10	9	11	11	11	10	10	10
BW, g	397 ± 38	391 ± 33	414 ± 21	425 ± 16	392 ± 32	413 ± 23	431 ± 25	422 ± 26	420 ± 17	418 ± 20
HR, beats/min	408 ± 20	399 ± 20	402 ± 23	416 ± 25	399 ± 28	418 ± 20	413 ± 24	412 ± 20	406 ± 21	401 ± 19
LVESP, mm Hg	118 ± 10	108 ± 9	102 ± 6	101 ± 8	107 ± 9	104 ± 7	105 ± 7	100 ± 7	103 ± 8	105 ± 7
LVEDP, mm Hg	6 ± 1	17 ± 2*	16 ± 4*	14 ± 3*	18 ± 4*	19 ± 3*	20 ± 3*	17 ± 4*	16 ± 5*	16 ± 5*
LVW/BW, mg/g	2.19 ± 0.19	2.93 ± 0.29*	2.84 ± 0.47*	2.89 ± 0.37*	3.22 ± 0.68*	3.07 ± 0.18*	3.09 ± 0.21*	2.80 ± 0.43*	2.92 ± 0.35*	2.78 ± 0.41*
RVW/BW, mg/g	0.54 ± 0.05	0.70 ± 0.13*	0.56 ± 0.07†	0.58 ± 0.07‡	0.72 ± 0.14*	0.66 ± 0.07*	0.68 ± 0.05*	0.52 ± 0.06§	0.55 ± 0.07§	0.54 ± 0.08§
LungW/BW, mg/g	4.16 ± 0.52	5.46 ± 0.62*	4.47 ± 0.42†	4.52 ± 0.38‡	5.18 ± 0.53*	5.09 ± 0.36*	5.15 ± 0.42*	4.59 ± 0.45§	4.62 ± 0.30§	4.52 ± 0.38§
+dP/dt, mm Hg/s	8,326 ± 1,032	4,173 ± 895*	6,778 ± 695†	6,573 ± 428*‡	5,234 ± 646*	4,872 ± 881*	3,876 ± 473*	6,382 ± 485*§	6,763 ± 238*§	6,392 ± 315*§
−dP/dt, mm Hg/s	5,656 ± 961	3,034 ± 671*	4,577 ± 482*†	4,635 ± 423*‡	2,988 ± 598*	2,700 ± 733*	2,275 ± 564*	4,291 ± 282*§	4,425 ± 382*§	4,297 ± 419*§
Infarct size, %	—	42 ± 5	42 ± 6	40 ± 5	41 ± 6	44 ± 9	41 ± 3	40 ± 5	40 ± 6	42 ± 6
Glucose, mg/dl	90 ± 8	88 ± 9	95 ± 10	89 ± 10	88 ± 12	92 ± 8	94 ± 8	95 ± 8	92 ± 11	93 ± 10
Insulin, μu/ml	21 ± 10	49 ± 15*	53 ± 17*	55 ± 10*	125 ± 16*§	128 ± 22*§	119 ± 21*§	45 ± 12*	51 ± 10*	48 ± 13*

Values are mean ± standard deviation. *$p < 0.005$ compared with sham; †$p < 0.007$ compared with infarcted groups treated with vehicle and nicorandil (Nic) + glibenclamide (Glib); ‡$p < 0.007$ compared with infarcted groups treated with vehicle and pinacidil (Pin) + Glib; §$p < 0.007$ compared with vehicle.
BW = body weight; HR = heart rate; LungW = lung weight; LVEDP = left ventricular end-diastolic pressure; LVESP = left ventricular end-systolic pressure; LVW = left ventricular weight; Rap = rapamycin; RVW = right ventricular weight.

Table 2 Characteristics of Isolated Cardiomyocytes

Parameters	Sham	Infarction Treated With								
		Vehicle	Nic	Pin	Glib	Nic + Glib	Pin + Glib	Rap	Rap + Nic	Rap + Pin
Number of animals	5	4	5	5	5	5	5	5	5	5
Border zone										
Myocyte length, μm	132 ± 5	179 ± 11*	158 ± 8*†	151 ± 12*‡	172 ± 12*	177 ± 11*	182 ± 10*	161 ± 11*§	156 ± 14*§	153 ± 9*§
Myocyte width, μm	21 ± 3	24 ± 3*	21 ± 2†	22 ± 2‡	27 ± 3*	26 ± 2*	25 ± 3*	21 ± 3§	22 ± 2§	21 ± 3§
Measured myocyte areas, μm^2	2,863 ± 92	4,664 ± 187*	3,721 ± 153*†	3,760 ± 85*‡	4,782 ± 162*	4,920 ± 143*	5,090 ± 86*	3,963 ± 186*§	3,894 ± 132*§	3,595 ± 126*§
Remote zone										
Myocyte length, μm	135 ± 6	143 ± 13‖	141 ± 10‖	136 ± 11‖	146 ± 13‖	143 ± 9‖	144 ± 12‖	145 ± 11	138 ± 11‖	143 ± 11
Myocyte width, μm	20 ± 2	19 ± 2‖	19 ± 3	20 ± 2	18 ± 2‖	19 ± 2‖	20 ± 2‖	19 ± 2	20 ± 3	19 ± 2
Measured myocyte areas, μm^2	2,861 ± 97	2,959 ± 89‖	2,985 ± 102‖	3,104 ± 98‖	2,851 ± 96‖	2,911 ± 88‖	3,092 ± 75‖	3,008 ± 114‖	2,983 ± 86‖	3,197 ± 105

Values are mean ± standard deviation. *$p < 0.005$ compared with the sham-operated group; †$p < 0.007$ compared with infarcted groups treated with vehicle and Nic + Glib; ‡$p < 0.007$ compared with infarcted groups treated with vehicle and Pin + Glib; §$p < 0.05$ compared with vehicle; ‖$p < 0.05$ compared with respective data from border zones within the same group.

Abbreviations as in Table 1.

Figure 1 Cardiac Fibrosis at the Remote Zone

(Top) Representative sections from the remote zone with sirius red staining (**red**, magnification 400×) at 4 weeks after infarction. Collagen deposition within the left ventricle is reduced after administering either nicorandil (Nic) or pinacidil (Pin). The line length corresponds to 50 μm. **(Bottom)** Left ventricular collagen area fraction (%). Each column and bar represents mean ± standard deviation. *$p < 0.007$ compared with vehicle-, glibenclamide (Glib)-, Nic + Glib-, and Pin + Glib-treated groups. **(A)** Sham; **(B)** vehicle; **(C)** Nic; **(D)** Pin; **(E)** Glib; **(F)** Nic + Glib; **(G)** Pin + Glib; **(H)** rapamycin (Rap); **(I)** Rap + Nic; **(J)** Rap + Pin.

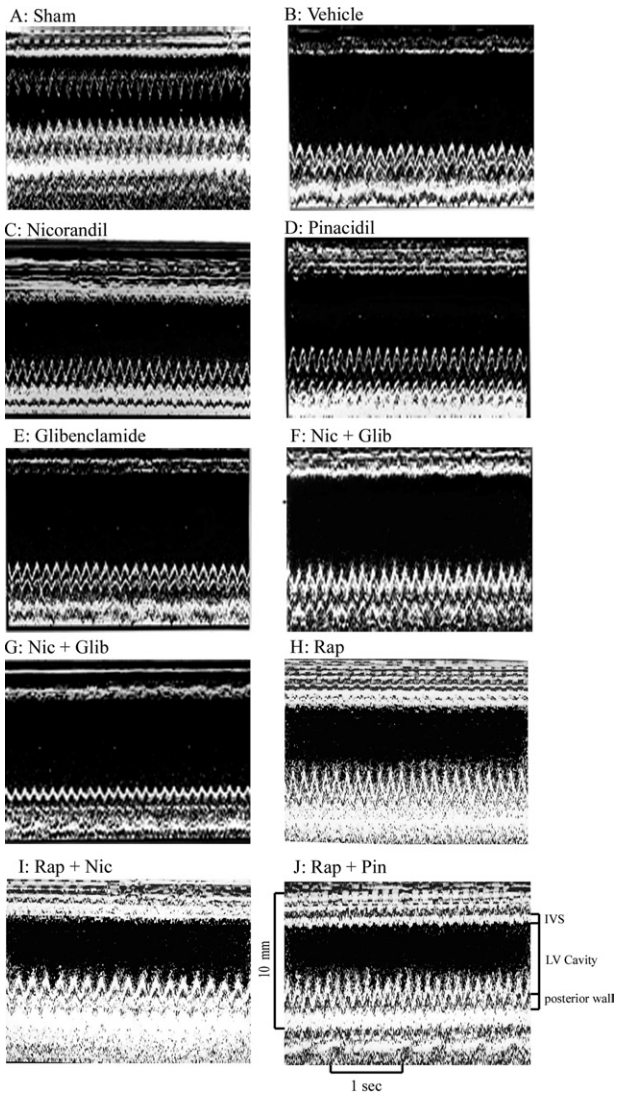

Figure 2 **Echocardiogram in Infarcted Rats**

Representative M-mode image reveals a hypokinetic-to-akinetic anterior wall and left ventricular (LV) dilation in the infarcted hearts **(B to J)** in contrast to normal anterior wall motion in sham-operated heart **(A)**. There are markedly dilated LV end-diastolic diameter and LV end-systolic diameter in groups treated with vehicle **(B)**, Glib **(E)**, Nic + Glib **(F)**, and Pin + Glib **(G)** compared with those in groups treated with Nic **(C)** and Pin **(D)**. Besides, infarcted rats treated with Rap **(H)**, Rap + Nic **(I)**, and Rap + Pin **(J)** showed a significant decrease of LV end-diastolic diameter and LV end-systolic diameter compared with vehicle. IVS = interventricular septum thickness; other abbreviations as in Figure 1.

tricular fractional shortening was significantly higher in the nicorandil- or pinacidil-treated group compared with the vehicle. Conversely, the rats to which glibenclamide was administered developed impaired LV systolic function and progressive LV dilation more than that in the nicorandil- and pinacidil-treated groups alone. These data were corroborated by the results that +dP/dt and −dP/dt were significantly improved in the nicorandil- and pinacidil-treated groups compared with the combined groups.

Immunohistochemical analyses, Western blot, and real-time PCR of p70S6 kinase. Immunohistochemical analysis of the infarcted myocardium revealed the presence of phospho-p70S6 kinase immunoreactivity in the myocardial tissue (Fig. 4). Stronger phospho-p70S6 kinase signals at the border zone of vehicle-treated rats were observed than in the same region of sham rats. The intensity of the immu-

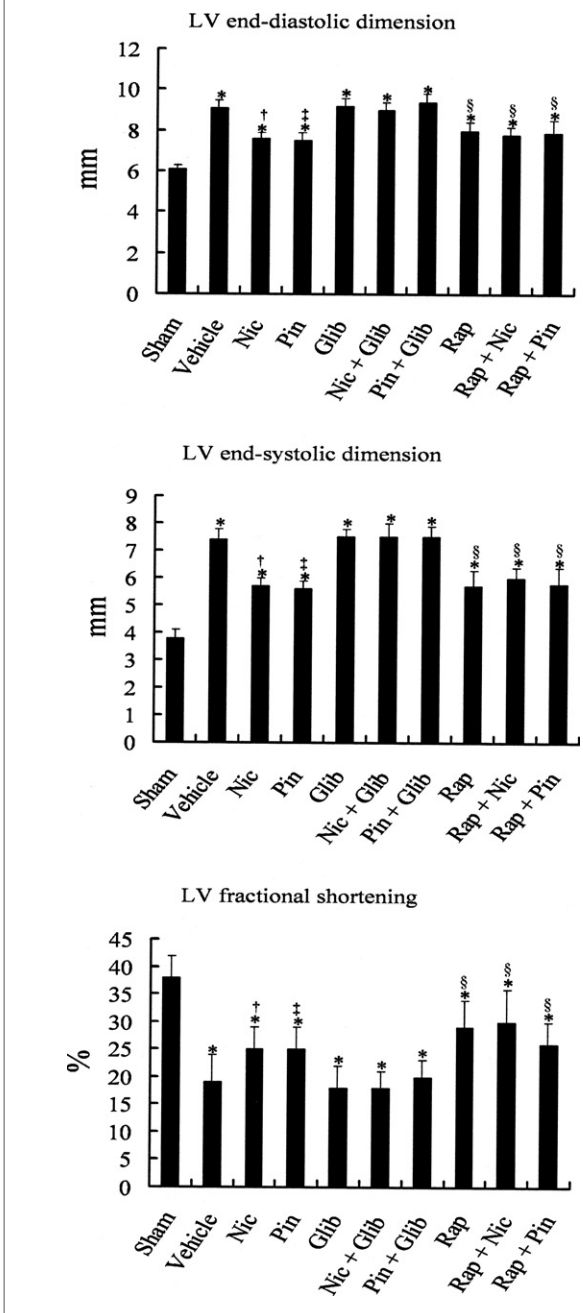

Figure 3 **Comparison of LV Remodeling and Function by 2D Echocardiography Study**

Values are mean ± standard deviation. *p < 0.005 compared with the sham group; †p < 0.007 compared with infarcted groups treated with vehicle and Nic + Glib; ‡p < 0.007 compared with infarcted groups treated with vehicle and Pin + Glib; §p < 0.007 compared with vehicle. LV = left ventricular; 2D = 2-dimensional; other abbreviations as in Figure 1.

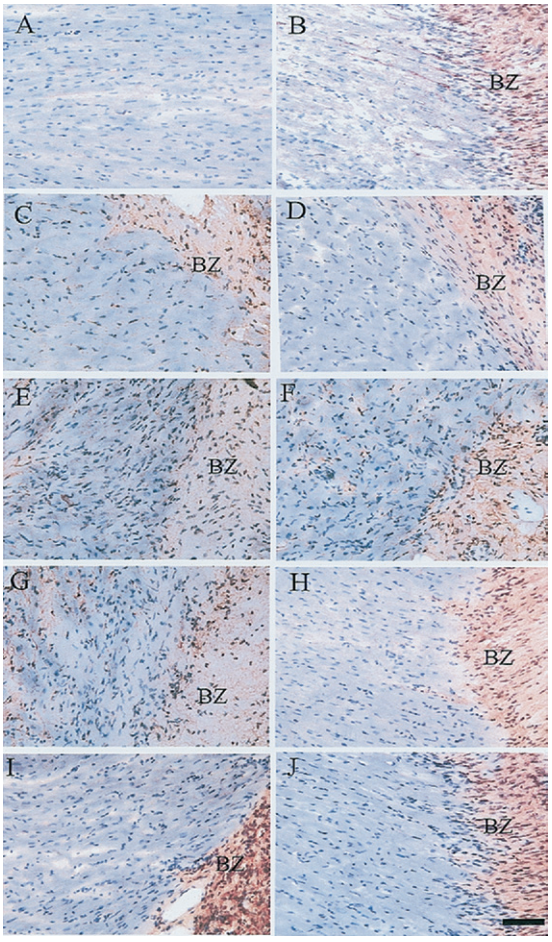

Figure 4 **Immunohistochemical Microscopy of p70S6 Kinase at the BZ in Different Treated Rats**

Positive staining for phospho-p70S6 kinase (p70S6 kinase) (magnification 200×) **(brown color)** in myocytes is significantly higher in groups treated with vehicle **(B)**, glibenclamide **(E)**, and a combination of nicorandil + glibenclamide **(F)** and pinacidil + glibenclamide **(G)** than those in sham **(A)**, nicorandil- **(C)**, pinacidil- **(D)**, rapamycin- **(H)**, rapamycin + nicorandil- **(I)**, and rapamycin + pinacidil–treated rats **(J)**. The line length corresponds to 200 μm. BZ = border zone.

noreaction was reduced in the nicorandil- and pinacidil-treated groups compared with that in the vehicle. Nicorandil- and pinacidil-induced beneficial effects were reversed by the addition of glibenclamide, implicating K_{ATP} channels as the relevant target.

Western blot shows that the levels of LV phospho-p70S6 kinase were significantly more up-regulated 1.9-fold at the border zone in the vehicle than in sham-operated rats ($p < 0.0001$), whereas total p70S6 kinase remained constant (Fig. 5). Compared with vehicle in nicorandil- and pinacidil-treated rats, LV phospho-p70S6 kinase levels were significantly lower at the border zone. The rats to which glibenclamide was administered showed significantly higher phospho-p70S6 kinase levels than those in the nicorandil- and pinacidil-treated groups alone. To elucidate the role of mitochondrial K_{ATP} channels in modulating p70S6 kinase, 5-HD was assessed in an in vitro model. Figure 6 shows that 5-HD significantly inhibited attenuated expression of phospho-p70S6 kinase compared with either nicorandil or pinacidil alone, confirming the role of mitochondrial K_{ATP} channels in mediating p70S6 kinase.

The mRNA levels of p70S6 kinase showed a 5.4 ± 1.2-fold up-regulation at the border zone in the vehicle compared with the sham-operated rats ($p < 0.0001$) (Fig. 7). Thus, the mRNA levels of p70S6 kinase changed in parallel to the tissue peptide levels, implying that the production of p70S6 kinase mRNA is a critical regulation step for its local activation.

Discussion

This study demonstrates for the first time that chronic treatment for 4 weeks with K_{ATP} channel agonists leads to favorable ventricular remodeling, probably through attenuated activity of phospho-p70S6 kinase. These results were concordant for beneficial effects of K_{ATP} channel agonists, as documented structurally by reduction in myocyte sizes and cardiac fibrosis, molecularly, by myocardial p70S6 kinase protein and mRNA levels, and functionally, by

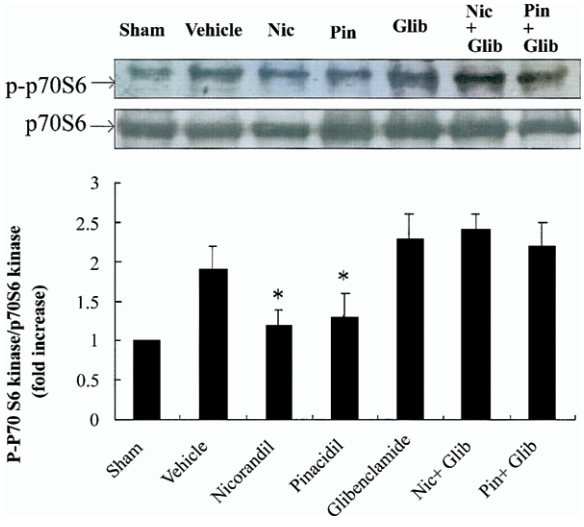

Figure 5 **Western Blot of Phospo-p70S6 Kinase and Total p70S6 Kinase in an In Vivo Study**

(Top) Western blot analysis of p70S6 kinase showing the effect of K_{ATP} channels on immunorecognition of p70S6 kinase in homogenates of the LV from the border zone. p70S6 kinase activity was measured with phospho-specific antibody against phosphorylated p70S6 kinase **(top band)** and a total p70S6 kinase antibody **(bottom band)**. Ventricular remodeling after myocardial infarction was associated with marked increase of phospho-p70S6 kinase. A significantly reduced phospho-p70S6 kinase had taken place in the groups treated with either nicorandil or pinacidil administration compared with that seen in vehicle. **(Bottom)** Densitometric quantification of phosphorylation levels was expressed as the ratio of the density of phosphorylated band over total p70S6 kinase. Each point is an average of 3 separate experiments. *$p < 0.007$ compared with vehicle-, Glib-, Nic + Glib-, and Pin + Glib–treated groups. Abbreviations as in Figure 1.

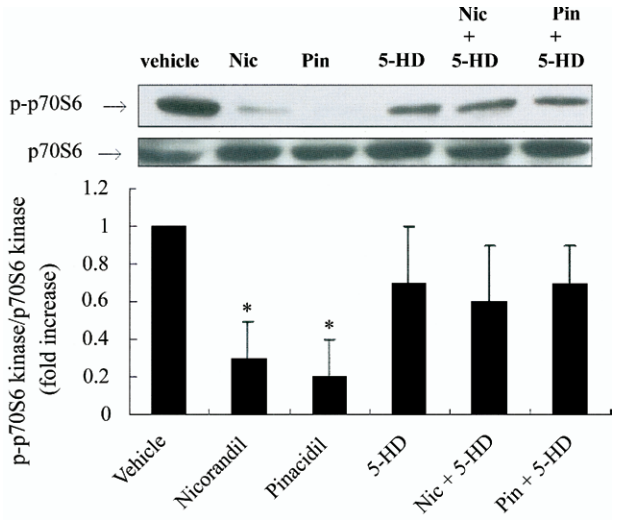

Figure 6 **Western Blot of Phospo-p70S6 Kinase and Total p70S6 Kinase in an In Vitro Study**

(Top) Western blot analysis of p70S6 kinase to furthermore confirm the mitochondrial K_{ATP} channels on p70S6 kinase activity in homogenates of the left ventricle from the border zone in a rat isolated heart model. P70S6 kinase activity was measured with phospho-specific antibody against phosphorylated p70S6 kinase **(top band)** and a total p70S6 kinase antibody **(bottom band)**. A significantly increased phospho-p70S6 kinase is noted in the groups treated with a combination of K_{ATP} channel agonists and 5-hydroxydecanoate (5-HD) compared with that seen in the groups treated with K_{ATP} channel agonists alone. **(Bottom)** Densitometric quantification of phosphorylation levels was expressed as the ratio of the density of phosphorylated band over total p70S6 kinase. Each point is an average of 3 separate experiments. $*p < 0.007$ compared with vehicle-, 5-HD-, Nic + 5-HD-, and Pin + 5-HD–treated groups. Abbreviations as in Figure 1.

improvement of echocardiography-derived systolic function. These new observations strengthen the concept that K_{ATP} channels play a central role in the remodeling process and may improve our understanding of the beneficial effect of early administration of K_{ATP} channel agonists in post-infarction remodeling.

The beneficial effect of K_{ATP} channels on LV remodeling was demonstrated as evidenced by 3 observations. First, K_{ATP} channel-related LV remodeling was noted after infarction. Administration of K_{ATP} channel agonists attenuates the dysfunction of chronically infarcted hearts. Cardiomyocyte sizes, cardiac fibrosis, and echocardiographically derived LVESD and LVEDD were significantly smaller in either the pinacidil- or nicorandil-treated group than those in the vehicle. Our results were consistent with the findings of Coromilas et al. (22), showing that K_{ATP} channels at the border zone remain functional and can be activated by K_{ATP} channel agonists. The involvement of K_{ATP} channels is further supported by the antagonizing action of glibenclamide. Second, either pinacidil or nicorandil administration can attenuate cardiomyocyte hypertrophy with a similar potency at the border zone. A similar potency to attenuate hypertrophy by pinacidil and nicorandil may suggest that mitochondrial K_{ATP} channels play a role in regulating cardiomyocyte hypertrophy. Although dissimilar structures, pinacidil and nicorandil appear to share a common mediator, p70S6 kinase, in which transcription levels of p70S6 kinase may play a role in the signal transduction pathway. Third, the involvement of p70S6 kinase in mitochondrial K_{ATP} channel inhibition-related ventricular remodeling after infarction was further confirmed by administering a mitochondrial K_{ATP} antagonist (5-HD). The pharmacologic selectivity of the used mitochondrial K_{ATP} channel blocker, at the conventional inhibitory concentrations (20) used in the present study may indicate the important role of the mitochondrial K_{ATP} channel in modulating p70S6 kinase activity, leading to attenuate cardiac hypertrophy. Furthermore, addition of rapamycin attenuated ventricular remodeling and did not have additional beneficial effects compared with rats treated with either nicorandil or pinacidil alone, confirming the critical role of p70S6 kinase in ventricular remodeling. Indeed, our results were consistent with the observation that cell hypertrophy is regulated by mitochondrial K_{ATP} channel-dependent p70S6 kinase (23,24).

It appears from our study that the attenuated ventricular remodeling is related to an activation of K_{ATP} channels. The mechanisms by which K_{ATP} channel agonists attenuate ventricular remodeling remain to be defined. However, several factors can be excluded: 1) hemodynamics: neither pinacidil nor nicorandil exerted any hemodynamic effects at the dose used in this study. Nicorandil has been shown to reduce hypertrophy in animals, which has been assumed to be due to the drug's blood-pressure-lowering effect (25). The observation was not consistent with our stable hemodynamics throughout the study in nicorandil-treated rats. Indeed, in rats, Xu et al. (26) have shown that oral dose of nicorandil (6 mg/kg/day), a dose much higher than that

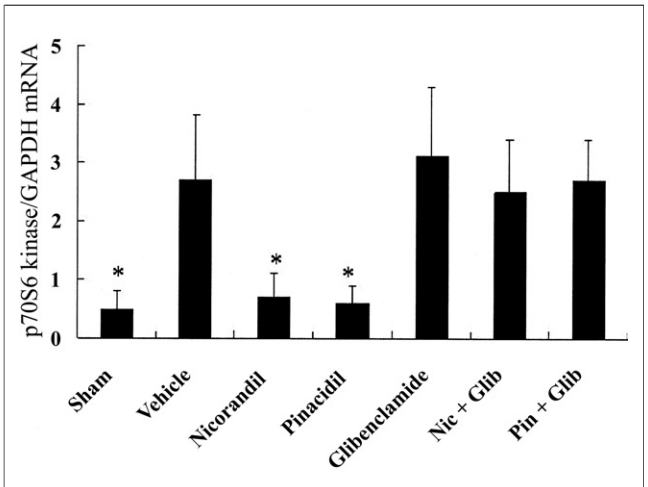

Figure 7 **Western Blot of p70S6 Kinase in an In Vivo Study**

Each mRNA was corrected for an mRNA level of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). Each column and bar represents mean ± standard deviation. $*p < 0.007$ compared with vehicle-, Glib-, Nic + Glib-, and Pin + Glib–treated groups. LV = left ventricular; other abbreviations as in Figure 1.

used in our study (0.1 mg/kg/day), did not lower blood pressure. 2) Differences in MI size: MI size is an important determinant of LV remodeling (27). However, there is a similar MI size among the infarcted groups.

Other mechanisms. Although the present study suggests that the mechanisms of K_{ATP} channel agonist-induced attenuated ventricular remodeling may be related to inhibition of p70S6 kinase, there are possible other candidates modulating the antihypertrophic effects of K_{ATP} channel agonists, such as endothelin-1 and angiotensin. First, blockade of endothelin-1 alleviated the development of cardiac hypertrophy (28). Activation of K_{ATP} channels has been shown to attenuate cardiac hypertrophy by inhibition of endothelin-1 (29). Thus, K_{ATP} channel agonists may attenuate cardiac hypertrophy by attenuated production of endothelin-1. Second, blunting by K_{ATP} channel agonists of antihypertropic effect may also result from attenuated effects of angiotensin; K_{ATP} channel agonists have been shown to inhibit angiotensin II-induced increase in cell protein content (30). Complex interactions between endothelin-1, angiotensin II, and K_{ATP} channels exist that could affect cardiac hypertrophy. Therefore, a variety of upstream regulators could interfere with hypertrophic signaling pathways. The development of cardiac hypertrophy is a multigenic, integrative response involving signal integration of multiple pathways. The failure to completely abrogate the hypertrophic process was not surprising in view of the underlying complex of hypertrophic process, which is unlikely to be amendable to one therapeutic intervention.

Clinical implications. After acute MI, patients remain at high risk for recurrent cardiovascular events and mortality (31). Previous studies have demonstrated that regional myocyte hypertrophy parallels regional myocardial dysfunction during post-infarct remodeling (32). Attenuation of ventricular remodeling prevents the transition for compensatory dilation to ventricular dysfunction and failure. Thus, the K_{ATP} channel agonists may have important biological effects that prevent occurrence of post-infarcted heart failure. Our results explained, at least in part, the clinical findings of the IONA (Impact Of Nicorandil in Angina) study group (33), showing that treatment with nicorandil improves outcome in terms of reducing events related to acute coronary disease and the associated requirement for admission to hospital.

Conclusions

These findings are consistent with a pathogenetic role of regional K_{ATP} channels in cardiac remodeling after MI. Early intervention with K_{ATP} channel agonists after MI can attenuate ventricular remodeling probably through inhibition of phospho-p70S6 kinase pathway. The pharmacologic profile of K_{ATP} channel agonists gives new perspectives in the early treatment of acute MI.

Reprint requests and correspondence: Prof. Nen-Chung Chang, Cardiology Section, Department of Medicine, Taipei Medical University and Hospital, 252, Wu-Hsing Street, Taipei, 110, Taiwan. E-mail: ncchang@tmu.edu.tw.

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