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Effect of pravastatin on left ventricular mass in the two-kidney, one-clip hypertensive rats

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Lee, Tsung-Ming, Mei-Shu Lin, Chang-Her Tsai, and Nen-Chung Chang. Effect of pravastatin on left ventricular mass in the two-kidney, one-clip hypertensive rats. *Am J Physiol Heart Circ Physiol* 291: H2705–H2713, 2006. First published June 23, 2006; doi:10.1152/ajpheart.00224.2006.—We have demonstrated that myocardial ATP-sensitive potassium (K_{ATP}) channels are implicated in the development of cardiac hypertrophy in hyperlipidemic rabbits. We investigated the effect of pravastatin on development of ventricular hypertrophy in male normolipidemic Wistar rats with two-kidney, one-clip (2K1C) hypertension and whether the attenuated hypertrophic effect was via activation of K_{ATP} channels. Twenty-four hours after the left renal artery was clipped, rats were treated with one of the following therapies for 8 wk: vehicle, nicorandil (an agonist of K_{ATP} channels), pravastatin, glibenclamide (an antagonist of K_{ATP} channels), hydralazine, nicorandil plus glibenclamide, or pravastatin plus glibenclamide. Systolic blood pressure, relative left ventricular (LV) weight, and cardiomyocyte sizes significantly increased in vehicle-treated 2K1C rats compared with those in sham-operated rats. Treatment with either nicorandil or pravastatin significantly attenuated LV hypertrophy/body weight compared with the vehicle, which was further confirmed by downregulation of LV atrial natriuretic peptide mRNA. Nicorandil-induced effects were abolished by administering glibenclamide. Similarly, pravastatin-induced beneficial effects were reversed by the addition of glibenclamide, implicating K_{ATP} channels as the relevant target. A dissociation between the effects of blood pressure and cardiac structure was noted because pravastatin and hydralazine reduced arterial pressure similarly. These results suggest a crucial role of cardiac K_{ATP} channel system in the development of ventricular hypertrophy in the 2K1C hypertensive rats. Pravastatin is endowed with cardiac antihypertrophic properties probably through activation of K_{ATP} channels, independent of lipid and hemodynamic changes.

hypertension; ion channels; statins

EPIDEMIOLOGICAL STUDIES have demonstrated that increased left ventricular (LV) mass is a risk factor of cardiac morbidity and mortality in patients with hypertension (27). Previous data revealed that LV mass regression reduced cardiovascular complications (10). Agents that cause the regression of ventricular hypertrophy have been shown to decrease cardiovascular complication (2). To more effectively prevent cardiac hypertrophy and more successfully apply therapeutic interventions, it is important to better understand the factor(s) involved in ventricular growth at the early stage of cardiac hypertrophy rather than at the established stage, and therapeutic interventions can be more successfully applied.

The systemic renin-angiotensin system plays an important role in cardiac remodeling during the development of 2-kidney, 1-clip (2K1C) hypertension. Renovascular hypertension in the 2K1C model is characterized by elevated ANG II expression caused by ischemia in the clipped kidney and shear stress in the nonclipped kidney. The process of cardiac remodeling in renin-dependent renovascular hypertension is characterized by an increased LV mass. Myocardial hypertrophy is characterized by altered phenotype; typically, this is manifest within the LV as a reexpression of fetal isoforms such as atrial natriuretic peptide (ANP) (2). However, although angiotensin-converting enzyme inhibitors can reduce blood pressure quite effectively, they cannot completely reverse LV mass in the 2K1C hypertensive rats (36), implying that other factors are undoubtedly involved in ventricular remodeling. Cardiac hypertrophy is a complex process involving numerous signaling pathways, such as myocardial ATP-sensitive potassium (K_{ATP}) channels. The ability of K_{ATP} channel openers to protect the ischemic and reperfused myocardium has been demonstrated (20, 25). However, little is known concerning the potential long-term effects of K_{ATP} channel opening. Recently, we showed an in vivo direct relation between inactivation of myocardial K_{ATP} channels and ventricular hypertrophy by administering K_{ATP} channel antagonists in hyperlipidemic rabbits (22). Xia et al. (42) have shown in vitro attenuated cardiomyocyte hypertrophy by activating K_{ATP} channels. Activation of K_{ATP} channels has been shown to attenuate cardiac hypertrophy by inhibition of 70-kDa S6 kinase (31) and Na/H exchanger isoform-1 (42), key triggers of protein synthesis for hypertrophic changes.

3-Hydroxy-3-methyglutaryl-CoA reductase inhibitor (statin) therapy has been shown to reduce cardiovascular morbidity and mortality, far surpassing the improvement of lipid profile (39). Epidemiological studies have demonstrated that the benefit of statin treatment extends to the patients with normocholesterolemia (39). Statins exert pleiotropic properties and interfere with signal pathways for hypertrophy, effects that may contribute to their beneficial effects on ventricular hypertrophy (39). We have demonstrated that pravastatin induces cardioprotection by increasing adenosine levels (24), an important mediator to trigger opening of K_{ATP} channels. Although we have shown that statins activate K_{ATP} channels in hypercholesterolemic rabbits (22), it remained unknown whether prav-

astatin would also prevent the ventricular hypertrophy in 2K1C renovascular hypertension, a process of hypertrophy induced by a different mechanism. The purpose of this study was to investigate the effect of pravastatin on LV mass and the role of myocardial K_{ATP} channels in 2K1C renovascular hypertension.

METHODS

Experimental Animals

Male normocholesterolemic Wistar rats that weighed 300–350 g were fed a normal sodium diet and offered tap water ad libitum. Twenty-four hours after the left renal artery was clipped, rats were randomly allocated to one of seven groups and treated for 8 wk: *1*) vehicle group; *2*) nicorandil (0.1 mg/kg per day, Chugai Pharmaceutical), a K_{ATP} channel agonist; *3*) pravastatin (5 mg/kg per day, Sankyo); *4*) glibenclamide (1.4 mg/kg per day), a K_{ATP} channel blocker; *5*) hydralazine (10 mg/kg per day); *6*) a combination of nicorandil and glibenclamide; and *7*) a combination of pravastatin and glibenclamide. Aged-matched controls received sham operation. The doses of nicorandil (7), pravastatin (22), and glibenclamide (1) used in this study have been shown to specifically modulate K_{ATP} channels without the interference of hemodynamics. The drugs were added to the drinking water, with careful monitoring of water consumption and body weight to ensure proper drug dosage. In each treated group, drugs were withdrawn ~24 h before the experiments to eliminate their pharmacological actions. To prevent hypoglycemic attacks during the administration of glibenclamide, glucose was supplied, and frequent glucose examinations were performed by the one-touch method. Since pravastatin has a blood pressure-lowering effect (9, 37), hydralazine was used to determine the degree to which this decrease in blood pressure is responsible for attenuating cardiac hypertrophy. The animals tolerated the treatment very well. 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, Tainan, Taiwan.

2K1C

To create the model, male rats were anesthetized with ketamine (90 mg/kg) intraperitoneally. Silver clips, 0.2-mm internal diameter, were slipped around the left renal artery as close as possible to its exit from the aorta. Sham-operated rats were exposed to the same surgical manipulations, except the clipping. The wound was sutured, and the animals were allowed to recover. After return to their cage, all rats were maintained on a regular diet for 8 wk.

Echocardiogram

At the end of the study, rats were lightly anesthetized with intraperitoneal injection of ketamine HCl (25 mg/kg). Echocardiographic measurements were done with a HP Sonos 5500 system with a 15–6L (6–15 MHz, SONOS 5500; Philips Medical System, Best) probe. M-mode tracing of the LV was obtained from the parasternal long-axis view to measure LV end-diastolic diameter dimension (LVEDD) and LV end-systolic diameter dimension (LVESD), posterior wall (PW), and interventricular septum (IVS), and fractional shortening (FS, %) was calculated. LV mass was calculated using standard formulas: LV mass = $1.04 \times [(\text{LVEDD} + \text{PW} + \text{IVS})^3 - \text{LVEDD}^3]$. FS (%) = (LVEDD − LVSD)/LVEDD × 100%. The hearts then quickly underwent hemodynamic measurement after systemic heparinization.

Hemodynamics

Functional parameters were measured in anesthetized rats at the end of the study. Using a 2-F micromanometer-tipped catheter (model SPR-407, Miller Instruments, Houston, TX) inserted through the right carotid artery, we measured LV systolic and diastolic pressure as the mean of measurements of five consecutive pressure cycles as previously described (21). Maximum and minimum first derivatives of LV pressure (+dP/d*t* and −dP/dt) were obtained from hemodynamic recordings. Next, the heart was then rapidly divided into right and left atria, right ventricles, and LV. Each cardiac tissue and both kidneys were then weighed individually. First, a midventricular section was immediately frozen in OCT for measuring tissue endothelin-1 levels. Another midventricular section of the LV was then cut perpendicularly to its longitudinal axis and fixed in phosphate-buffered 4% formaldehyde. Histological paraffin-embedded sections (5 μm) were then prepared.

Real-time RT-PCR

To further confirm the degree of ventricular hypertrophy, mRNA levels of ANP were measured by real-time quantitative RT-PCR from samples obtained from the border zone with the TaqMan system (Prism 7700 Sequence Detection System, PE Biosystems) as previously described (21). For ANP, the primers were 5′-GCCCTTGCG-GTGTGTCA and 5′-TGCAGCTCCAGGAGGGTATT. For glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), the primers were 5′-CTTCACCACCATGGAGAAGGC and 5′-GGCATGGACTGTG-GTCATGAG. For quantification, ANP 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.

Morphometric Determination of Myocyte Size

Because cardiac hypertrophy is a combination of reactive fibrosis and myocyte hypertrophy, we measured cardiomyocyte sizes besides using myocardial weight to avoid the confounding influence of nonmyocytes on cardiac hypertrophy. LV sections were stained with hematoxylin and eosin. The samples were obtained from the middle part of the LV, to exclude differences in regional cardiomyocyte size in different regions of the LV (34). For consistency of results, myocytes positioned perpendicularly to the plane of the section with a visible nucleus and cell membrane clearly outlined and unbroken were then selected for the cross-sectional area measurement (17). This area was determined by manually tracing the cell contour on a digitized image acquired on the image-analysis system at a magnification of ×400 using computerized planimetry (ImagePro Plus) as described previously (23). A total of 100 myocytes were selected in the LV of each heart and analyzed by an observer blinded to the experimental treatment.

Additionally, heart sections were stained with Masson's trichrome stain to distinguish areas of connective tissue. The percentage of blue staining, indicative of fibrosis, was measured (10 fields randomly selected on each section). The value was expressed as the ratio of trichrome-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, insulin, and cholesterol in ventricular hypertrophy, blood samples from the aorta were assayed at the end of the study. Plasma insulin concentration was measured by collecting 4 ml of blood in test tubes containing 2% EDTA (80 μl/ml of blood). Blood samples were immediately centrifuged at 3,000 *g* for 10 min, and the plasmas were stored at −70°C until further analysis. Insulin was measured by ultrasensitive rat enzyme immunoassay (Mercodia, Uppsala, Sweden).

Activation of K_{ATP} channels has been shown to attenuate cardiac hypertrophy by inhibition of endothelin-1 (40), a key mediator of protein synthesis for hypertrophic changes. To exclude the possible effect of pravastatin on LV mass by modulation of endothelin-1, we measured endothelin-1 levels. Because of a local release of endothe-

Table 1. *Cardiac morphology, hemodynamics, cholesterol, glucose, insulin, plasma and tissue ET-1, and ANG II concentrations 8 wk after clipping*

	2K1C Hypertensive Rats							
Parameters	Vehicle	Nicorandil	Pravastatin	Glib	Nic + Glib	Prava + Glib	Hydralazine	Sham Operation
No. of rats	10	12	10	11	10	10	10	12
BW, g	415±26	421±26	425±29	425±22	432±19	435±25	438±29	444±31
Heart weight, g	1.41±0.08	1.20±0.10*	1.18±0.10*	1.43±0.12	1.42±0.05	1.45±0.10	1.45±0.10	1.13±0.11*
LVW/BW, mg/g	3.04±0.36	2.41±0.26*	2.32±0.18*	2.93±0.44	3.10±0.30	2.97±0.27	3.08±0.17	2.16±0.17*
HR, beats/min	375±31	380±24	376±21	370±31	378±27	362±31	386±34	375±21
Left/right kidney weight ratio	0.71±0.13	0.70±0.13	0.70±0.13	0.69±0.11	0.74±0.11	0.74±0.12	0.72±0.12	0.98±0.05†
LVESP, mmHg	182±15	146±10*	143±8*	174±13	176±16	174±14	143±13*	121±10†
LVEDP, mmHg	6±2	5±2	6±1	5±2	6±1	6±1	7±2	6±2
+dP/dt, mmHg/s	8,748±573	8,091±675	7,895±524	8,829±482	8,805±518	9,506±522	9,489±551	6,264±446†
−dP/dt, mmHg/s	6,528±582	5,982±522	4,780±572	5,762±372	5,988±429	5,934±822	5,815±352	4,182±628
Cholesterol, mg/dl	40±11	41±10	48±12	42±11	45±10	41±10	42±10	49±15
Glucose, mg/dl	89±9	90±11	89±8	88±12	90±10	87±10	84±11	89±11
Insulin, μu/ml	17±9	18±8	22±11	79±15‡	56±19‡	68±18‡	15±11	20±7
Plasma ET-1, pg/ml	1.36±0.48	1.62±0.67	1.84±0.49	1.52±0.57	1.42±0.40	1.50±0.34	1.43±0.55	1.48±0.57
LV ET-1, pg/mg tissue	2.91±0.73	3.01±0.68	2.68±0.57	3.11±0.68	2.87±0.80	2.72±0.76	2.80±1.08	2.24±0.62
Plasma ANG II, pg/ml	142±13	113±14	152±23	142±15	137±15	148±21	129±18	27±6†

Values are means ± SD. BW, body weight; +dP/dt and −dP/dt, maximum and minimum first derivatives of LV pressure. ET-1, endothelin-1; Glib, glibenclamide; HR, heart rate; LVEDP, left ventricular (LV) end-diastolic pressure; LVESP, LV end-systolic pressure; LVW, LV weight; Nic, nicorandil; Prava, pravastatin. *$P < 0.05$ compared with two-kidney, one-clip (2K1C) groups treated with vehicle, Glib, Nic + Glib, Prava + Glib, and hydralazine; †$P < 0.05$ compared with 2K1C groups; ‡$P < 0.05$ compared with rats treated with vehicle, Nic, Prava, Hydralazine and compared with sham operation.

lin-1 and a poor correlation between plasma and tissue endothelin-1 levels, blood samples from the aortic root and the tissue from the LV were obtained for measurements of systemic and local endothelin-1 levels at the end of the study as described previously (21). For the measurement of cardiac endothelin-1 levels, the LV was immediately homogenized with a Polytron homogenizer for Triton X, boiled for 7 min, and centrifuged at 20,000 g for 30 min at 4°C. Following the measurement of the protein concentration (pg/mg), the supernatant was stored at −70°C until use. Plasma endothelin-1 concentration was measured by collecting 4 ml of blood in test tubes containing 2% EDTA (80 μl/ml of blood). Endothelin-1 was measured by immunoassay (R&D Systems, Minneapolis, MN). Plasma (1 ml) was acidified with 3 ml of 4% acetic acid, and endothelin-1 was extracted with a Sep-pak C$_{18}$ cartridge. The detection limit was 1 pg/ml for endothelin-1. Intra-assay and interassay coefficients of variation were 4.5% and 6.6%, respectively. ANG II concentrations were measured by immunoassay (Bohmann Diagnostic). The detection limit was 2 pg/ml for ANG II. Intra-assay and interassay coefficients of variation were 8.3% and 11%, respectively.

Statistical Analysis

Results are presented as means ± SD. A two-way ANOVA was used to search for possible effects of pravastatin and glibenclamide on the measurements of hemodynamics, cholesterol levels, and myocyte sizes, and, if an F value was found to be significant, a two-tailed Student's t-test for paired observation with Bonferroni's correction was used to test differences. Interaction term of pravastatin and glibenclamide effects was incorporated into the model. The significance level was assumed at the value of $P < 0.05$.

RESULTS

Because of the variable response of each rat to clipping, only rats with a ratio of left kidney weight to right kidney weight between 0.4 and 0.9 were included. The effectiveness of the 2K1C model was confirmed by the significant elevation of ANG II concentration and blood pressure in the clipped groups (Table 1). 2K1C hypertensive rats were not associated with increased insulin levels compared with sham-operated rats. Insulin concentrations were significantly increased in rats ad-

ministered with glibenclamide. Glucose, plasma endothelin-1, and tissue endothelin-1 levels did not differ among the groups (Table 1). Pravastatin does not lower serum cholesterol in rats, consistent with the notion that compensatory increases in hepatic enzyme production were observed in rats treated with statins. These data indicated the nonlipid effect of pravastatin on ventricular hypertrophy.

Systolic blood pressure measured under anesthesia was significantly higher ($P < 0.001$) in vehicle-treated 2K1C compared with sham-operated rats (Table 1). During the 8-wk treatment period, pravastatin decreased systolic blood pressure by 39 mmHg, without, however, normalizing it. Glibenclamide administration did not affect baseline hemodynamic parameters but markedly attenuated the hemodynamic actions of pravastatin and nicorandil. Chronic antihypertensive treatment of 8 wk with hydralazine led to a similarly lower systolic blood pressure when compared with pravastatin-treated 2K1C rats. Heart rate did not differ among all groups. The maximal rate of LV pressure rise (+dP/dt) was significantly increased in 2K1C rats compared with sham-operated rats. The ratio of left to right kidney weight was significantly decreased in 2K1C rats compared with sham-operated rats.

Chronic drug treatment did not modify the increase of body weight with age. Before treatment, the 2K1C groups had similar baseline body weights (data not shown). At the end of the treatment period, body weights were similar in the 2K1C groups (Table 1). After 8 wk, the 2K1C rats had a significantly higher ratio of LV weight to body weight than that of the sham-operated group (3.04 ± 0.36 vs. 2.16 ± 0.17 mg/g in sham-operated rats, $P < 0.0001$, Table 1). Compared with vehicle-treated 2K1C rats, nicorandil and pravastatin decreased LV weight/body weight ratios by 21% and 24% ($P = 0.0001$ and $P < 0.0001$, respectively). Conversely, the rats to which glibenclamide was administered developed ventricular hypertrophy greater than that in the pravastatin-treated rats alone. Despite producing similar antihypertensive effects, hydralazine

Table 2. *Echocardiographic data at the end of the study*

Parameters	2K1C Hypertensive Rats							
	Vehicle	Nicorandil	Pravastatin	Glib	Nic + Glib	Prava + Glib	Hydralazine	Sham Operation
LVEDD, mm	6.74 ± 0.21	$6.43 \pm 0.19^*$	$6.44 \pm 0.20^*$	6.67 ± 0.20	6.64 ± 0.39	6.62 ± 0.34	6.63 ± 0.33	$6.21 \pm 0.41^*$
LVESD, mm	3.12 ± 0.24	3.12 ± 0.22	3.17 ± 0.19	3.01 ± 0.23	3.04 ± 0.21	2.93 ± 0.33	3.00 ± 0.29	3.10 ± 0.34
IVS, mm	1.76 ± 0.14	$1.53 \pm 0.15^*$	$1.54 \pm 0.16^*$	1.85 ± 0.15	1.91 ± 0.24	1.87 ± 0.23	1.83 ± 0.25	$1.49 \pm 0.11^*$
LVPW, mm	1.81 ± 0.13	$1.58 \pm 0.18^*$	$1.61 \pm 0.17^*$	1.82 ± 0.10	1.78 ± 0.15	1.81 ± 0.14	1.88 ± 0.25	$1.49 \pm 0.13^*$
LVM, g	1.30 ± 0.12	$1.13 \pm 0.13^*$	$1.15 \pm 0.12^*$	1.27 ± 0.14	1.30 ± 0.11	1.32 ± 0.09	1.34 ± 0.14	$1.05 \pm 0.12^*$
LVFS, %	54 ± 4	52 ± 3	51 ± 3	55 ± 3	54 ± 3	56 ± 6	54 ± 4	50 ± 7

Values are means $\pm$ SD. IVS, interventricular septum; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; LVFS, LV fraction shortening; LVM, LV mass; LVPW, LV posterior wall. $^*P < 0.05$ compared with 2K1C groups treated with vehicle, Glib, Nic + Glib, Prava + Glib, and hydralazine.

given to 2K1C rats showed a significantly increased LV weight compared with those treated with pravastatin.

Eight weeks after clipping, echocardiography revealed a significant increase of LV wall thickness (interventricular septum and posterior wall), and, as a consequence, an 18% increase in echocardiogram-derived LV mass compared with sham-operated rats (Table 2). A good correlation was found between LV mass determined by echocardiography and LV mass measured at autopsy ($P = 0.002$).

To characterize the cardiac hypertrophy on a cellular level, morphometric analyses of LV sections were demonstrated from different treated groups (Fig. 1). Consistent with whole heart and echocardiographic data, 2K1C myocytes were hypertrophied compared with sham-operated myocytes. The cardiomyocyte cell areas of the cells from the vehicle-treated 2K1C group significantly increased by 34% compared with those from the same area of hearts from sham-operated rats ($P < 0.0001$). Nicorandil and pravastatin reduced cell areas 18% and 19% compared with the vehicle-treated 2K1C group (both $P = 0.002$). Conversely, the rats to which glibenclamide was administered developed greater cardiomyocyte hypertrophy than that in the pravastatin-treated group alone.

Fibrosis of the LV was examined in tissue sections after Masson's trichrome staining as shown in Fig. 2. 2K1C rats treated with vehicle had significantly larger areas of intense focal fibrosis compared with sham-operated rats ($4.3 \pm 0.9\%$ vs. $1.2 \pm 0.3\%$, $P < 0.05$). Treatment with either nicorandil or pravastatin in 2K1C hypertensive rats attenuated fibrosis as observed by reduced collagen staining (Fig. 2, *C* and *D*). Quantitative analysis showed that collagen formation in the LV was significantly increased in 2K1C rats treated with a combination of pravastatin and glibenclamide compared with pravastatin-treated rats alone.

Myocardial expression of ANP was measured by competitive RT-PCR (Fig. 3). ANP was markedly increased in vehicle-treated 2K1C rats compared with the sham-operated group. Compared with vehicle-treated 2K1C rats, nicorandil and pravastatin decreased ANP mRNA expression by 21% and 24% ($P = 0.0001$ and $P < 0.0001$, respectively).

DISCUSSION

The present study demonstrates for the first time that pravastatin prevents ongoing cardiac hypertrophy and fibrosis in the 2K1C rats, independent of hemodynamic and lipid changes via a K_{ATP} channel-dependent pathway. This result extends the findings of a recent study showing that chronic treatment with

a K_{ATP} channel agonist, iptakalim, preserves renal structure and function in spontaneously hypertensive rats (43). K_{ATP} channel agonists can also preserve cardiac structure and improve cardiac remodeling in 2K1C renovascular hypertension.

Our conclusions are supported by three lines of evidence. *1*) 2K1C renovascular hypertension was associated with the development of ventricular hypertrophy confirmed by LV gross weight, histological cardiomyocyte size and fibrosis, molecular LV ANP mRNA measurement, and echocardiographic parameters. *2*) Myocardial K_{ATP} channels play a role in preventing the pathogenesis of ventricular hypertrophy in 2K1C renovascular hypertension, which can be reversed by nicorandil administration. The effect of nicorandil was abolished by treatment with glibenclamide, further confirming the predominant role of K_{ATP} channels in this phenomenon. *3*) The cardioprotective effect of pravastatin was abolished by glibenclamide, suggesting that the inhibitory effects of the drug may result from activation of myocardial K_{ATP} channels. The results were consistent with previous studies, showing that pravastatin provides cardioprotection by enhanced activation of myocardial K_{ATP} channels (22, 24). These combined results indicate that pravastatin prevents progression of ventricular hypertrophy through activation of myocardial K_{ATP} channels in 2K1C renovascular hypertension.

Mechanisms: Four Excluded Factors

It appears from our study that the attenuated ventricular hypertrophy is related to a cholesterol-independent activation of K_{ATP} channels in response to statin treatment. The addition of glibenclamide to pravastatin-treated rats impaired their ability to attenuate cellular hypertrophy, implying that this effect is not a nonspecific action. The mechanisms by which pravastatin attenuates cardiac hypertrophy remain to be defined. However, several factors can be excluded.

1) Hemodynamics. Blood pressure did not play a major role in the process. Various statins have shown to reduce blood pressure in patients (9) and animals (37). Glorioso et al. (9) have shown hemodynamic improvement after 16-wk therapy with pravastatin in hypertensive patients. However, the mechanism by which pravastatin prevents cardiac hypertrophy is probably not due to its antihypertensive effect alone. In this study, we found that chronic treatment of 2K1C renovascular hypertension with hydralazine did not prevent ongoing cardiac hypertrophy even though it was effective as pravastatin in lowering blood pressure in 2K1C renovascular hypertension. Our results were consistent with previous findings of Buzello et
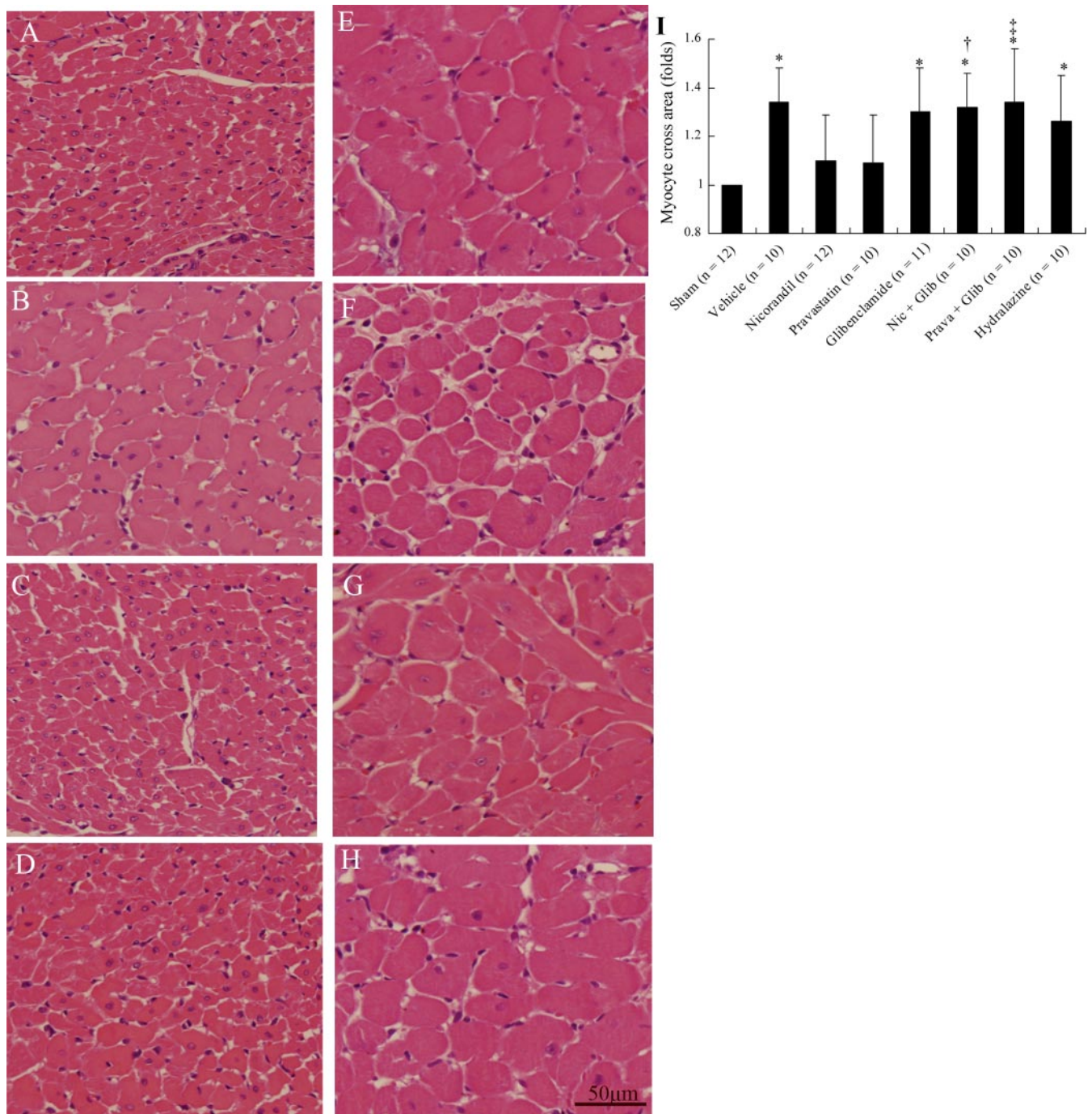
Fig. 1. Morphometric analyses of cardiac sections at 8 wk after clipping. Left ventricular (LV) cardiomyocyte cross-sectional areas in rats treated with indicated agents were examined by hematoxylin-eosin staining. Relative myocyte cross-sectional area was normalized to a mean value of sham-operated rats at the end of the study. *A–H*: representative staining images. *A*: sham-operated rats. *B*: two-kidney, one-clip (2K1C) rats treated with vehicle. *C*: 2K1C rats treated with nicorandil. *D*: 2K1C rats treated with pravastatin. *E*: 2K1C rats treated with glibenclamide (Glib). *F*: 2K1C rats treated with nicorandil (Nic) + glibenclamide. *G*: 2K1C rats treated with pravastatin (Prava) + glibenclamide. *H*: 2K1C rats treated with hydralazine. Bar = 50 μm. *I*: quantitative analysis of the cardiomyocyte sizes in different treated groups. The number of animals in each group is indicated in parentheses. *$P < 0.05$ compared with sham-operated and nicorandil-treated and pravastatin-treated groups; †$P < 0.05$ compared with nicorandil-treated group; ‡$P < 0.05$ compared with pravastatin-treated group.

al. (5), showing that cardiomyocyte hypertrophy was partly, but not completely, prevented by normalization of blood pressure with antihypertensive treatment with dihydralazine. These findings support the notion that pravastatin has direct tissular effects dissociated from its hemodynamic systemic effects.

2) Differences in insulin concentrations. Insulin secretion significantly increases in rats treated with glibenclamide com-

pared with vehicles as shown in this study. The increased insulin levels cannot be a confounding factor of cardiomyocytes hypertrophy. Although hyperinsulinemia has been shown to enhance cardiac hypertrophy (4), similar cardiomyocyte hypertrophy was observed in 2K1C groups treated with vehicle and glibenclamide, suggesting that factors other than insulin may contribute to the pathogenesis of cardiac hypertrophy.
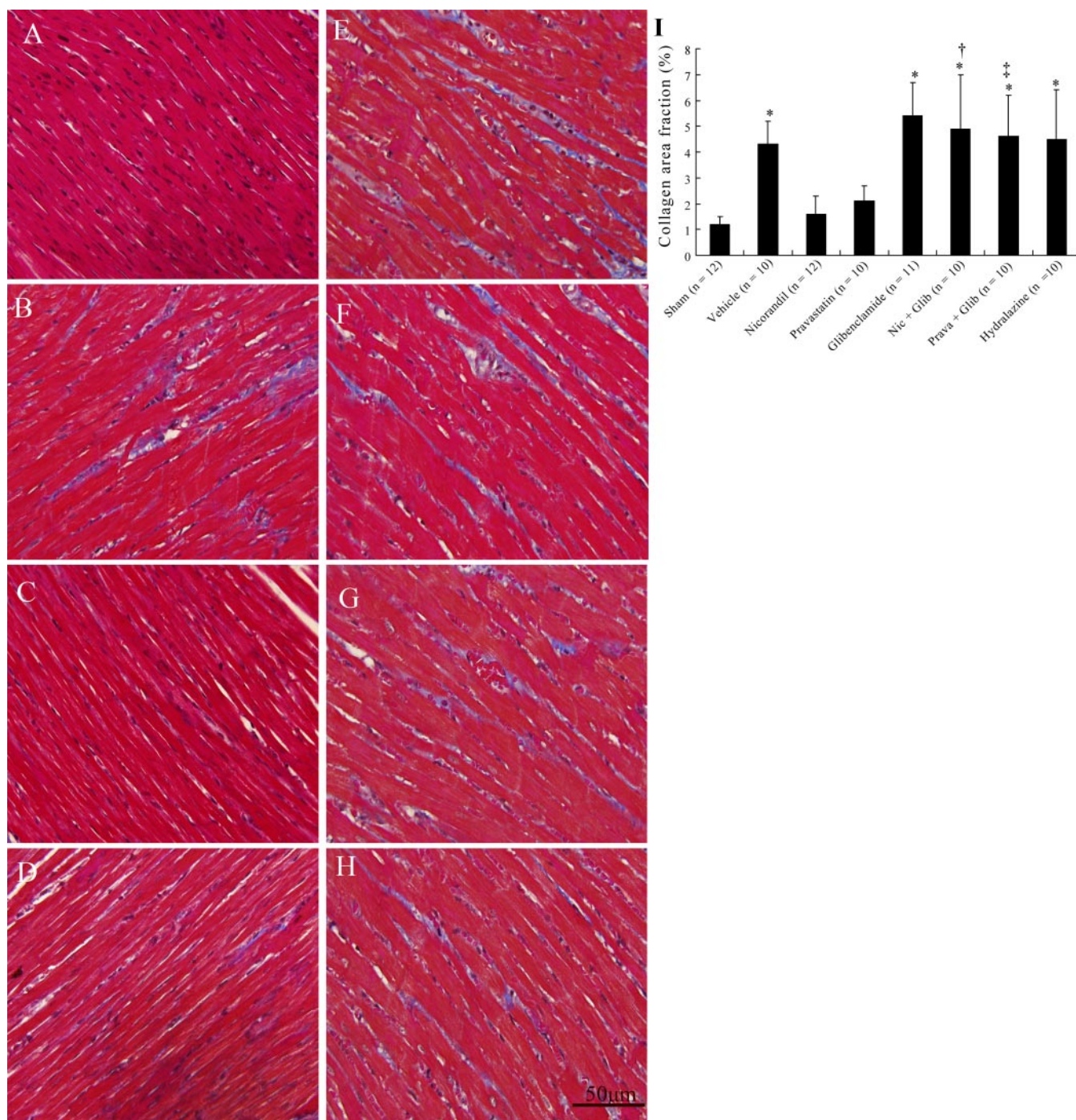
Fig. 2. *A–H*: representative sections with Masson's trichrome staining (blue; magnification, ×400) at 8 wk after clipping. Collagen deposition within the LV is reduced after administering either nicorandil or pravastatin. *A*: sham-operated rats. *B*: 2K1C rats treated with vehicle. *C*: 2K1C rats treated with nicorandil. *D*: 2K1C rats treated with pravastatin. *E*: 2K1C rats treated with glibenclamide. *F*: 2K1C rats treated with nicorandil + glibenclamide. *G*: 2K1C rats treated with pravastatin + glibenclamide. *H*: 2K1C rats treated with hydralazine. The line length corresponds to 50 μm. *I*: LV collagen area fraction (%). Each column and bar represent mean ± SD. *$P < 0.05$ compared with sham-operated and nicorandil-treated and pravastatin-treated groups; †$P < 0.05$ compared with nicorandil-treated group; ‡$P < 0.05$ compared with pravastatin-treated group.

3) Differences in glucose concentrations. Hyperglycemia has been shown to adversely affect subsequent cardiomyocyte sizes (30). However, it is unlikely because blood glucose concentrations maintained stable during the course of the experiments. Furthermore, nicorandil improved the degree of ventricular hypertrophy without significant changes in blood glucose levels.

4) Endothelin-1. Endothelin-1 acts as a key autocrine/paracrine mediator to trigger the hypertrophic signaling pathways by activation of extracellular signal-regulated kinase in myocardium via activation of ET A/B receptors (44). However, it is unlikely because either plasma or tissue endothelin-1 concentrations remained similar in all 2K1C groups. The results were consistent with previous findings of Hocher et al. (12),
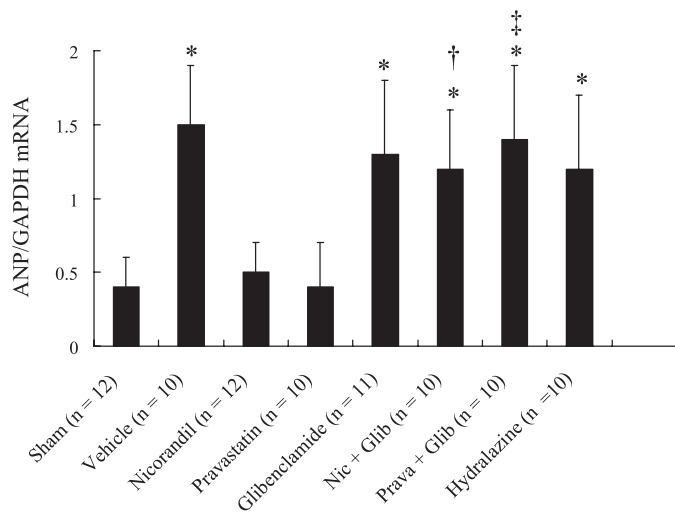
Fig. 3. LV atrial natriuretic peptide (ANP) mRNA measured by competitive RT-PCR. Compared with vehicle-treated 2K1C rats, rats treated with nicorandil and pravastatin significantly decreased ANP mRNA expression. *$P < 0.05$ compared with sham-operated and nicorandil-treated and pravastatin-treated groups; †$P < 0.05$ compared with nicorandil-treated group; ‡$P < 0.05$ compared with pravastatin-treated group. The number of animals in each group is indicated in parentheses.

showing that cardiac endothelin system did not contribute to ventricular hypertrophy even in the late stage of 2K1C renovascular hypertensive rats.

Proposed Mechanisms

Our observations showed that pravastatin-related K_{ATP} channel activation may play crucial roles in attenuation of ventricular hypertrophy. The role of K_{ATP} channel activation in preventing the pathogenesis of ventricular hypertrophy was further confirmed by directly blocking K_{ATP} channels. Exactly how the activation of K_{ATP} channels leads to attenuated cardiac hypertrophy cannot be determined from this study. Two mechanisms were proposed. First, the activation of K_{ATP} channels has been shown to attenuate myocardial hypertrophy by inhibiting activity of 70-kDa S6 kinase (31). Activation of 70-kDa S6 kinase phosphorylates the 40S ribosomal protein S6 and plays an important role in progression through the G_1 phase of the cell cycle (18). Microinjection of anti-S6 kinase antibodies into quiescent rat embryo fibroblasts abolishes serum-induced entry into S phase of the cell cycle and inhibits overall protein synthesis (18). Second, K_{ATP} channel activation is related to attenuate Na/H exchanger isoform-1 expression (42). Increased cardiac protein expression levels of Na/H exchanger isoform-1 appear to be involved in the ventricular hypertrophy (14). Blockade of Na/H exchanger isoform-1 has been shown to be highly beneficial in preventing inducible cardiac hypertrophy in animals (6). Taken together, regardless of the relative importance of each mechanism, all of the changes caused by pravastatin are compatible with our understanding of the beneficial effects on prevention of ventricular hypertrophy.

Other Mechanisms

Although the present study suggests that the mechanisms of pravastatin-induced cardioprotection may be related to activa-

tion of K_{ATP} channels, there are possible other candidates modulating the antihypertrophic effects of pravastatin, such as free radicals, nitric oxide, and angiotensin. First, blockade of free radicals alleviated the development of cardiac hypertrophy (19). Previous studies have demonstrated that statins decrease myocardial oxidative stress by inhibiting Rac-induced NAD(P)H oxidase activity (35). Increased production of free radicals may induce cardiac hypertrophy via activation of mitogen-activated protein kinases (35). Renovascular hypertension generated by clipping the left renal artery was associated with increased free radical production (41). Thus pravastatin may attenuate cardiac hypertrophy by attenuated production of free radicals. Second, blunting by pravastatin of antihypertrophic effect may also result from attenuation of nitric oxide inhibition. Liu et al. (28) have shown increased LV mass in endothelial nitric oxide synthase knockout mice. Finally, concerning the mechanisms involved in activation of pravastatin-induced K_{ATP} channels, it is attractive also to consider a potential role of ANG II, which may interact with statins. In vitro studies have demonstrated crosstalk between angiotensin system and the K_{ATP} channel system (11). ANG II has been shown to inhibit K_{ATP} channel activation in vitro (35). Lovastatin has been shown to inhibit activity of ANG II (29), thus accounting for K_{ATP} channel activation. However, pravastatin-induced angiotensin attenuation is unlikely because of similar ANG II concentrations among the 2K1C groups, which was consistent with the notion that pravastatin has little effect on cardiac angiotensin activity (16). Complex interactions between free radical, nitric oxide, ANG 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 amenable to one therapeutic intervention. However, the effect of pravastatin was abolished by treatment with glibenclamide, implying a pivotal role of K_{ATP} channels in this phenomenon. Extensive investigation of such pathways and the mechanisms by which pravastatin attenuates cardiac hypertrophy require additional studies.

Study Limitations

There were potential limitations in the present study. First, it is not clear whether the number or affinity of K_{ATP} channels in the myocardium decreases with 2K1C hypertensive rats. Although the function of K_{ATP} channels is impaired in smooth muscle cells from aorta of deoxycorticosterone acetate-salt hypertensive rats (8), no data are available regarding changes in myocardial K_{ATP} channels with 2K1C hypertensive rats or their functional consequences. Further studies are needed to confirm whether the beneficial effects of pravastatin on cardiac remodeling in renovascular hypertension can be explained by impaired baseline activity of cardiac K_{ATP} channels. Second, glibenclamide used as an antagonist of K_{ATP} channels has many potential nonspecific targets of glibenclamide, including the opening of Ca^{2+} channels (15). Glibenclamide can enhance the resting and stimulation-evoked release of norepinephrine, an effect unrelated to K_{ATP} channel blockade (33). These

alternative effects are associated with increased ventricular mass (26, 32) and could confound the interpretation of the present study. Use of a more selective antagonist of K_{ATP} channels, 5-hydroxydecanoate or HMR-1098 (38), would have strengthened the hypothesis. Finally, there are mechanistic differences of the renin-angiotensin system between acute and chronic 2K1C renovascular hypertension (13). Thus the results in this model of acute 2K1C hypertension may not be fully applicable to chronic renovascular hypertension in humans.

Conclusions

The present study shows that pravastatin treatment can attenuate ventricular hypertrophy through activation of K_{ATP} channel pathway, independent of lipid and hemodynamic changes. The finding can be rationalized mechanistically suggesting a pathogenetic role of K_{ATP} channels in preventing the development of myocardial hypertrophy. Treatment aimed at activation of K_{ATP} channels could have advantages over current therapy for renovascular hypertension. The long-term clinical benefit of the LV mass reduction obtained after chronic treatment with pravastatin has to be elucidated in clinical studies.

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