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AT_1 receptor antagonist therapy preferentially ameliorated right ventricular function and phenotype during the early phase of remodeling post-MI

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1 The influence of AII on contractile dysfunction, regulation of the tyrosine kinase-dependent signaling molecule extracellular signal-regulated kinase (ERK), and natriuretic peptide gene expression were examined in the noninfarcted left ventricle (NILV) and right ventricle (RV) during the early phase of remodeling post-myocardial infarct (MI) in the rat. The selective AT_1 receptor antagonist irbesartan was administered <10 h following coronary artery ligation, and rats were killed either at 4-day or 2-week post-MI.

2 At 4 days post-MI, left ventricular systolic pressure (LVSP: sham $=125\pm12$, MI $=91\pm4$ mmHg) was decreased, whereas left ventricular end-diastolic pressure (LVEDP: sham $=9\pm2$, MI $=17\pm2$ mm Hg), right ventricular systolic (RVSP: sham $=26\pm1$, MI $=34\pm2$ mm Hg), and end-diastolic pressures (RVEDP: sham $=3\pm0.5$, MI $=7\pm1$ mm Hg) were increased. ERK phosphorylation was significantly elevated in the NILV and RV.

3 Irbesartan (40 mg kg^{-1}/day^{-1}) administration did not improve left ventricular function, or suppress increased ERK phosphorylation in the 4-day post-MI rat. By contrast, irbesartan therapy normalized RVSP (MI + irbesartan $=25\pm1$ mm Hg), RVEDP (MI + irbesartan $=3\pm0.3$ mm Hg), and reduced ERK1 (MI $=3.0\pm0.6$, MI + irbesartan $=2.0\pm0.3$-fold increase), and ERK2 (MI $=3.8\pm0.8$, MI + irbesartan $=2.2\pm0.5$-fold increase) phosphorylation.

4 In 2-week post-MI rats, biventricular dysfunction was associated with increased prepro-ANP, and prepro-BNP mRNA expression. Irbesartan therapy normalized RVSP, attenuated RVEDP, and abrogated natriuretic peptide mRNA expression (prepro-ANP; MI $=9\pm2$, MI + irbesartan $=2\pm1$-fold increase, prepro-BNP; MI $=6\pm2$, MI + irbesartan $=1\pm1$-fold increase), whereas both transcripts remained elevated in the NILV despite the partial attenuation of LVEDP.

5 These data suggest that the therapeutic benefit of irbesartan treatment during the early phase of remodeling post-MI was associated with the preferential amelioration of RV contractile function and phenotype.

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Abbreviations: AII, angiotensin II; AT_1, angiotensin II receptor type I; ACE, angiotensin-converting enzyme; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; ERK, extracellular signal-regulated kinase; LV, left ventricle; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; MI, myocardial infarct or infarction; NILV, noninfarcted left ventricle; PI3-K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PCNA, proliferating cell nuclear antigen; RV, right ventricle; RVSP, right ventricular systolic pressure; RVEDP, right ventricular end-diastolic pressure

Introduction

It has been established that left ventricular failure is the most prevalent cause of secondary pulmonary hypertension, resulting in right ventricular dysfunction (Cody *et al*., 1992). A recent study has shown that right ventricular ejection fraction is an independent risk factor in patients with moderate-to-severe heart failure (Ghio *et al*., 2001). Clinically, the beneficial effects of angiotensin-converting enzyme (ACE) inhibitor therapy in heart failure patients have been well described (Pfeffer *et al*., 1992, 1995; Hennekens *et al*., 1996). Likewise, at least in the myocardial infarct (MI) rat model, angiotensin II type 1 (AT_1) receptor antagonist treatment mimicked the therapeutic effect of ACE inhibitors on left ventricular dysfunction (Smits *et al*., 1992; Ju *et al*., 1997; Richer *et al*., 1999). Despite these latter observations, the therapeutic benefit of either ACE inhibitors or AT_1 receptor antagonists on right ventricular contractile function and remodeling post-MI remains undefined.

Cardiac myocyte hypertrophy and fibroblast proliferation in the noninfarcted left ventricle (NILV) and right ventricle (RV) are predominant cellular events post-MI (Pfeffer *et al*., 1995).

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Myocyte hypertrophy is characterized by an increase in cell size and the re-expression of the fetal gene prepro-atrial natriuretic peptide (prepro-ANP), whereas uncontrolled cardiac fibroblast proliferation leads to the disproportionate synthesis and secretion of the extracellular matrix proteins collagen and fibronectin, resulting in interstitial fibrosis (Chien *et al*., 1991; Pfeffer *et al*., 1995; Weber, 1997). The prerequisite signaling events coupled to cardiac cell growth, and phenotype post-MI remains undefined, albeit several in vivo and in vitro studies have identified a potential role for the serine/threonine kinases extracellular signal-regulated kinase (ERK), and protein kinase B (PKB). The manipulation of ERK activity with transgenic mice revealed marked cardiac hypertrophy (Bueno *et al*., 2000, 2001). Consistent with this latter observation, ERK is recruited by several well-defined hypertrophic stimuli, including the sympathetic system, and peptide growth factors (Sadoshima & Izumo, 1993; Xiao *et al.,* 2001). In addition, ERK activation was identified as a prerequisite event of both human and rat cardiac fibroblast proliferation (Hafizi *et al*., 1999; Moriguchi *et al*., 1999; Kim *et al.,* 2002). In contrast to ERK, PKB represents a putative downstream target of the enzyme phosphatidylinositol 3-kinase (PI3-K), and its activation in cardiac myocytes was sufficient to induce a hypertrophic phenotype (Morisco *et al.,* 2000; Schubert *et al.,* 2000). In transgenic mice, PKB overexpression in cardiac myocytes caused an increase in cell size and promoted a molecular phenotype consistent with hypertrophy (Condorelli *et al.,* 2002). Moreover, PKB represents a potent antiapoptotic molecule, and its potential activation post-MI in the NILV may act as a protective mechanism to limit cardiac myocyte apoptosis (Matsui *et al.,* 1999). In cardiac fibroblasts, a PI3-K pathway has been implicated in cell proliferation, albeit the role of PKB in this process remains undefined (Colombo *et al.,* 2001; Kim *et al.,* 2002). Lastly, in ischemic or idiopathic failing human hearts, ERK, and PKB activity was increased (Haq *et al.,* 2001). Thus, both ERK and PKB may represent early signal events required to elicit the full complement of cellular and molecular events associated with cardiac remodeling. However, the activation of either ERK or PKB, or the stimuli (e.g. hormonal or mechanical stress) implicated in their regulation in the myocardium post-MI remains undefined.

The primary objective of the present study was to examine whether the early phase of remodeling post-MI in the NILV was associated with ERK and PKB recruitment, and a pattern of gene expression consistent with cardiac myocyte hypertrophy, and fibroblast proliferation. In parallel, the cellular and molecular phenotype of the RV in the post-MI rat was examined to identify whether the pattern of remodeling was similar or disparate to the NILV. Lastly, and as previously mentioned, ACE inhibitors and AT_1 receptor antagonists improved ventricular function in the post-MI rat, albeit the underlying mechanisms remain equivocal. In vitro studies have demonstrated that AII can increase ERK activity in cardiac myocytes and fibroblasts, and elicit qualitatively similar molecular events characteristic of hypertrophy, and proliferation, respectively (Sadoshima & Izumo, 1993; Booz *et al.,* 1994; Moriguchi *et al*., 1999). In vascular smooth muscle cells, AII treatment increased PKB activity (Takahashi *et al*., 1999). However, the recruitment of PKB in cardiac cells by AII remains undefined. Moreover, it remains to be demonstrated that the reported increase of local and circulating levels of AII represents an integral factor during the early phase of cardiac remodeling post-MI (Pfeffer *et al.,* 1995). Thus, the potential role of AII in cellular and molecular remodeling, and the therapeutic effect on LV and RV contractility was assessed via the administration of the selective AT_1, receptor antagonist irbesartan <10 h following coronary artery ligation.

Methods

Experimental protocol

MI was induced in male Wistar rats (7 – 9 weeks old; Charles Rivers, St. Constant, Quebec, Canada) by ligating the left anterior descending coronary artery as previously described (Pfeffer *et al*., 1987). Rats underwent either a sham operation or coronary artery ligation and were subsequently randomized into four groups: (1) sham-operated + soya oil; (2) sham-operated + irbesartan; (3) MI + soya oil; and (4) MI + irbesartan. Two separate protocols were established as rats were killed either at 4 days or 2 weeks following surgery. If an infarct was not present in a rat that underwent coronary artery ligation, the animal was relegated to either the sham or sham + irbesartan group. Regardless of the infarct size, all MI and MI + irbesartan treated rats were analyzed. In the untreated 4-day post-MI group, scar/body weight ratio was variable with a range of 0.182 – 0.52. Despite this variability, scar/body weight ratio correlated with LVSP, LVEDP, and RVSP in the 4-day post-MI rats (see Results section). The scar/body weight ratio of 2-week post-MI rats was 0.32 – 0.42, indicative of a large MI (Nguyen *et al*., 2001). A dose of $40\,mg\,kg^{-1}/day^{-1}$ of irbesartan was dissolved in soya oil, and administered by gavage <10 h following sham-operation or coronary ligation, and continued daily. Irbesartan (kindly provided by Bristol Myers, Canada; Dr Eleonora Muratti) is a long-acting novel non-peptidic selective AT_1 receptor antagonist (Burnier, 2001). To confirm the efficacy of the dose employed in the present study, the effect of irbesartan on AII-mediated increase of mean arterial pressure (MAP) was examined. Normal male Wistar rats were gavaged either with 20 or $40\,mg\,kg^{-1}$ of irbesartan, and $0.3\,\mu g\,kg^{-1}\,min^{-1}$ of AII was infused 90 minutes later (Nava *et al.,* 2000). In the AII-treated rats, MAP was increased 1 min following infusion and reached a plateau at 5 min (50 ± 9 mm Hg increase versus baseline at time zero; $n=3$). In the 20 and $40\,mg\,kg^{-1}$ irbesartan-treated rats, the AII-mediated increase of MAP was reduced by 50% (24 ± 1 mm Hg; $n=2$), and 82% (9 ± 3 mm Hg; $n=2$) respectively, at 5 min. The antagonistic action of irbesartan was maintained during the entire period of AII infusion (10 min). This latter dose of irbesartan has been previously shown to improve cardiac hemodynamics in the MI rat model (Ambrose *et al*., 1999; Richer *et al*., 1999). Lastly, the use and care of laboratory rats was according to the Canadian Council for Animal Care and approved by the Animal Care Committee of the Montreal Heart Institute.

Hemodynamic measurements

Rats were anesthetized with a gas mixture of 2% halothane in 100% oxygen and was reduced to 0.5 – 0.8% 15 min before left and right ventricular hemodynamics were measured by a microtip pressure transducer catheter (model SPR-407, 2F,

Millar instrument, Houston, TX, U.S.A.), as previously described (Nguyen *et al*., 2001). Following hemodynamic measurements, the heart was removed and separated into the RV, NILV, and scar. The NILV and RV were immediately weighed, and stored at −80°C. The infarct region was weighed, and the surface area calculated by planimetry, as previously described (Nguyen *et al.,* 2001).

Immunoblot analysis of extracellular-signal-regulated kinase (ERK), protein kinase B (PKB), and expression of proliferating cell nuclear antigen (PCNA)

Following hemodynamic measurements, left and right ventricles of the sham rat, and the noninfarcted left and right ventricular tissue of 4-day post-MI rats were homogenized in a buffer containing 10 mM TRIS (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 0.5 mM phenylmethylsulfonyl flouride, 1 mM sodium vanadate, 1% triton X-100, 0.5% nonidet P-40, and 1 $\mu g\,ml^{-1}$ of leupeptin and aprotinin. The homogenate was centrifuged for 5 min, and the supernatant was frozen and stored at − 80°C. The BioRad assay was used to determine the protein content. A 100 μg of ventricular lysate was subjected to SDS – polyacrylamide gel (10%) electrophoresis, and subsequently transferred to Hybond-C membrane (Amersham Biosciences Baie d'Urfe Quebec, Canada). Equal loading of the samples was confirmed by Ponceau S staining. Immunoblot analysis was performed as previously described (Colombo *et al*., 2001). The membrane was preincubated in 10 mM TRIS pH 7.4, 150 mM NaCl, 0.1% Tween (v/v) (TBS-T), containing 3% skim milk for 1 – 2 h at room temperature, and subsequently incubated overnight with TBS-T containing 5% bovine serum albumin at 4°C with either a rabbit polyclonal antibody (1 : 1000) directed against phosphorylated ERK1 and ERK2 (recognizes Thr^{202}/Tyr^{204}; Cell Signaling, Beverley, MA, U.S.A.), rabbit polyclonal antibodies (1 : 1000) directed against the phosphorylated residues of PKBα $serine^{473}$ and $threonine^{308}$ (Cell Signaling, Beverly, MA, USA), or a rabbit polyclonal antibody directed against proliferating cell nuclear antigen (PCNA; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Following overnight incubation, the membrane was washed 3 × with TBS-T, and reprobed with an anti-rabbit conjugated horseradish peroxidase antibody in TBS-T containing 3% skim milk (1 : 10,000; Santa Cruz Biotechnology) for 1 – 2 h at room temperature. The membranes were washed 3 × with TBS-T, and the bands were subsequently detected by autoradiography utilizing the ECL detection kit (Amersham Canada Limited).

Northern hybridization

Following hemodynamic measurements, total RNA from left and right ventricle of the sham rat, and the noninfarcted left and right ventricular tissue of 2-week post-MI rats was isolated by a modification of guanidine thiocyanate – phenol – chloroform extraction method, and Northern hybridization were performed as previously described (Colombo *et al*., 2001). The cDNA probes used were a 0.7-kb fragment of rat prepro-ANP (courtesy of Dr M. Boluyt), a 0.6-kb fragment of rat prepro-BNP (courtesy of Dr M. Nemer), a 0.6-kb fragment of rat fibronectin (courtesy of Dr R.O. Hynes), and a 1.2-kb fragment of rat GAPDH (American Type Culture Collection, Rockville, MD, U.S.A.).

Statistics

Data are expressed as the mean ± s.e.m. Statistical comparison of the data between sham, sham + irbesartan, MI, and MI + irbesartan was evaluated by a two-way ANOVA and significant difference was determined by the Neuman – Keuls test. $P<0.05$ was considered as statistically significant. With regard to the immunoblotting and Northern hybridization experiments, the data obtained in the sham-treated irbesartan rats was identical to untreated sham rats. In this regard, sham and irbesartan-treated sham rats were arbitrarily designated as 1, and changes in the MI and MI + irbesartan-treated rats were calculated as fold increase.

Results

Morphometric measurements at 4-day and 2-week post-MI, and the effect of irbesartan (Table 1)

The infarct region was clearly visible 4 days following coronary artery ligation, and associated with a concomitant

Table 1 Morphological parameters of the myocardial infarct (MI) rat and the effect of irbesartan (IRB)

	BW (g)	*LV (g)*	*RV (g)*	*Scar (g)*	*LV/ BW × 10⁻³*	*RV/ BW × 10⁻³*	*Scar/ BW × 10⁻³*	*Lung (g)*	*Lung/ BW × 10⁻³*
4 days									
Sham ($n=7$)	294 ± 7	0.57 ± 0.02	0.17 ± 0.008	—	1.98 ± 0.09	0.57 ± 0.03	—	1.41 ± 0.06	4.77 ± 0.16
Sham + IRB ($n=6$)	267 ± 5*	0.52 ± 0.01	0.16 ± 0.004	—	1.95 ± 0.07	0.58 ± 0.02		1.34 ± 0.05	5.01 ± 0.13
MI ($n=10$)	254 ± 8*	0.52 ± 0.04	0.17 ± 0.012	0.08 ± 0.01	2.02 ± 0.09	0.66 ± 0.05	0.29 ± 0.03	2.05 ± 0.17*	7.94 ± 0.66*
MI + IRB ($n=7$)	273 ± 7*	0.55 ± 0.01	0.15 ± 0.006	0.08 ± 0.01	2.00 ± 0.04	0.55 ± 0.02	0.28 ± 0.05	1.71 ± 0.11	6.06 ± 0.32**
2 weeks									
Sham ($n=7$)	348 ± 12	0.69 ± 0.01	0.20 ± 0.01	—	1.98 ± 0.01	0.58 ± 0.01	—	1.53 ± 0.03	4.42 ± 0.07
Sham + IRB ($n=5$)	321 ± 17	0.60 ± 0.02	0.19 ± 0.01	—	1.86 ± 0.01	0.59 ± 0.01		1.50 ± 0.08	4.68 ± 0.21
MI ($n=6$)	296 ± 12*	0.48 ± 0.02*	0.31 ± 0.03*	0.12 ± 0.01	2.01 ± 0.06	1.05 ± 0.11*	0.39 ± 0.02	2.70 ± 0.33*	9.16 ± 1.11*
MI + IRB ($n=10$)	271 ± 3*	0.37 ± 0.02*,**	0.21 ± 0.02**	0.12 ± 0.01	1.82 ± 0.04**	0.78 ± 0.06**	0.44 ± 0.03	1.75 ± 0.17**	6.50 ± 0.65**

BW, body weight; LV, left ventricle; RV, right ventricle; data are presented as mean ± s.e.m.*Represents $P<0.05$ Versus sham; and **Represents $P<0.05$ *versus* MI.

decrease in the absolute left ventricular weight. The infarct size (scar/body weight) of the 4-day post-MI rats was variable (range 0.18 – 0.52). Left ventricular to body weight ratio (LV/BW), and right ventricular to body weight ratio (RV/BW) in the 4-day post-MI rat was similar to sham. By contrast, lung to body weight ratio (lung/BW) was significantly increased in the 4-day post-MI rat, as compared to sham. In 2-week post-MI rats, scar/body weight ratio was relatively homogenous (0.32 – 0.42), and indicative of a large infarct. LV/BW ratio in the 2-week post-MI rat was similar to sham, despite scar formation, thereby suggestive of hypertrophy in the NILV, whereas RV/BW and lung/BW ratios were significantly increased, as compared to sham.

Irbesartan therapy did not reduce scar size or scar/body weight rat is either in 4-day or 2-week-treated post-MI rats. To further confirm this latter finding, the scar surface area was calculated by planimetry and found to be similar between MI and MI + irbesartan-treated rats in the 4-day ($MI = 0.65 \pm 0.05$, $MI + irbesartan = 0.62 \pm 0.1\,cm^2$) and 2-week ($MI = 1.01 \pm 0.05$, $MI + irbesartan = 1.15 \pm 0.07\,cm^2$) protocols. In 2-week post-MI rats, absolute left ventricular weight and LV/BW ratio were significantly decreased in the irbesartan-treated MI rat, as compared to untreated MI rats. Likewise, irbesartan therapy in 2-week post-MI rats had normalized absolute right ventricular weight, and significantly attenuated RV/BW ratio, as compared to untreated MI rats. Lastly, lung weight and lung/BW ratio were markedly reduced in both the 4-day and 2-week post-MI rats with irbesartan therapy, as compared to untreated MI rats.

Hemodynamic measurements at 4-day and 2-week post-MI rats, and the effect of irbesartan (Table 2)

Consistent with scar formation, left ventricular systolic pressure (LVSP), the rate of pressure development ($+dp/dt$), and decline ($-dp/dt$) were decreased, whereas left ventricular end-diastolic pressure (LVEDP) was increased in the 4-day post-MI rats, as compared to sham. Further analysis revealed that the variable infarct size of 4-day post-MI rats correlated significantly with LVSP ($r = 0.8112$, $P = 0.004$; $n = 10$), and LVEDP ($r = 0.7241$; $P = 0.017$; $n = 10$) (Figure 1). Left ventricular contractile dysfunction of the 4-day post-MI rat was associated with significant increases in right ventricular systolic (RVSP) and end-diastolic pressures (RVEDP), and RVSP correlated significantly with scar/body weight ratio ($r = 0.7935$; $P = 0.006$; $n = 10$) (Figures 1 and 2). In the 2-week protocol, left ventricular contractility was further diminished (Table 2), and RVSP, and RVEDP (Figure 2) were significantly increased in the post-MI rats.

In the 4-day post-MI rat, irbesartan therapy did not improve LVSP, dp/dt indices, or ameliorate LVEDP, whereas a partial reduction of LVEDP was observed in the 2-week post-MI rat, as compared to sham. By contrast, in both the 4-day and 2-week post-MI rats, irbesartan therapy normalized RVSP, and significantly reduced RVEDP (Figure 2).

The phosphorylation of ERK, PKB, and PCNA expression in the 4-day post-MI rat, and the effect of irbesartan (Table 3 and Figure 3)

In the NILV, the phosphorylation level of the Thr^{202}/Tyr^{204} residues of ERK1 and ERK2 were significantly increased, as compared to sham. Likewise, ERK1/2 phosphorylation was significantly increased in the RV, demonstrating that ERK recruitment represented a conserved event in the NILV and RV post-MI. The increased phosphorylation state of ERK was not a result of elevated protein expression, as Western blot analysis revealed that total protein content of ERK1 and ERK2 in the MI was identical to sham rats (data not shown). Moreover, the level of ERK1/2 phosphorylation did not correlate with either LVSP (ERK1: $r = -0.15$, $P = 0.69$, $n = 10$; ERK2: $r = -0.18$, $P = 0.62$, $n = 10$) or LVEDP (ERK1: $r = -0.47$, $P = 0.2$, $n = 10$; ERK2: $r = -0.1$, $P = 0.85$, $n = 10$). Likewise, ERK1/2 phosphorylation in the RV did not correlate with contractile function in the 4-day post-MI rat (data not shown). In contrast to the increased state of ERK1/2 phosphorylation, PKB $serine^{473}$ and $threonine^{308}$ phosphorylation in the NILV and RV lysates of 4-day post-MI rats was variable; a modest nonsignificant increase was observed, as compared to sham. The inconsistent level of PKB $serine^{473}$ and $threonine^{308}$ phosphorylation may be due in part to the variable scar size of the 4-day post-MI rats, and to the subsequent effect on contractility. Thus, the influence of ventricular function of the 4-day post-MI rat on PKB $serine^{473}$ and $threonine^{308}$ phosphorylation was examined. PKB $serine^{473}$ phosphorylation ($r = 0.10$, $P = 0.78$, $n = 10$) did not correlate with LVSP. By contrast, a negative correlation between PKB $serine^{473}$ phosphorylation and LVEDP was

Table 2 Hemodynamic parameters of the myocardial infarct (MI) rat and the effect of irbesartan (IRB)

	LVSP (*mmHg*)	*LVEDP* (*mmHg*)	*LV*+d*P*/d*t* (*mmHg s*$^{-1}$)	*LV*−d*P*/d*t* (*mmHg s*$^{-1}$)	*RV*+d*P*/d*t* (*mm Hg s*$^{-1}$)	*RV*-d*P*/d*t* (*mmHg s*$^{-1}$)
4 days						
Sham ($n=7$)	125±12	9±2	6664±524	5220±469	1171±66	853±28
Sham + IRB ($n=6$)	127±7	8±1	5883±440	4933±444	1208±67	874±59
MI (n= 10)	91±4*	17±2*	4397±423*	3292±345*	1206±59	930±58
MI + IRB ($n=7$)	100±7*	18±2*	5042±404*	3335±261*	1043±87	762±39
2 weeks						
Sham ($n=7$)	117±1	3±1	6479±169	6129±132	1000±62	857±65
Sham + IRB ($n=5$)	118±2	1±1	6533±240	6367±318	1200±153	1017±44
MI ($n=6$)	92±4*	29±2*	3800±423*	2733±368*	1367±84	1217±111
MI + IRB ($n=10$)	84±4*	15±2*,**	3985±218*	3100±154*	1170±118	960±97

LVSP, left ventricular systolic pressure; LVEDP, left ventricular end- diastolic pressure; RVdenotes right ventricle; $+dP/dt$, rate of contraction; $-dP/dt$, rate of relaxation; data are presented as mean±s.e.m; *Represents $P<0.05$ *versus* sham; and ** represents $P<0.05$ *versus* MI.

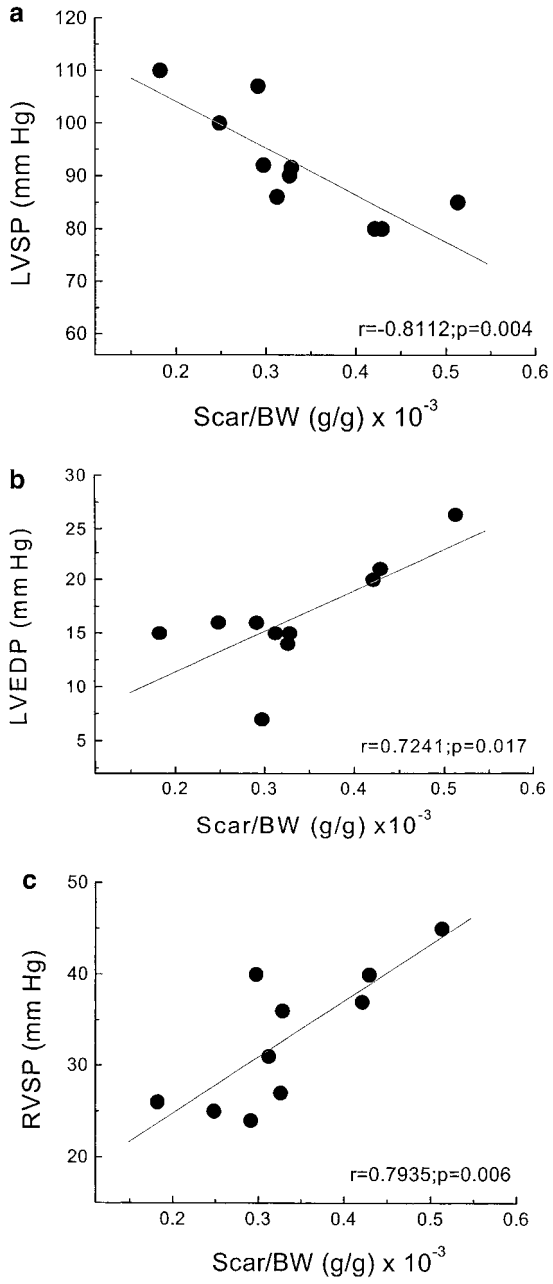

Figure 1 Correlation between scar size and ventricular function in untreated 4-day post-MI rats. A significant correlation was observed between scar/body weight ratio and LVSP (a), LVEDP (b), and RVSP (c).

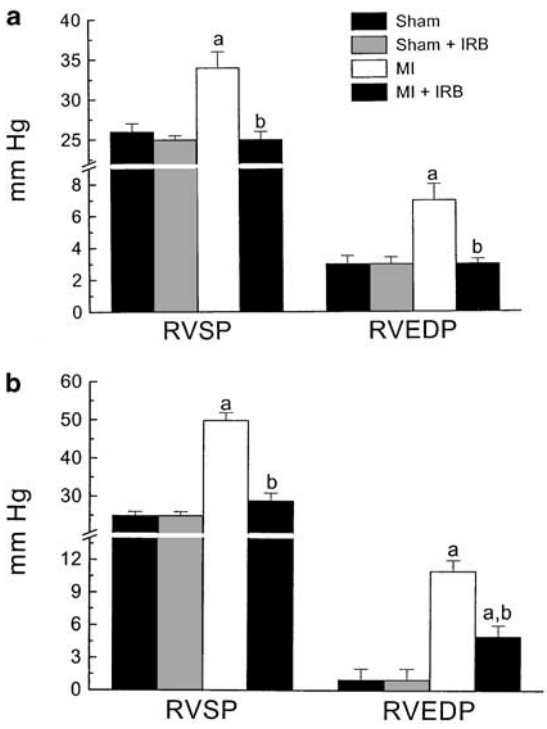

Figure 2 Right ventricular contractile function and the effect of irbesartan. RVSP and RVEDP were increased in the 4-day (a), and 2-week post-MI rats (b), and irbesartan therapy normalized RVSP, and ameliorated RVEDP. (a) Represents $P<0.05$ *versus* sham; (b) represents $P<0.05$ *versus* MI; and (n) in each group is indicated in Table 2.

observed ($r=-0.61$, $P=0.065$, $n=10$). Likewise, a modest negative correlation was observed between PKB threonine^{308} phosphorylation and LVEDP ($r=-0.56$, $P=0.08$, $n=10$), and not LVSP ($r=-0.02$, $P=0.96$, $n=10$). Moreover, previous studies have demonstrated that PKB serine^{473} and threonine^{308} phosphorylation can be differentially regulated. Indeed, in some but not all of the 4-day post-MI rats, the increased phosphorylation state of PKB serine^{473} in the NILV was associated with either no change or a reduction in PKB threonine^{308} phosphorylation, relative to sham. In contrast to the NILV, neither RVSP ($r=0.19$, $P=0.63$, $n=9$) nor RVEDP ($r=0.38$, $P=0.30$, $n=9$), influenced PKB serine^{473} phosphorylation in the 4-day post-MI rat. Likewise, PKB threonine^{308} phosphorylation did not correlate with RV function (data not shown). Lastly, PCNA protein expression was significantly increased in both the NILV (2.7±0.5-fold-

Table 3 The phosphorylation of extracellular signal-regulated kinase (ERK1/2), and protein kinase B (PKB) in the non-infarcted left ventricle (NILV), and right ventricle (RV) of the 4 day post-MI rat, and the effect of irbesartan (IRB)

	NILV		*RV*	
	MI (n=10)	*MI + IRB (n=7)*	*MI (n=9)*	*MI + IRB (n=7)*
ERK1	2.6±0.30*	2.2±0.30**	3.0±0.60*	2.00±0.30**
ERK2	1.8±0.20*	3.0±0.60*,**	3.8±0.80*	2.20±0.50**,***
PKB (Threonine^{308})	1.7±0.60	1.60±0.40	1.5±0.40	1.1±0.30
PKB (Serine^{473})	1.6±0.30	1.80±0.30	1.7±0.40	1.1±0.10

The phosphorylation level of ERK1/2, and PKB (threonine^{308} or serine^{473}) in the MI group is presented as fold increase±s.e.m. *versus* sham rat. In the MI+IRB group, data is presented as fold increase±s.e.m. *versus* IRB-treated sham rats.*Represents $P<0.05$ *versus* sham rat; **Represents $P<0.05$ *versus* IRB-treated sham rat; ***Represents $P<0.05$ *versus* untreated MI rat; and (n) denotes number of rats examined.

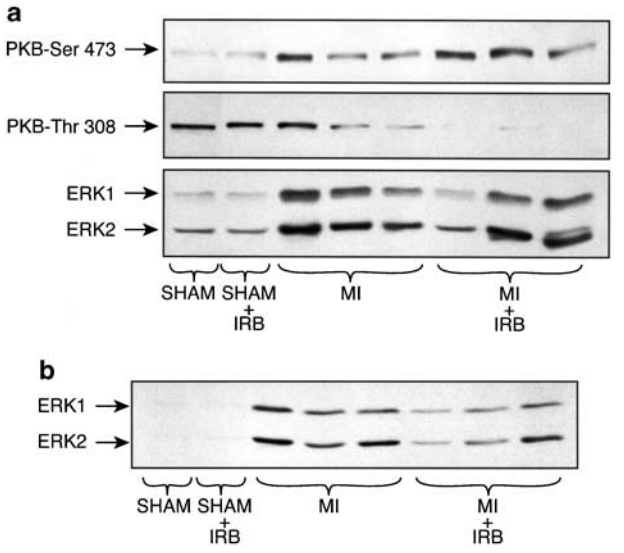

Figure 3 Phosphorylation pattern of extracellular signal-regulated kinase (ERK1/2), and protein kinase B (PKB) in 4-day post-MI rats. (a) ERK and PKB-theonine[308], and -serine[473] phosphorylation in the NILV, and the effect of irbesartan (IRB). (b) Phosphorylation of ERK1/2 in the RV, and the effect of IRB.

Table 4 mRNA expression in the non-infarcted left ventricle (NILV), and right ventricle (RV) of the 2 week post-MI rat, and the effect of irbesartan (IRB)

	NILV		*RV*	
	MI (n=4)	*MI+IRB (n=4–7)*	*MI (n=4–5)*	*MI + IRB (n=5–9)*
prepro-ANP mRNA	12±5*	25±3**	9±2*	2±1***
prepro-BNP mRNA	10±2*	17±4**	6±2*	1±1***
Fibronectin mRNA	5±1*	8±2**	4±1*	4±1**

mRNA data in the myocardial infarct (MI) group is presented as fold increase±s.e.m. versus sham rat. MI+IRB mRNA data is presented as fold increase±s.e.m. versus IRB-treated sham rats.*Represents $P<0.05$ versus sham rat; **Represents $P<0.05$ versus IRB-treated sham rat; ***Represents $P<0.05$ versus untreated MI rat; mRNA expression was normalized to GAPDH mRNA; and (n) denotes number of rats examined.

increase, $P<0.05$, $n=10$) and RV (1.7±0.4-fold-increase, $P<0.05$, $n=9$) of the 4-day post-MI rat, as compared to sham.

Irbesartan therapy did not reduce the increased phosphorylation state of the Thr[202]/Tyr[204] residues of ERK1/2 in the NILV. By contrast, a significant attenuation of ERK1/2 phosphorylation state was observed in the RV with irbesartan treatment. In the NILV, PKB serine[473], and threonine[308] phosphorylation in the irbesartan-treated MI rats, was quantitatively, similar to untreated MI rats. In the RV, irbesartan therapy normalized RVSP and RVEDP, and was associated with a partial reduction of PKB serine[473] and threonine[308] phosphorylation, as compared to untreated MI rats. Lastly, in irbesartan-treated MI rats, PCNA expression remained elevated in the NILV (2.8±0.5-fold increase, $n=7$), whereas a modest nonsignificant reduction was observed in the RV (1.4±0.25-fold increase, $n=7$).

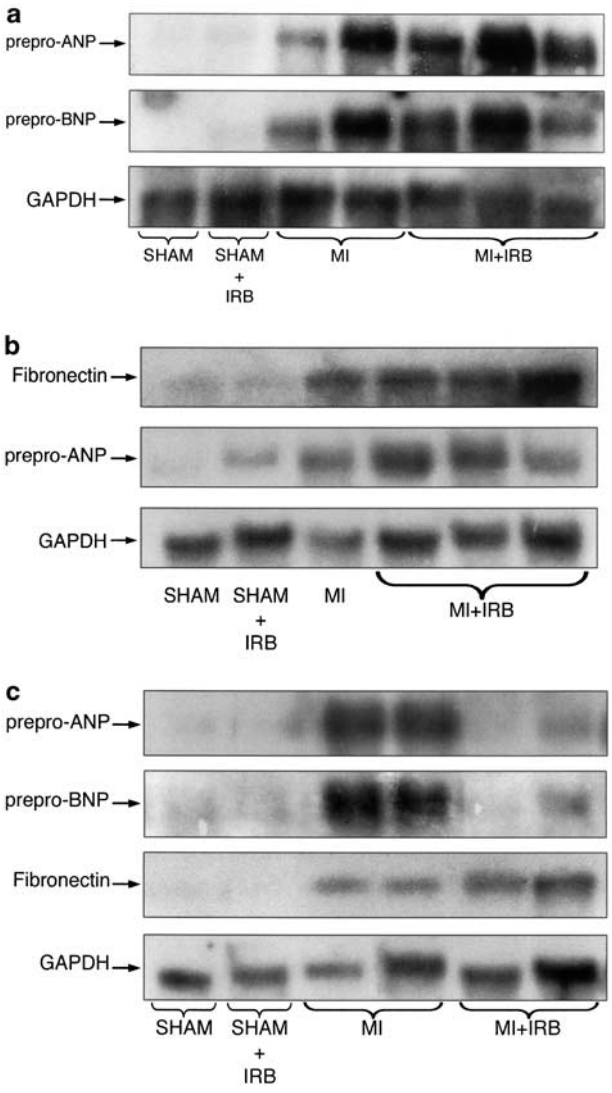

Figure 4 Pattern of gene expression in the NILV and RV of the 2-week post-MI rat. (a) *(NILV)* The steady-state mRNA levels of prepro-ANP, and prepro-BNP were increased in the MI rat, as compared to sham. IRB therapy had no effect on natriuretic peptide mRNA levels. (b) *(NILV)* Fibronectin and prepro-ANP mRNA levels were increased in the MI rat, as compared to sham. Irbesartan treatment of MI rats did not alter the expression of either transcript. (c) *(RV)* The mRNA levels of prepro-ANP, prepro-BNP, and fibronectin were increased in the MI rat. Irbesartan therapy abrogated natriuretic peptide mRNA expression, whereas fibronectin mRNA remained elevated in the MI rat.

The ventricular pattern of gene expression in the 2-week post-MI rat and the effect of irbesartan (Table 4 and Figure 4)

In the NILV of 2-week post-MI rats, the steady-state mRNA level of prepro-ANP, -BNP, and fibronectin were significantly increased, as compared to sham. Despite the partial attenuation of LVEDP, irbesartan treatment of MI rats did not reduce the elevated expression of either natriuretic peptide or fibronectin mRNAs.

In the untreated 2-week post-MI rat, right ventricular cardiac hypertrophy was associated with an increase in prepro-ANP and -BNP mRNA levels, as compared to sham. Irbesartan therapy abrogated natriuretic peptide mRNA

expression in the RV of the MI rat. Concomitant with an increased natriuretic peptide mRNA expression, the steady-state mRNA level of fibronectin was increased. However, fibronectin mRNA expression remained elevated in the RV of irbesartan-treated MI rats.

Discussion

The presented study has demonstrated that the pattern of cellular and molecular remodeling in the RV and NILV of the 4-day and 2-week post-MI rat were qualitatively similar. The administration of the AT_1 receptor antagonist irbesartan normalized RVSP, ameliorated RVEDP, attenuated ERK phosphorylation, and suppressed natriuretic peptide mRNA expression. By contrast, a temporal delay in the amelioration of LVEDP was observed in the MI rat with irbesartan therapy, whereas elevated ERK1/2 phosphorylation and increased natriuretic peptide mRNA expression in the NILV remained unchanged. These data support the premise that the selective improvement of right ventricular contractile function, and remodeling at the cellular and molecular level, represent in part the underlying features associated with therapeutic benefit of irbesartan in the post-MI rat.

In the 4-day post-MI rat, irbesartan therapy did not reduce elevated LVEDP, whereas a partial attenuation was observed in the 2-week post-MI rat. The observation of a temporal-dependent therapeutic action of the AT_1 receptor antagonist irbesartan on LVEDP in the MI rat has not been previously described. However, these data are consistent with a previous study demonstrating that AII did not influence left ventricular function or remodeling during the acute post-MI phase (Hanatani *et al*., 1995). This latter premise was further demonstrated in the 1-week post-MI AT_{1A} knockout mouse, as the relative increase in left ventricular wall thickness, depressed LVSP, elevated LVEDP, and right ventricular hypertrophy were identical to the wild-type post-MI mouse (Harada *et al*., 1999). It is tempting to speculate that the apparent therapeutic delay of AT_1 receptor antagonists may in part be because of the overwhelming hemodynamic and inflammatory reaction that accompanies the acute post-MI phase. Thus, these data suggest that AII, acting via the AT_1 receptor was not the primary driver influencing either left ventricular diastolic dysfunction or remodeling during the acute (4 days) post-MI phase in the rat. By contrast, irbesartan did significantly improve LV function in the 2-week post-MI rat as reflected by a decrease in LVEDP. This latter effect could be the result of a direct antagonism of the AT_1 receptor or secondary to the improvement of RV function.

Left ventricular failure is the most prevalent cause of secondary pulmonary hypertension, resulting in right ventricular hypertrophy, and dysfunction (Cody *et al*., 1992). In the 4-day and 2-week post-MI rats, RVSP, and RVEDP were significantly elevated. Right ventricular hypertrophy was evident in 2-week post-MI rats, whereas lung/body weight ratio was significantly increased in both the 4-day and 2-week post-MI rats. To date, the efficacy of AT_1 receptor antagonists and ACE inhibitors to attenuate right ventricular hypertrophy in the MI rat remains equivocal, and the therapeutic effect on right ventricular contractility undefined (Litwin *et al*., 1991; Belichard *et al*., 1994; Ju *et al*., 1997; Wollert *et al*., 1997; Makino *et al*., 1996). In the present study, the early administration of irbesartan normalized RVSP, ameliorated RVEDP, and coincided with the attenuation of both right ventricular hypertrophy and lung/BW ratio of post-MI rats. Moreover, since LVEDP remained elevated in the 4-day post-MI rat with irbesartan therapy, the normalization of RVSP was not secondary to a reduction of LV filling pressures. However, as previously discussed, it is conceivable that the normalization of RV function because of a decrease in afterload (pulmonary hypertension) may have secondarily improved LVEDP in the 2-week post-MI rat. The mechanism by which irbesartan preferentially improved RV rather than LV contractile function during the early phase of remodeling post-MI remains presently undefined, but it is possible that the progression of pulmonary hypertension post-MI may have been in part dependent on AII. Several studies have demonstrated that the vasomotor tone of the pulmonary circulation was extremely sensitive to the vasoconstrictor action of AII, and acting via the AT_1 receptor, was involved in the early pathogenesis of hypoxia-induced pulmonary hypertension (Lipworth & Dagg, 1994; Zhao *et al*., 1996). Thus, these data highlight a novel and preferential therapeutic benefit of irbesartan on right ventricular function, which may have occurred in part via the reduction of pulmonary hypertension during the early phase of remodeling post-MI.

ERK represents a conserved early intracellular signaling event of putative growth-promoting factors. Several studies have demonstrated that the pharmacological inhibition of ERK attenuated the subsequent sympathetic-mediated hypertrophy of cardiac myocytes and proliferation of cardiac fibroblasts (Xiao *et al*., 2001; Kim *et al.,* 2002). Consistent with this latter premise, the manipulation of the ERK pathway with transgenic mice revealed an integral role in cardiac hypertrophy. (Bueno *et al*., 2000; 2001). Collectively, these data support the premise that the early phase of remodelling post-MI may be associated with ERK recruitment. Indeed, the phosphorylation state of ERK1/2 in the RV and NILV was significantly increased in the 4-day post-MI rat. Interestingly, neither LV or RV contractile status influenced ERK1/2 phosphorylation in the 4-day post-MI rat. However, the latter conclusion may be related in part to the protocol employed, as ERK1/2 phosphorylation was measured in cardiac lysates reflecting changes occurring in both cardiac myocytes and fibroblasts. As previously discussed, AII stimulation of cardiac myocytes and fibroblasts increased ERK1/2 activity *in vitro*, however, irbesartan therapy did not attenuate increased ERK phosphorylation in the NILV. Thus, neither ventricular function, nor AII acting via the AT_1 receptor was implicated in ERK1/2 phosphorylation in the NILV during the early phase of remodeling post-MI. By contrast, a significant reduction of ERK1/2 phosphorylation was observed in the RV of 4-day post-MI rats treated with irbesartan. Presently, it is not possible to discern whether the irbesartan-mediated partial attenuation of ERK1/2 phosphorylation was related to a direct inhibition of AII, and/or a secondary effect associated with a normalization of right ventricular pressures. Moreover, the continued presence of ERK1/2 phosphorylation in the RV of 4-day post-MI rats following irbesartan therapy supports the involvement of a yet unidentified stimulus (e.g. sympathetic system) which may also be critical in regulating ERK1/2 phosphorylation in the NILV. Lastly, the present study did not ascertain the cellular source of increased ERK1/2 phosphorylation in the hearts of 4-day post-MI rats, albeit recruitment

of the serine/threonine kinase is consistent with its role as an early signaling event implicated in the induction of cardiac myocyte hypertrophy and fibroblast proliferation during the early phase of remodeling post-MI.

In cardiac myocytes, PKB can function as an antiapoptotic molecule in response to hypoxia, as well as an intermediate signaling event involved in prepro-ANP mRNA expression, and hypertrophy (Matsui *et al*., 1999; Morisco *et al*., 2000; Hiraoka *et al*., 2001; Condorelli *et al.,* 2002). In cardiac fibroblasts, the PI3-K pathway has been identified as a growth-promoting pathway, albeit the downstream targets implicated remain undefined. Thus, PKB activation during the early phase of remodeling post-MI may represent a prerequisite event, acting in concert with ERK to promote the cellular and molecular events associated with cardiac myocyte hypertrophy and fibroblast proliferation. However, its recruitment and stimuli implicated in PKB regulation during the early phase of remodeling post-MI remain undefined. PKB activation requires the dual phosphorylation of both serine^{473} and threonine^{308} residues via a PI3-K-dependent pathway (Schubert *et al*., 2000). In the present study, PKB serine^{473} and threonine^{308} phosphorylation were modestly increased in the NILV and RV 4-day post-MI, which may have been in part influenced by the variability in scar size and the subsequent effect on contractile function. Indeed, both PKB serine^{473} and threonine^{308} phosphorylation in the NILV negatively correlated with LVEDP, suggesting that PKB regulation may have occurred preferentially in cardiac myocytes of 4-day post-MI rats. However, this latter premise remains to be verified. Moreover, based on the observation that scar size correlated with LVEDP would support the premise that rats with large infarcts were associated with a greater hemodynamic burden. Thus, it is possible that maximal PKB serine^{473} and threonine^{308} phosphorylation in the NILV of rats with large infarcts may have occurred at an earlier time point, rather than at 4-day post-MI. A second salient finding regarding PKB regulation was the disparate pattern of serine^{473} and threonine^{308} phosphorylation in both the NILV and RV. Consistent with this latter observation, the treatment of TF-1 cells with ceramide was associated with a differential pattern of PKB serine^{473} and threonine^{308} phosphorylation (Schubert *et al*., 2000). The underlying mechanism implicated in this latter phenomenon in the MI rat remains presently undefined, however, the discordant regulation of serine^{473} and threonine^{308} phosphorylation would compromise PKB activation, and subsequent physiological role.

Third, irbesartan therapy of MI rats did not significantly alter the magnitude of PKB phosphorylation in either the NILV or RV. Thus, at least during the early phase of remodeling post-MI, the AT_1 receptor does not appear to regulate cardiac PKB phosphorylation. Lastly, as noted for ERK, the cellular source of PKB regulation in the myocardium of the MI rat was not identified. However, it is tempting to speculate that the inability to recruit PKB in cardiac myocytes during the acute post-MI phase in at least rats with large infarcts may result in an inadequate hypertrophic response. Thus, possibly predisposing these cells to apoptotic stimuli, thereby leading to further scar expansion, and deleterious ventricular remodeling (Matsui *et al*., 1999).

The inability of irbesartan therapy to influence PKB and ERK phosphorylation in the NILV and the partial attenuation of ERK in the RV of 4-day post-MI rats supports the premise that AII, acting via the AT_1 receptor may not be directly implicated in the subsequent induction of a hypertrophic phenotype in cardiac myocytes. To investigate this latter premise, molecular events characteristic of cardiac myocyte hypertrophy and the effect of irbesartan were examined in 2-week post-MI rats because RV hypertrophy was established (data in present study; RV/BW ratio), and hypertrophic markers were previously shown to be induced during this time frame in the NILV (Hanatani *et al*., 1995). Prepro-ANP and -BNP mRNAs were induced in the RV, and NILV of 2-week post-MI rats, thereby indicative of cardiac myocyte hypertrophy, and decreased ventricular contractile function, respectively (Chien *et al.,* 1991; Troughton *et al*., 2000). In the RV, irbesartan therapy normalized natriuretic peptide mRNA expression, whereas these transcripts remained elevated in the NILV. It is highly unlikely that the disparate action of irbesartan on natriuretic peptide mRNA expression was associated with a preferential direct effect of AII on the RV. By contrast, the normalization of right ventricular pressures, as a consequence of a reduced hemodynamic burden (attenuation of pulmonary hypertension) may represent the primary mechanism. It remains to be determined whether the reduction of ERK phopshorylation in the RV of irbesartan-treated 4-day post-MI rats may have contributed in part to the suppression of natriuretic peptide mRNA expression. Moreover, with regard to hemodynamic load as a potential mechanism influencing natriuretic peptide mRNA induction in the RV, the irbesartan-mediated decrease of LVEDP may not have been sufficient to attenuate either prepro-ANP, or -BNP mRNA expression in the NILV. Alternatively, AII acting via the AT_1 receptor may not play a direct role during the early phase of remodeling, as increased prepro-ANP mRNA expression in the NILV of 1-week post-MI AT_{1A} receptor knockout mice was quantitatively similar to that observed in wild-type post-MI mice (Harada *et al*., 1999). Likewise, the AT_1 receptor antagonist TCV-116 did not suppress the elevated expression of prepro-ANP mRNA in NILV 1-week post-MI (Hanatani *et al*., 1995).

Reactive fibrosis represents a phenotypic event of post-MI remodeling and is characterized by uncontrolled cardiac fibroblast proliferation and accompanied by the disproportionate synthesis and secretion of the extracellular matrix proteins collagen and fibronectin (Weber, 1997). In addition to a potential role in cardiac myocyte hypertrophy, ERK, and the PI3-K pathway, possibly acting via PKB, have been identified as integral signaling events of both human and adult rat cardiac fibroblast proliferation (Hafizi *et al*., 1999; Kim *et al*., 2002). However, the absence of an effect of irbesartan on either kinase in the NILV, and a partial attenuation of ERK in the RV suggests that AII stimulation of the AT_1 receptor may not be directly implicated in either cardiac fibroblast proliferation or extracellular matrix protein expression. In this regard, the effect of irbesartan on the expression of the cell cycle protein proliferating cell nuclear antigen (PCNA), and fibronectin mRNA were examined in the 4-day and 2-week post-MI rat, respectively. A previous study demonstrated that PCNA, a DNA polymerase accessory factor required for DNA synthesis, was significantly increased primarily in the nonmyocyte fraction (predominantly fibroblasts) of the NILV of the post-MI rat, and unaffected by losartan therapy. (Prelich *et al*., 1987; Taylor *et al*., 1998). In the present study, PCNA protein expression was significantly increased in the NILV and RV 4

days post-MI, and irbesartan therapy did not significantly influence the expression of the cell cycle protein. With regard to interstitial fibrosis, collagen mRNA, and protein content were significantly increased in the NILV as early as 1 – 2 weeks post-MI (Makino *et al*., 1996; Richer *et al*., 1999). In at least three separate studies, collagen α_1 and α_3 mRNA expression in the NILV remained elevated following AT_1 receptor antagonist therapy 1 – 2 weeks post-MI (Hanatani *et al*., 1995; Ju *et al*., 1997; Harada *et al*., 1999). Moreover, captopril therapy for 8 weeks failed to suppress elevated collagen α_1 and α_3 mRNA expression in the NILV (Jin *et al*., 2001). By contrast, collagen protein content was either suppressed, partially attenuated, or remained elevated in the NILV following either ACE inhibitor or AT_1 receptor antagonist therapy (Smits *et al*., 1992; Makino *et al*., 1996; Ju *et al*., 1997; Taylor *et al*., 1998; Jin *et al*., 2001). There exists a paucity of data regarding fibronectin mRNA expression in the MI rat and its potential modulation by AII. In isolated rat and human cardiac fibroblasts, AII administration either increased or had no effect on fibronectin expression, respectively (Crabos *et al*., 1994; Kawano *et al*., 2000; Kupfahl *et al*., 2000). In the present study, fibronectin mRNA expression was increased in the RV, and NILV. Despite the amelioration of ventricular function, and the suppression of natriuretic peptide mRNA expression in the RV, fibronectin mRNA levels remained elevated in both ventricles of the irbesartan-treated MI rats. Likewise, 8 weeks of captopril therapy ameliorated left ventricular function, but did not suppress increased fibronectin mRNA expression in the NILV (Jin *et al*., 2001). These data suggest that neither a hemodynamic burden nor an AT_1 receptor-dependent mechanism contributed to ventricular fibronectin mRNA expression in the MI rat.

This study is the first to highlight the preferential therapeutic effect of the selective AT_1 receptor antagonist irbesartan on right ventricular contractility, cellular, and molecular phenotype during the early phase of remodeling post-MI. By contrast, irbesartan therapy caused a modest decrease in LVEDP in the 2-week post-MI rat, but did not alter either ERK or PKB phosphorylation, nor inhibit natriuretic peptide mRNA expression in the NILV. These latter observations suggest that AII activation of the AT_1 receptor may not represent an integral factor influencing left ventricular contractile dysfunction or phenotype during the early phase of remodeling in the post-MI rat.

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