Isoproterenol and Angiotensin I-Converting Enzyme in Lung, Left Ventricle, and Plasma During Myocardial Hypertrophy and Fibrosis

*†María Paz Ocaranza, ‡Guillermo Díaz-Araya, *Mario Chiong, †David Muñoz, †Juan Pablo Riveros, *Roberto Ebensperger, §Sebastián Sabat, §Pablo Irarrázaval, †Jorge E. Jalil, and *Sergio Lavandero

*Departamentos de *Bioquímica y Biología Molecular and ‡Química Farmacológica y Toxicológica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile; †Departamento de Enfermedades Cardiovasculares, Hospital Clínico; and §Facultad de Ingeniería, P. Universidad Católica de Chile, Santiago, Chile*

Summary: This study investigated whether long-term administration of isoproterenol (ISO) induces differential expression of angiotensin-converting enzyme (ACE) in lung, plasma, and left ventricle (LV) during development of left ventricular hypertrophy (LVH) and myocardial fibrosis. Male Sprague-Dawley rats (n = 7–9 per group) were treated with isoproterenol (ISO) 5 mg/kg per day for 10 days or saline and examined at 1, 15, and 33 days after the last injection. ISO stimulated the development of left ventricular hypertrophy (LVH); relative LV weight (mg LV 100/body weight), LV protein content, and LV β-myosin heavy chain levels increased at day 1. LVH regressed at days 15 and 33. ISO also increased myocardial fibrosis (assessed by hydroxyproline content and morphometry) at days 15 and 33. There no were changes in arterial blood pressure. Long-term β-adrenergic stimulation with ISO increased ACE expression in lung, LV, and plasma during development of LVH and myocardial fibrosis. However, time courses were markedly different. ISO stimulated a sustained increase in lung and plasma ACE activities, whereas ISO induced a high LV ACE. Plasma ACE activity paralleled lung ACE activity. LV ACE activity correlated with ACE mRNA levels and paralleled development of LVH. Our data suggest long-term β-adrenergic stimulation induced a differential temporal expression of LV, lung, and plasma ACE in rat during development of LVH and myocardial fibrosis. **Key Words:** Angiotensin—β-adrenergic—Fibrosis—Hypertrophy—Renin-angiotensin system.

Left ventricular hypertrophy (LVH) is a crucial adaptive process during mechanical overload, resulting in growth of cardiac myocytes and epidemiologically associated with increased cardiovascular risk (1,2). There may also be a structural remodeling of the myocardial extracellular space, characterized by an increase in fibrillar collagen (fibrosis) (3–5). These changes have been observed in LVH induced by different forms of mechanical overload, by myocardial injury, or by exposure to supraphysiological levels of catecholamines (6–14).

The activation of the renin-angiotensin system (RAS) and the adrenergic system induces LVH and fibrosis both in vivo and in vitro (15,16). Increased sympathetic activity has been observed as a central feature in patients with heart failure (17,18), and the extent of elevation of plasma norepinephrine correlates with the severity of the LV dysfunction and mortality (19,20). Experimental administration of isoproterenol (ISO), a β-adrenergic agonist, results in LVH, myocyte necrosis and apoptosis, fibroblast proliferation, collagen accumulation, endomyocardial fibrosis, and in the development of mild heart failure (21–24).

The RAS has long been considered as a circulating humoral system responsible for blood pressure regulation and salt and water homeostasis. Angiotensin-I converting enzyme (ACE) and other RAS components have been found in cardiac tissue, indicating their local synthesis (25–27) or a system generating angiotensin II (Ang II) locally (28). The intracardiac RAS could be involved in the development of cardiac remodeling, since Ang II itself stimulates cardiac myocyte hypertrophy and fibroblast hyperplasia (11,15,29,30). ACE is a membrane-bound zinc-containing dipeptidyl carboxypeptidase, which is widely expressed at the luminal surface of the vascular endothelium (specially in lung), inflammatory cells, fibroblasts, cardiac myocytes, and renal proximal tubule (28,31,32), while being in the soluble form in plasma. ACE is responsible for the conversion of angiotensin I (Ang I) to Ang II. Bradykinin (BK) and angiotensin-(1–7) [Ang-(1–7)] are also degraded and inactivated by ACE (33), and an increase in ACE activity therefore leads not only to higher Ang II production but also to an increase in BK and Ang-(1–7) degradation.

The regulation of ACE expression depends on the particular cell type and environment. In vitro studies have shown that ACE gene expression is regulated by steroid hormones (dexamethasone, estrogen), fibroblast growth factor, and β-adrenergic agonists (34–37). In rats with experimental myocardial infarction, with renovascular hypertension or after ISO administration, increased cardiac ACE activity has also been found (22,23,29, 38–40).

There are no in vivo studies exploring the regulation of the expression of tissue and circulating ACE by β-adrenergic agonists during the development and regression of LVH and fibrosis. We have investigated whether long-term β-adrenergic stimulation by ISO induces differential expression of plasma, lung, and cardiac ACE during the development and regression of LVH and myocardial fibrosis in the rat.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (baseline body weight, 95 ± 3 g) were obtained from the Animal Breeding Facility (University of Chile, Santiago). Animals were individually housed in a 12-h dark-and-light–cycle controlled room and maintained on standard rat chow with water ad libitum. They were handled according to animal welfare regulations of the University of Chile. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH publication No. 85–23, revised 1996).

Experimental groups

Fibrosis and heart hypertrophy were induced by ISO administration as previously described (41). Briefly, rats received subcutaneous injection of (±) isoproterenol hemisulfate (ISO; Sigma, St. Louis, MO), 5 mg/kg body weight (BW) per day for 10 days at the same time. A matched group of control rats received saline injections. In pilot experiments, 5 mg ISO/kg per day for 10 days was the most effective dose to induce significant myocardial hypertrophy and fibrosis along with an acceptable survival. After 1, 15, and 33 days, animals were killed by decapitation and trunk blood was collected into prechilled tubes containing heparin for the determination of plasma ACE activity. Hearts and lungs were removed, were freed of connective tissue, and placed on ice. The left and right ventricles were separated, washed extensively with saline to remove all contaminating blood, dried, and weighed. The tissues were quick-frozen in liquid nitrogen and stored at −80°C until analyzed for ACE mRNA, ACE activity, and hydroxyproline content. Hearts were also fixed in neutral buffered formalin for collagen and morphometric analysis.

Hemodynamic and morphologic indices

Systolic blood pressure was measured by the tail-cuff method 1 day before animals were killed (42). The degree of LVH was quantified by the relationship between the left ventricular (LV) weight and the body weight, the

relative left ventricular weight ratio (RLVH; LV weight 100/body weight) and LV protein (LVP) content at 1, 15, and 33 days after last ISO or saline injection. LVP concentration was determined by Bradford's method (43).

Western blot analysis for β-myosin heavy chain

Proteins (100 μg per lane) were separated by SDS-polyacrylamide gel electrophoresis on 10% (w/v) poly-acrylamide gels and transferred electrophoretically to nitrocellulose. Nonspecific binding sites were blocked with 5% (w/v) nonfat milk powder in PBST (in mM: NaH$_2$PO$_4$ 20 [pH 7.5], Na$_2$HPO$_4$ 80, NaCl 100) containing 0.05% (v/v) Tween-20 for 30 minutes, and the blots were incubated with β-MHC antibody (1:200 dilution in blocking solution) overnight at 4°C. After washing in PBST (3 × 5 minutes), the blots were incubated with horseradish peroxidase-linked anti-mouse IgG antibodies (1:5,000 dilution in PBST containing 1% (w/v) nonfat milk powder) for 1 hour at room temperature. The blots were washed again in PBST (3 × 5 minutes), and the bands were detected using ECL with exposure to film. Blots were quantified by laser scanning densitometry.

Measurement of left ventricular hydroxyproline content

A 100-mg sample of frozen tissue, corresponding to the middle portion of the left ventricle, was hydrolyzed in HCl 6 M for 24 hours at 110°C. Hydroxyproline content was measured spectrophotometrically at 600 nm as previously described (44). Hydroxyproline values were determined directly from a standard curve (Sigma, St. Louis, MO). Collagen concentration was calculated from the assumption that hydroxyproline makes up 12.7% of the total collagen. Collagen content was obtained by multiplication of collagen concentration by left ventricle weight. The p-aminobenzaldehyde and chloramine T were purchased from Merck Química Chile (Santiago, Chile).

Collagen morphology and morphometric analysis

A 2-mm–thick coronal section was taken from the equator of each heart and fixed in neutral buffered formalin. Formalin-fixed sections were dehydrated through a graded series of alcohol and xylene and embedded in paraffin. Paraffin sections (5 μm thick) were stained with hematoxylin and eosin for histologic evaluation. Sections from the myocardium located at the equator of the left ventricle were treated for 2 minutes with phosphomolybdic acid (0.2%) and stained with the collagen-specific stain Sirius red F3BA (Pfaltz & Bauer Inc., Stanford, CO) in saturated aqueous picric acid (pH 2.0) for 90 minutes. Thereafter, the picrosirius red-prepared sections

were examined by a single investigator who was unaware of the nature of the experimental groups. Each section was viewed in its entirety. ISO-induced fibrosis was located primarily within the endocardium, with extensions of fibrous tissue reaching the midwall. For each heart, 20 sections were examined, and collagen was quantified by computer-assisted morphometry. The automated system included an image-analysis processor based on mathematical morphology software connected to a PC computer. Each field sent to the image analyser was transmitted by a video camera connected to a microscope and transformed into a digital image.

Processing of tissue and solubilization of membranes

Membranes were prepared at 4°C as previously described, but care was taken to ensure minimal handling to preserve ACE activity (45). A representative sample of LV or lung tissue was homogenized using an Ultraturrax (Janke and Kunkel AG, Germany) in 1 ml of ice-cold extraction buffer (50 mM tris HCl, pH 8.0; containing 1% NaCl). Each homogenate was centrifuged at 1,000 g for 15 minutes at 4°C, and the resulting supernatants were centrifuged again at 15,000 g for 60 minutes at 4°C. Supernatants were discarded, and pellets were washed once with 1 ml of ice-cold extraction buffer. The resultant pellet was resuspended in 1 ml of solubilization buffer (50 mM Tris HCl, pH 8.0; containing 1% NaCl and 8 mM CHAPS) and frozen at −80°C. The protein content of the membrane fraction was determined by Bradford's method (43).

Assessment of tissue and plasma ACE activity

Tissue or plasma ACE activity was assayed by following the release of His-Leu from the synthetic substrate Z–Phe-His-Leu with or without enalapril according to the fluorometric assay described by Cushman et al. (44). A portion of the homogenized sample or plasma was then incubated with Z-Phe-His-Leu (Bachem Bioscience Inc., King of Prussia, PA) for 20 minutes (for lung and plasma) or 45 minutes (for cardiac tissue) at 37°C in a shaking water bath. The reaction was stopped by adding 100 μL of cold 10% TCA and 280 mM NaOH, respectively. Phthalaldehyde (0.1%) was then added to the samples and incubated for 10 minutes at 37°C before reaction was stopped with 3M HCl. The fluorescence at 486 nm was measured with excitation at 364 nm (Fluorescence Spectrophotometer, Perkin Elmer). Plasma and tissue ACE activity was expressed as nanomole of His–Leu liberated per minute per milliliter of plasma or per milligram of protein at 37°C, respectively.

Determination of ACE mRNA

Total RNA was isolated from the left ventricle according to Chomczinsky and Sacchi (46). RNA pellets were suspended in distilled water and their concentrations were quantified by UV spectroscopy, assuming 40 mg/ml for 1 absorbance unit. RNA integrity was assessed from the intensity of the staining with ethidium bromide of 18 and 28S ribosomal RNA after agarose electrophoresis. The expression of tissue ACE was done by RT-PCR. For reverse transcription, 1 μg RNA was incubated with or without reverse transcription (Gibco-BRL, Gaithersburg, MD) in a mixture containing random hexamers, deoxynucleotides, and RNAsin in reverse transcription buffer. For the amplification of the resulting cDNA, 1 μM ACE specific primers, 0.2 mM deoxynucleotides, 1.5 mM MgCl$_2$, and 1.5 U *Taq* polymerase (Gibco-BRL, Gaithersburg, MD) were added to 3 μl of each RNA sample in 50 μl. ACE sequences of the sense and antisense primers were 5′–CGCTACAACTTC-GACTGGTGG–3′ and 5′–TATTTCCGGGATGTGGC-CAT–3′, yielding an amplification fragment of 881–bp (24). Amplification conditions were as follows: denaturation at 94°C for 1 minute, annealing at 62°C for 1 minute, and elongation at 72°C for 1 minute for 28 cycles, with a final elongation step at 72°C for 7 minutes. Pilot experiments showed that in 28 cycles we did not bring to the plateau phase of this PCR. After PCR, the amplification products were fractionated on a 1.5% (w/w) agarose gel and visualized by staining with ethidium bromide. Band intensities were quantified by computerized densitometry and normalized with respect to 18S RNA.

Statistical analysis

All data are presented as means ± SEM. The differences in each parameter were evaluated by an analysis of variance (ANOVA), followed by Scheffé *F* test to compare the effect of different conditions on these parameters. An ANOVA was also used to analyze the combined effects of dose and time. Significance was accepted at the level of $p < 0.05$.

RESULTS

Time-course effect of isoproterenol administration on systolic blood pressure and LVH

Systolic blood pressures of the ISO group were similar to that of age-matched control rats (Fig. 1). ISO decreased the body weights, but not significantly lower than controls, despite similar baseline body weights. There was an increase in the RLVW and LVP at day 1
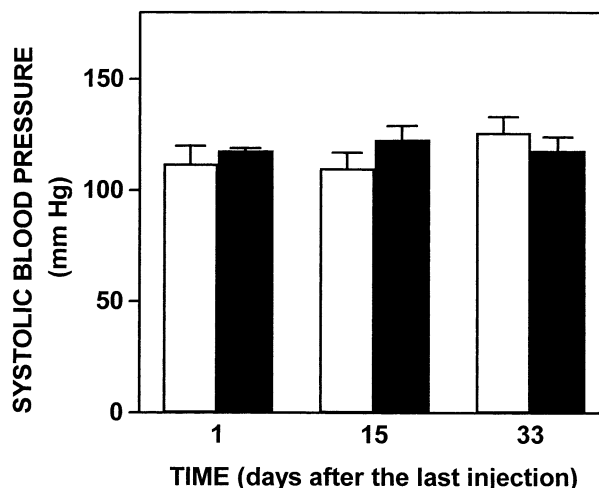

FIG. 1. Effect of isoproterenol (ISO) administration on systolic blood pressure. Rats were injected subcutaneously with saline (open bar) or 5 mg ISO per kilogram per day (black bar) for 10 days. After 1, 15, or 33 days after last injection, systolic blood pressure was measured by the tail-cuff method. Data represent mean ± SEM of duplicate determinations (n = 7–9 rats).

after the last ISO injection. This effect was dose dependent (data not shown). Table 1 also shows that the maximal degree of LVH (26% in RLVW and 37% LVP) was attained with 5 mg ISO/kg per day at day 1 after last injection ($p < 0.01$). Nevertheless, LVH regressed in the ISO-treated groups 15 days after finishing treatment and was similar in the corresponding controls (Table 1). This effect was specific for the LV since no change in RW weight was observed in response to ISO (data not shown).

Another characteristic feature of LVH is the increase in contractile protein such as β-MHC. As shown in Figure 2, ISO increased LV β-MHC levels (31.6% with respect to controls) only at day 1 post last injection. There were no differences in LV β-MHC levels between ISO and control groups at days 15 and 33 (data not shown).

Time-course effect of isoproterenol administration on myocardial fibrosis

Collagen content estimated by the hydroxyproline content (Fig. 3A) was higher in all ISO groups than in controls after 15 and 33 days after treatment ($p < 0.01$). To investigate the distribution of collagen, picrosirius red-prepared cardiac sections were examined by morphometry. Figure 3B shows that at day 15 after treatment with 5 mg ISO/kg per day (but not at day 1), there was a marked connective tissue accumulation in the LV subendocardium in comparison to control groups. No in-

TABLE 1.
Left ventricular hypertrophy development induced by isoproterenol

Day after the last injection	Isoproterenol	BW	HW	LV/RV	RLVW	LVP
1	0	162 ± 3	0.62 ± 0.03	3.7 ± 0.1	308 ± 14	1.7 ± 0.1
	5	155 ± 7	0.72 ± 0.03	4.1 ± 0.1*	380 ± 15*	2.8 ± 0.2*
15	0	302 ± 9	0.94 ± 0.04	3.4 ± 0.1	241 ± 4	3.5 ± 0.4
	5	276 ± 20	0.97 ± 0.04	2.9 ± 0.2	269 ± 29	3.4 ± 0.3
33	0	471 ± 11	1.16 ± 0.04	4.0 ± 0.2	205 ± 6	4.8 ± 0.4
	5	403 ± 16	1.10 ± 0.04	3.5 ± 0.2	212 ± 4	5.2 ± 0.3

Rats were injected subcutaneously with isoproterenol (5 mg/kg/d) or saline (control) for 10 days. At day 1, 15, and 33 after treatment, body weight (BW, g), heart weight (HW, g), left ventricle weight to right ventricular weight ratio (LV/RV), relative left ventricular weight (RLVW; mg LV/100 g BW), and left ventricular protein (LVP, mg protein, mg) were determined. Values are mean ± SEM, n = 7–9; *p < 0.01 versus control.

crease in fibrosis was detected in the epicardium. Myocardial fibrosis in subendocardium was 57% higher than control levels after 15 days after treatment with 5 mg ISO/kg per day for 10 days.

Time-course effects of isoproterenol administration on lung, plasma, and left ventricular ACE activities

As shown in Figure 4A, lung ACE activities after 1, 15, and 33 days of posttreatment with 5 mg ISO/kg per day were significantly increased. Plasma ACE activity increased 1 day after daily ISO injection and remained high until the end of the study (Fig. 4B). Plasma ACE activity correlated with lung ACE activity, and both activities showed similar time courses. As Figure 4C depicts, LV ACE activity showed different time-course behavior in response to ISO compared with plasma and lung ACE activities. ISO-induced ACE activity in the LV was higher 1 day after repetitive treatment of 10 days with ISO (p < 0.001; Fig. 4C). Later on, at days 15 and 33, LV ACE activity in the ISO-treated rats decreased compared with that of day 1 but remained higher than in control animals.

We also investigated whether β-adrenergic stimulation involves the participation of local or systemic factors by measuring ACE activity in LV and RV homogenates of control and ISO-treated rats. ISO administration increased LV and RV ACE activities 2.7- and 1.9-fold, respectively (p < 0.01) at day 1 after treatment.

A significant linear correlation between LV ACE and RLVM was observed ($r = 0.59$; p < 0.001). No correlation was, however, observed between RLVM and plasma ACE.

Effect of isoproterenol administration on LV ACE mRNA levels

ACE mRNA levels were measured by RT-PCR in LV of control- and ISO-treated rats to verify that increased ACE mRNA levels correlated with increased ACE activity. Densitometric analysis of amplified ACE cDNAs showed that the relative amount of LV ACE mRNA increased 1.9-fold at day 1 after last ISO administration compared with controls (Fig. 5). There were no differences in ACE mRNA levels between ISO and control groups at days 15 and 33 (Fig. 5). This effect was selective for ACE mRNA since no changes were observed in the mRNA levels for Ang II AT1 receptor (data not shown).
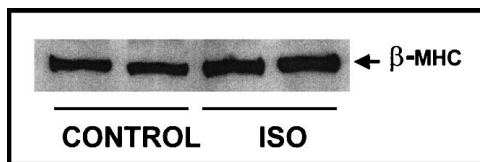
DISCUSSION

ACE is a critical regulator of many physiological responses, including blood pressure regulation and salt and water homeostasis. There are two ACE isoforms transcribed from a single ACE gene by two alternate promoters (47). Somatic ACE is synthesized by vascular endothelial cells as well as in several types of epithelial cells. A soluble form of ACE exists in plasma and other

FIG. 2. Effect of isoproterenol on cardiac β-myosin heavy chain levels. Rats were injected subcutaneously with saline (control) or 5 mg isoproterenol per kilogram per day (ISO) for 10 days. One day after last injection, cardiac β-myosin heavy chain (β-MHC) levels were determined by Western blot as described in Materials and Methods. The result is representative from three separate experiments.
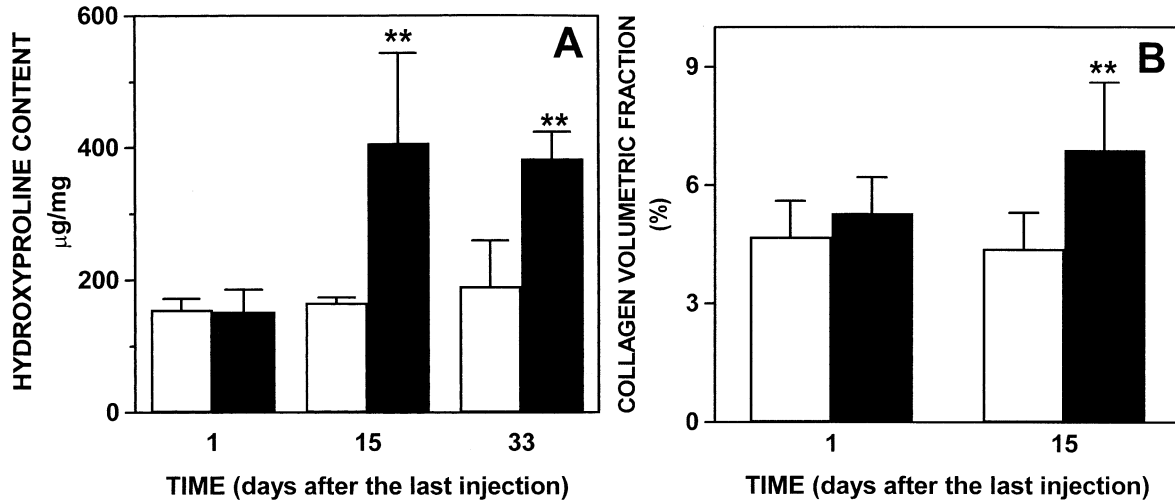
FIG. 3. Development of myocardial fibrosis induced by isoproterenol. Rats were injected subcutaneously with saline (open bar) or 5 mg isoproterenol (ISO) per kilogram per day (black bar) for 10 days. At day 1, 15, and 33 after the last injection, left ventricular (LV) hydroxyproline contents (A) were determined as described in Methods. LV picrosirius red sections were also prepared, and subendocardial collagen volume fraction (B) was quantified by computer-assisted morphometry as described in Materials and Methods. Results are mean ± SEM from 7–9 rats. **$p < 0.01$ vs. saline.
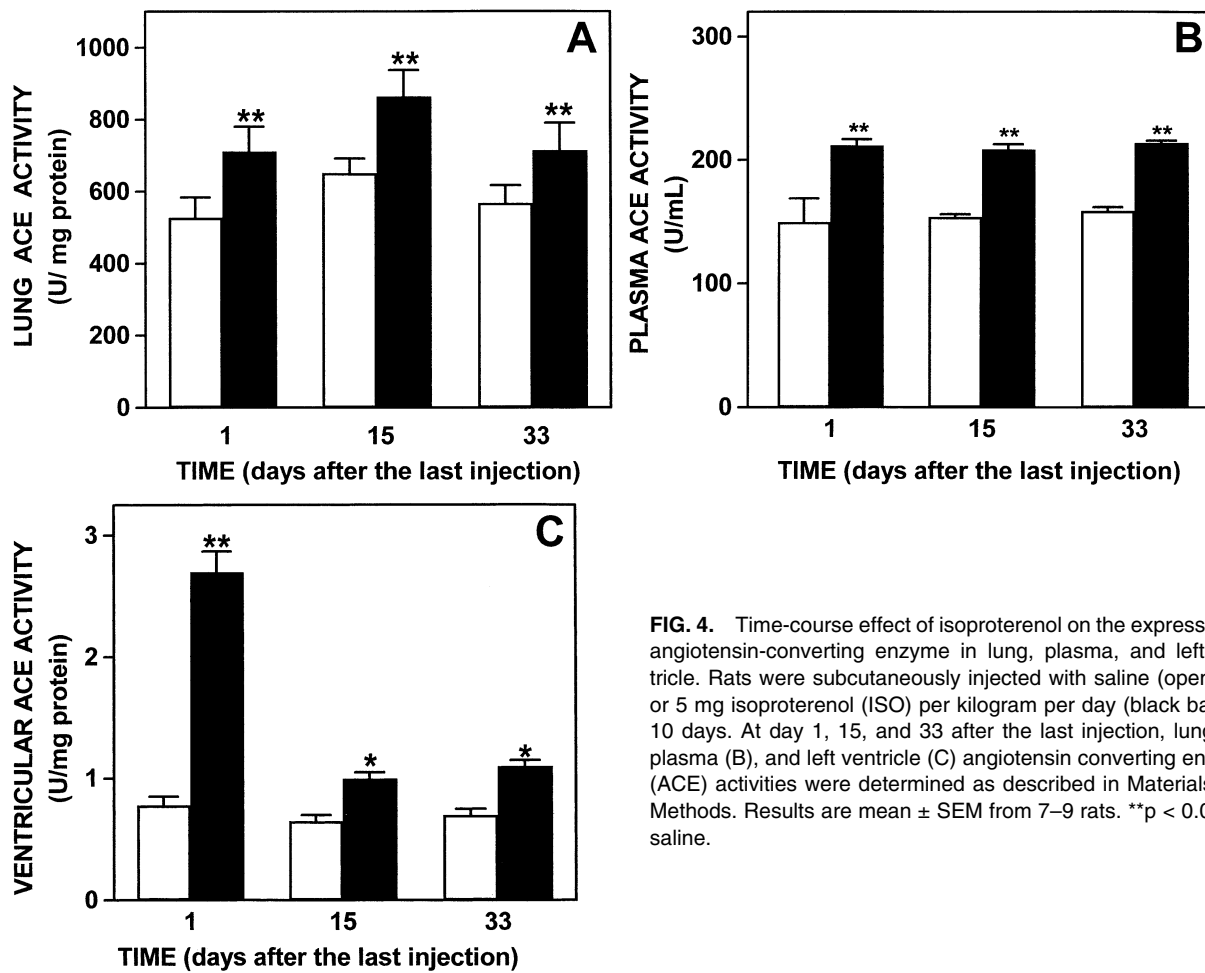

FIG. 4. Time-course effect of isoproterenol on the expression of angiotensin-converting enzyme in lung, plasma, and left ventricle. Rats were subcutaneously injected with saline (open bar) or 5 mg isoproterenol (ISO) per kilogram per day (black bar) for 10 days. At day 1, 15, and 33 after the last injection, lung (A), plasma (B), and left ventricle (C) angiotensin converting enzyme (ACE) activities were determined as described in Materials and Methods. Results are mean ± SEM from 7–9 rats. **$p < 0.01$ vs. saline.

body fluids derived from endothelial cells by posttranslational cleavage. However, there is minimal information on the in vivo regulation of the expression of tissue and circulating ACE by β-adrenergic agonist during the development of LVH and fibrosis. The principal finding of the current study was that long term β-adrenergic stimulation with ISO increased ACE expression in LV, lung, and plasma during development of LVH and myocardial fibrosis in the rat. However, the time courses were markedly different: ISO stimulated a sustained increase in lung and plasma ACE activities, whereas it induced a transient expression of ACE in the LV. Plasma ACE activity paralleled lung ACE activity. LV ACE expression requires a continued presence of ISO, a requirement that was not necessary for lung and plasma. LV ACE expression paralleled changes in ACE activity and LVH development. ACE activities increased both in the right ventricle and LV after ISO treatment, although in different magnitude, indicating that systemic and local factors may be involved in the regulation of ACE expression in the heart.

ACE gene expression is regulated by dexamethasone, estrogen, fibroblast growth factor, and β-adrenergic agonist (34–37). There are several pathologic conditions, such as experimental myocardial infarction or renovascular hypertension, in which increased cardiac ACE activity has been found (29,38,40,48,49). In our experimental model, Bussato et al. (50) recently reported that ISO increased ACE activity and produced hypertrophy in both ventricles. We found significantly higher ACE activity in the hypertrophied LV relative to the control hearts.

However, our data also show that increased ACE activity did not parallel with hypertrophy in the RV. Grimm et al. (22) reported a close positive correlation between LV/BW ratio and LV ACE activity in rats treated with a single subcutaneous injection of 150 mg ISO/kg. However, they found only a 22% increase in LV ACE activity 2 weeks after ISO treatment and no change after 4 months (22). Low plasma ACE activity, decreased pulmonary ACE activity as well as decreased ACE gene expression in the lung, have been observed in rats with large myocardial infarctions and systolic dysfunction (51). Our results agreed with previous work in which ISO or cAMP and analogs in cultured rabbit and bovine aortic endothelial cells increased ACE expression (37). Evidence for a transcriptional role of cAMP on ACE gene expression has also been described, including an intracellular signal transduction pathway linking β-adrenergic stimulation to ACE promoter induction. ISO induced LVH and myocardial fibrosis without changes in long-term arterial blood pressure. Other stud-

ies have shown either no change or a slight reduction in mean systolic blood pressure in rats treated with β-adrenergic agonists, suggesting that afterload is not elevated during ISO treatment (52–54).

We also confirmed by different criteria that ISO stimulated LVH development and increased myocardial fibrosis. However in this experimental model, different time courses for the generation of LVH and myocardial fibrosis were observed. LVH was earlier than fibrosis. LVH was maximal at day 1, while maximal fibrosis was attained at day 15 post last ISO injection, respectively. Although we did not evaluate myocyte hypertrophy by histologic analysis, other hypertrophic markers, such the left ventricular total protein (LVP) and β-myosin heavy chain (β-MHC) levels, demonstrated the development of LVH. The decrease in LV weight despite a persistent fibrosis can be explained by the effect of ISO on cardiac protein levels rather than cell death effect. ISO also induces an early cardiac apoptotic process. This effect was demonstrated by an increase in DNA fragmentation in paraffin-embedded sections using terminal deoxynucleotide transferase-mediated dUTP nick end labeling (TUNEL) and confirmed by an independent ligase assay (24). No evidence of apoptosis was detected later. The presence of ISO was required to maintain LVH, probably regulating cardiac protein levels.

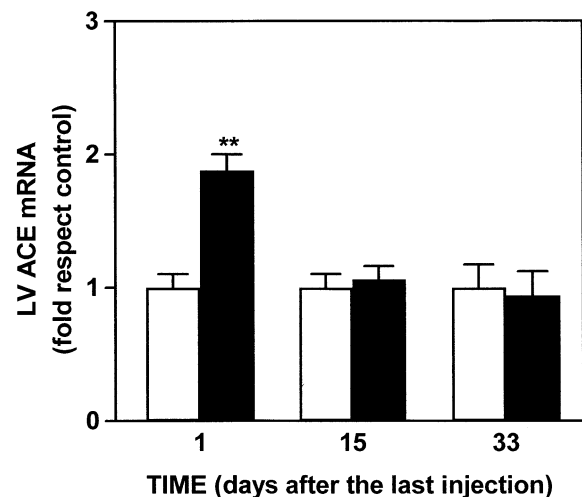The precise nature of the molecular stimuli respon-

FIG. 5. Effect of isoproterenol on left ventricular angiotensin-converting enzyme mRNA levels in the left ventricle. Rats were injected subcutaneously with saline (open bar) or 5 mg isoproterenol (ISO) per kilogram per day (black bar) for 10 days. At day 1, 15, and 33 after the last injection, left ventricular angiotensin converting enzyme (LV ACE) mRNA levels were determined by reverse transcription (RT)-polymerase chain reaction (PCR) as described in Materials and Methods. Results are mean ± SEM from 7–9 rats. **p < 0.01 vs. control.

sible for the fibrotic response in the myocardium is only slowly coming to light. Grimm et al. (22) observed that concentrations of plasma aldosterone, atrial natriuretic peptide, and renin activity were doubled in ISO treated groups compared with control rats. Nicoletti et al. (55) obtained evidence in the rat Goldblatt model suggesting that aldosterone stimulates type III collagen deposition independently of hypertension, but does not alter type I collagen accumulation. On the contrary, Ang II seems to enhance type I collagen deposition and LVH in association with its hypertensive activity (55).

In the present work, no measurements of LV function were performed. However, we previously did a similar study with lower ISO doses (41). An elevation in the slope of the diastolic stress-strain relation was observed. The developed systolic stress-strain relation (a measurement of contractility), which was elevated at day 10, declined with the reduction in myocardial muscle mass. Those findings paralleled the previously observed changes in LVH and myocardial collagen as well as in LV diastolic and systolic function and explained why progressive ventricular dysfunction accompanies chronic myocardial disease with myocardial fibrosis (41).

CONCLUSIONS

Our data are consistent with the hypothesis that there is a differential regulation of ACE expression in plasma, lung, and left ventricle during the development of cardiac remodeling in cardiovascular diseases.

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