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European Journal of Pharmacology 520 (2005) 135–141

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Chronic β-adrenoceptor stimulation and cardiac hypertrophy with no induction of circulating renin

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Received 2 November 2004; received in revised form 11 July 2005; accepted 21 July 2005
Available online 29 August 2005

Abstract

The hemodynamic and cardiac effects of isoproterenol were examined in rats submitted to chronic salt loading (1% NaCl as drinking water) to prevent activation of the systemic renin–angiotensin system. Isoproterenol treatment for 1 week resulted in 36% and 44% ($P<0.05$) increase in left ventricle mass in both control and chronic salt loading rats and induction of cardiac angiotensin-converting enzyme activity and expression ($P<0.05$) with no changes in serum angiotensin-converting enzyme. Plasma renin activity decreased significantly with chronic salt loading and failed to increase by isoproterenol treatment, whereas it increased 2.33 fold ($P<0.05$) in animals kept on regular chow. Isoproterenol treatment leads to transient increase in heart rate and cardiac output while blood pressure remained unchanged. Altogether, these data provide evidence that isoproterenol induced hypertrophy is associated with cardiac induction of angiotensin-converting enzyme and daily transient hemodynamic overload even in the absence of systemic activation of renin–angiotensin system.

Keywords: Angiotensin-converting enzyme; Myocardial hypertrophy; Renin–angiotensin system; Isoproterenol; β-adrenoceptor

1. Introduction

A great body of evidence indicates that the bulk of angiotensin II synthesized in the heart appears to be locally generated in the cardiac interstitium (van Kats et al., 1998). Despite the existence of alternative angiotensin II pathways or blood borne enzymes, cardiac angiotensin-converting enzyme appears to play a major role in this process in vivo which is further supported by the efficient blockade of cardiac conversion of angiotensin I to angiotensin II by angiotensin-converting enzyme inhibitors indicating that local levels of the enzyme activity reflect cardiac angiotensin II concentration (Zisman et al., 1995). Despite its wide distribution in the endothelium, a variety of epithelia, and inflammatory cells, angiotensin-converting enzyme is predominantly a local enzyme, which is consistent with the finding that genetic modified mice producing only a soluble form of the enzyme behave similarly to animals lacking this gene (Esther et al., 1997). Indeed there is marked local angiotensin-converting enzyme induction in a variety of pathological models of cardiac injury suggesting a key role for this enzyme in the generation of angiotensin II under pathological conditions (Fabris et al., 1990; Pieruzzi et al., 1995; Schunkert et al., 1990). Neuro-hormones, including norepinephrine, and increased wall stress are well characterized stimuli associated to cardiac hypertrophy and angiotensin-converting enzyme activation in vivo as well as protein synthesis and cell growth in vitro (Baker and Aceto, 1990; Fabris et al., 1990; Geisterfer et al., 1988; Sadoshima et al., 1993). Hormonal and physical factors interact in a complex manner influencing the cardiovascular system and especially during pathological states it becomes difficult to single out their relative contribution. Accordingly, β-adrenoceptor stimulation induced cardiac hypertrophy may involve local and/or systemic activation of the renin–angiotensin system leading to production of pleiotropic peptides which can act directly on cardiac cells or indirectly via hemodynamic alterations contributing for development of the final cardiac phenotype

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doi:10.1016/j.ejphar.2005.07.026

(Golomb et al., 1994; Leenen et al., 2001; Nagano et al., 1992).

This work was undertaken to investigate the influence of β-adrenoceptor stimulation on the control of angiotensin-converting enzyme expression/activity, which can potentially influence the cardiac trophic response. We also employed a chronic physiological salt loading maneuver to prevent systemic activation of renin in response to β-adrenoceptor stimulation.

2. Materials and methods

Male normotensive Wistar rats (200–250 g) were used and handled according to approved Institutional guidelines. The rats were assigned randomly into four groups: control (olive oil), isoproterenol (0.3 mg/kg, sc, daily for 7 days), chronic salt loading (1% NaCl as drinking water), chronic salt loading plus isoproterenol ($n=6$, for each group). Control group received only vehicle (olive oil). In the salt–isoproterenol group, salt treatment began 7 days prior to isoproterenol administration and continued thereafter. Placement of arterial catheters and aortic probes was made under anesthesia (thiopental sodium, 100 mg/kg i.p.). Twenty four hours following the last isoproterenol administration, conscious animals were decapitated, blood and tissue samples were harvested, frozen and stored at −80 °C. In pilot studies to assess the contribution of angiotensin-converting enzyme activation to the cardiac hypertrophic response, two other groups were included: angiotensin-converting enzyme inhibitor (enalapril maleate, 60 mg/l in drinking water), and enalapril plus isoproterenol ($n=6$, for each group). In the angiotensin-converting enzyme inhibitor group, enalapril treatment was initiated 7 days in advance. Indeed, left ventricle/total body weight ratio, which was used as an index of hypertrophy, increased only 12% in angiotensin-converting enzyme inhibitor–isoproterenol treated groups, however, it is important to emphasize that animals from group angiotensin-converting enzyme inhibitor–isoproterenol showed signs of poor health state which is consistent with a high mortality rate, approximately 50%, observed during this short period. Therefore, the data from these animals were considered unreliable and were not used for further analysis.

2.1. Morphological analysis

The heart was dissected to obtain the left ventricle, which corresponds to the remaining organ upon removal of both atria and the free wall of the right ventricle. The weight of each cardiac chamber was normalized by the total body weight of the animal. For morphometric analysis, left ventricle samples obtained from the free wall, at the level of papillary muscle, were fixed in 6% formaldehyde and embedded in paraffin, cut in 5 μm sections and subsequently stained with hematoxylin and eosin. Two randomly selected sections from each animal were visualized by light microscopy using an oil immersion objective with a calibrated magnification (400×). Myocytes with visible nucleus and intact cellular membranes were chosen for diameter determination. The width of individually isolated cardiomyocyte displayed on a viewing screen was manually traced, across the middle of the nuclei, with a digitizing pad and determined by a computer assisted image analysis system (Quantimet 520; Cambridge Instruments). For each animal, approximately 20 visual fields were analyzed.

2.2. Angiotensin-converting enzyme activity assay

Angiotensin-converting enzyme activity was determined by a fluorometric assay based on the rate of generation of His-Leu by hydrolysis of Hippuryl-His-Leu substrate (Sigma Chemical, St Louis, USA) (Oliveira et al., 2000). In brief, tissues were homogenized in 0.32 M sucrose in 0.4 M buffer borate pH 7.2 (1 g tissue: 10 ml buffer). Homogenates were centrifuged (3.000 rpm × 10 min) and the supernatants were used for fluorometric assay of angiotensin-converting enzyme activity. Serum (10 μl) and supernatants from homogenized tissues (20 μl) were incubated with 490 or 480 μl of assay buffer, respectively, containing 5 mM Hip-His-Leu in 0.4 M sodium borate buffer, pH 8.3 and 0.9 M NaCl for 15 or 30 min at 37 °C. The reaction was halted by the addition of 1.2 ml of 0.34 N NaOH. *o*-phthaldialdehyde (100 μl, 20 mg/ml, Sigma Chemical) was then added to the aliquots, stop the reaction with 3 N HCl and the fluorescence of the His-Leu product was measured at 495 nm with an excitation wavelength of 365 nm (Luminescence Spectrometer, SLM-AMINCO, Rochester, NY, USA). All assays were performed in triplicate. Protein was measured via the method of Bradford (1976), using bovine albumin as a standard. Activity calculations were based on Michaelis–Menten first-order kinetics.

2.3. Plasma renin activity assay

Plasma renin activity was determined by radioimmunoassay using commercially available kits according to the manufacture instructions (Plasma renin activity: REN-CIS).

2.4. Northern blot analysis

Frozen tissue samples (150 to 200 mg) were homogenized in guanidinium thiocyanate solution (4 mol/l) and RNA was isolated according to the method of Chomczynski and Sacchi (1987). RNA samples were analyzed by Northern blot. Briefly, aliquots of total RNA (20 μg) were denatured and size-fractionated by electrophoresis on 1% agarose gels under denaturing conditions. RNA was transferred to nylon membrane (GeneScreen Plus, NEN) and immobilized with ultraviolet irradiation. Hybridization with cDNA probes was performed overnight at 42 °C in buffer containing 50% formamide, 5× SSC buffer (1× SSC contains 0.15 mol/l NaCl, 0.015 mol/l sodium citrate), 5× Denhardt's solution

(5× contains 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 0.2% Ficoll), 5% dextran sulfate, 0.1% sodium dodecyl sulfate (SDS), and 100 μg/ml denatured salmon sperm DNA. Rat angiotensin-converting enzyme, atrial natriuretic peptide and glyceraldehyde-3-phosphate dehydrogenase cDNA probes were radioactively labeled with (^{32}P)dCTP (Amersham Bioscience) using random-primer DNA labeling kit (Ready To Go from Amersham Pharmacia Biotech Inc). After hybridization for 16 h, membranes were washed twice with 2× SSC, 0.1% SDS at room temperature for 10 min and twice with 0.2× SSC, 0.1% SDS at 55 °C for 15 min. All membranes were exposed at −80 °C for varying time periods to X-Omat X-ray film (Eastman Kodak Co) with intensifier screens. Quantification of Northern blots was obtained on the basis of the integrated absorbance increase over background absorbance in a rectangular region of interest. The levels of and glyceraldehyde-3-phosphate dehydrogenase cDNA probes mRNA expression in the same lane normalized the densitometric score of angiotensin-converting enzyme and atrial natriuretic peptide mRNA. Following each hybridization, probes were stripped off from membranes by boiling in 0.01× SSC, 0.1% SDS for 10 min.

2.5. Hemodynamics

2.5.1. Arterial blood pressure

Twenty-four hours after the last isoproterenol administration and under anesthesia a cannula (PE-50) was inserted into the femoral artery and emerged through the back of the rat. Twenty-four hours after the surgery the arterial blood pressure was recorded on a beat-a-beat basis (AT/CODAS) at a frequency of 100 Hz for 30 min in quiet, conscious, unrestrained rats. During the experimental session, this cannula was connected to a strain-gauge transducer (P23 Db; Gould-Statham). The data reported indicate the average of all values of systolic, diastolic, and mean arterial pressure over the entire recording time of 30 min.

2.5.2. Cardiac output

An ultrasonic perivascular flow probe (2SB; Transonic Systems) was placed around the ascending aorta just above the coronary arteries emerging through the back of the rat via a transversal incision performed on the right side of the chest wall in the second intercostal space just below the armpit area. Different layers of muscle were dissected following fiber orientation and the pectoral musculature was opened carefully avoiding bleeding. Upon opening the chest a rodent ventilator (model 683; Harvard) was connected (60 breaths/min at 2.5 ml/breath) during all surgical procedure. Body temperature was maintained stable (37 °C) by heating surgical pad. During the experimental session, the microprobe was connected to an ultrasonic flowmeter (model T206; Transonic Systems). The signal from the flowmeter was fed into an amplifier and a 16-channel analog-to-digital converter and then to a microcomputer for a direct cardiac output measurement.

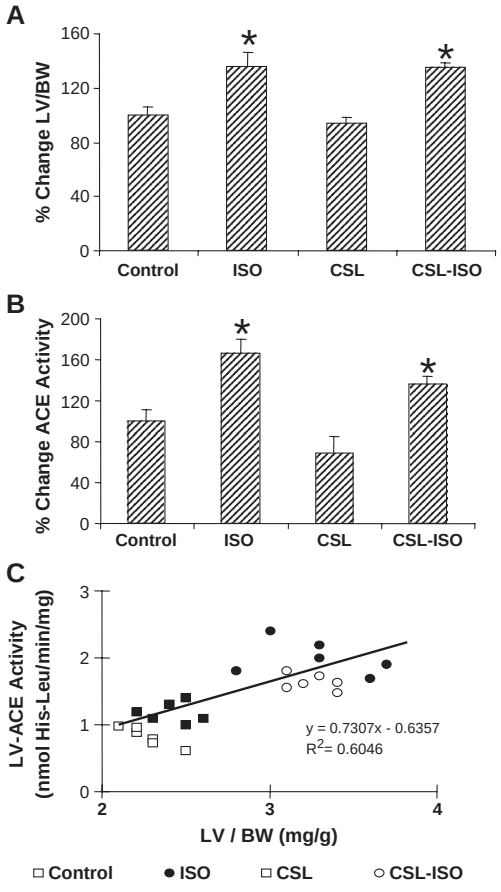

Fig. 1. Effects of isoproterenol (ISO) alone or in combination with chronic salt loading (CSL–ISO) in Wistar rats. A) Left ventricle (LV) weight normalized by total body weight change (% Change LV/BW, mg/g); B) LV angiotensin-converting enzyme activity (% Change LV angiotensin-converting enzyme, nmol His-Leu/min/mg); C) Correlation of LV angiotensin-converting enzyme activity and left ventricle/body weight (mg/g) (r=0.78; P<0.0001). *P<0.05 compared to control group. Data in panels A and B are shown as percentage change. Hundred percent was considered the control values for LV/BW (2.4±0.15 mg/g) and for ACE activity (1.2±0.13 nmol His-Leu/min/mg), respectively. Please note that statistical analysis from data shown in panels A and B was performed using the raw data.

Cardiac output was recorded on a beat-to-beat basis at a frequency of 100 Hz for 30 min in quiet, conscious, unrestrained rats. Stroke volume was measured simultaneously with arterial blood pressure. Heart rate was calculated from arterial blood pressure pulses and cardiac output from the product cardiac output × stroke volume.

2.5.3. Statistics

Data are expressed as mean±s.d. Unpaired Student's t test was used to compare angiotensin-converting enzyme and atrial natriuretic peptide gene expressions between two groups. Angiotensin-converting enzyme and renin activity, cardiac hypertrophy (atrium, right ventricle and left ventricle), total body weight, systolic blood pressure, diastolic blood pressure, mean arterial pressure and cardiac output data were compared between groups using 2-way analysis of the

Table 1
The effect of isoproterenol (ISO), chronic salt loading (CSL) and CSL–ISO on body weight (g) and the weight of cardiac chambers

Treatment	Atrium/BW	RV/BW	LV/BW	BW
Control	0.17±0.03	0.59±0.07	2.40±0.15	253±6.9
Isoproterenol	0.22±0.03 [a]	0.72±0.07 [a]	3.27±0.32 [a]	249±7.5
Salt	0.14±0.01	0.59±0.09	2.25±0.11	294±12.1
Salt+Iso	0.20±0.04 [a]	0.76±0.08 [a]	3.24±0.11 [a]	256±8.6

The weight of cardiac chambers, atrium, right ventricle (RV) and left ventricle (LV) was normalized by body weight (mg/g) in all groups. Data are expressed as mean±s.d. $N=6$ for each group.
[a] $P<0.05$ compared to control group.

variance (ANOVA) with a post hoc testing by Tukey. Please note that statistical analysis shown in Fig. 1A and B was performed using the raw data and the transformation to percent change was used only to facilitate visualization of the results. Hemodynamic values (mean arterial pressure, cardiac output, cardiac output and stroke volume) in response to isoproterenol treatment were analyzed by 1-way ANOVA and Tukey's post hoc testing. Differences were considered statistically significant at a level of $P<0.05$.

3. Results

Body weight before and after isoproterenol or saline treatments was similar among all studied groups whereas normalized heart and left ventricle weights increased following isoproterenol treatment with or without chronic salt loading (Table 1 and Fig. 1A). Left ventricle/total body weight ratio, which was used as an index of hypertrophy, increased 36% in isoproterenol, 35% in chronic salt loading–isoproterenol treated groups (Table 1). The myocardial hypertrophy was accompanied by an elevation in cardiac angiotensin-converting enzyme activity (Fig. 1B). The increase in left ventricle/total body weight ratio observed in the isoproterenol treated groups was further confirmed by a 28% increase in left ventricle myocyte diameter (data not shown). Interestingly, considering the data either from control or salt loaded groups isolated or combined from all groups, there is a close positive correlation between left ventricle angiotensin-converting enzyme activity and left

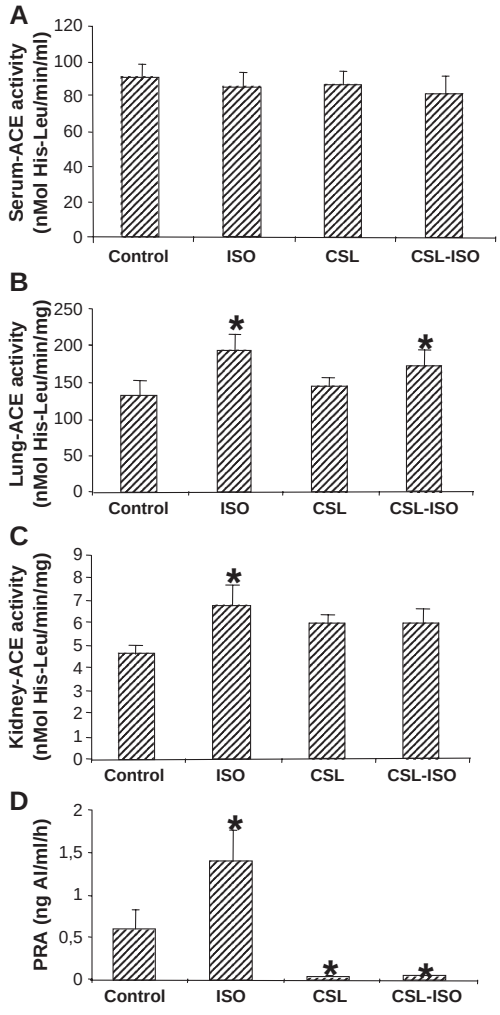

Fig. 2. The effect of isoproterenol (ISO), chronic salt loading (CSL) or chronic salt loading plus isoproterenol (CSL–ISO) treatments on serum, lung, and kidney angiotensin-converting enzyme activity and plasma renin angiotensin (PRA). Data are expressed as mean±s.d. $N=6$ for each group. *$P<0.05$ compared to control group.

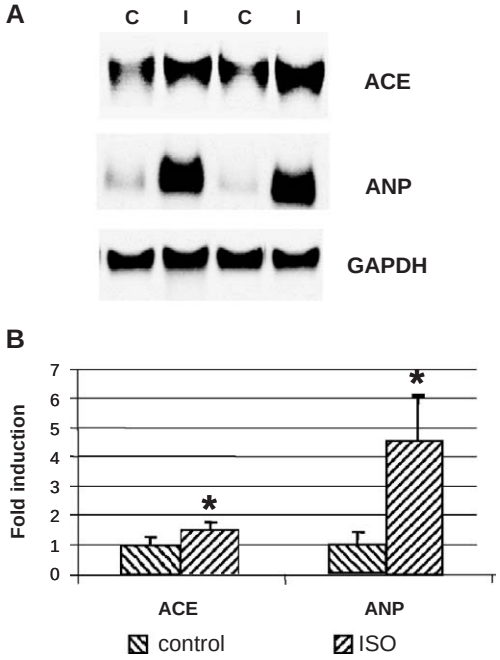

Fig. 3. Effect of isoproterenol (ISO) treatment on cardiac angiotensin-converting enzyme (ACE) and atrial natriuretic peptide (ANP) gene expressions. In A, the photomicrograph shows a representative Northern blot analyses. Control (C) and isoproterenol (I). The result of the densitometric analysis of the blots ($N=3$ for each group) are shown in B. Data signals, in arbitrary units, normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression are expressed as means±s.d. *$P<0.05$ compared to control group.

Table 2
The effect of isoproterenol (ISO), chronic salt loading (CSL) and CSL–ISO on systolic (SBP), diastolic (DBP), mean arterial pressure (MAP) (in mm Hg), and heart rate (HR) (bpm)

Treatment	SBP	DBP	MAP	HR
Control	133.0±13.6	98.3±16.1	109.9±15.1	334.3±25.3
Isoproterenol	131.0±13.7	96.8±11.0	107.3±14.8	315.3±12.7
Salt	137.8±15.9	95.5±15.9	109.6±16.4	323.8±11.6
Salt+Iso	128.2±4.7	97.2±8.8	106.5±4.3	341.0±32.4

Data are expressed as mean±s.d. $N=6$ for each group.

ventricle/total body weight ratio ($r^2=0.60$; $P<0.001$ for the combined data) (Fig. 1C). These data are consistent with an isoproterenol effect on the correlation regardless of the salt intake status of the animals. β-adrenoceptor stimulation via isoproterenol treatment resulted in no change in serum angiotensin-converting enzyme levels despite activation of lung angiotensin-converting enzyme activity (Fig. 2A and B). Renal angiotensin-converting enzyme levels increased only in animals not treated with salt (Fig. 2C). As expected, β-adrenoceptor stimulation resulted in activation of plasma renin activity, which was inhibited by salt treatment in both the control as well as in the isoproterenol treated group (Fig. 2D). Thus, β-adrenoceptor stimulation by isoproterenol treatment resulted in activation of local as well as systemic components of the renin–angiotensin system whereas superimposed salt loading plus isoproterenol lead to local activation of lung and cardiac angiotensin-converting enzyme. There was a significant induction of cardiac angiotensin-converting enzyme mRNA levels (1.5 times vs. control) and cardiac atrial natriuretic peptide (4.7 times vs. control), a molecular marker of hypertrophy (Fig. 3).

Mean blood pressure and heart rate remained unchanged following seven days of treatment in all groups (Table 2).

The hypertrophic response in this model can be attributable to direct effect of β-adrenoceptor stimulation on cellular growth and activation of renin–angiotensin system components, as previously suggested by others, and/or to indirect increases in the cardiac load via a hyperdynamic state. Since isoproterenol treatment was performed by single daily injections, the potential hemodynamic load was assessed in a pilot study, which showed a rise in cardiac output via increase in cardiac output lasting for about 2–4 h following the injection. All variables returned to basal levels by 6 h postinjection (data not shown). Then, we performed an additional study where the instrumented animals were evaluated for seven days (3 days vehicle and 4 days isoproterenol) and hemodynamic variables were determined throughout the first 2 h postinjection and the final 2 h prior to new injection next day. Upon β-adrenoceptor stimulation, mean blood pressure tended to decrease acutely (first period=first 2 h after isoproterenol injection) and remained virtually unchanged in the late period (last period=2 h before the next injection) compared to vehicle values (Fig. 4A). In contrast, cardiac output increased by approximately 56% in the first period and showed no difference from control in the last period (Fig. 4B). The increase in cardiac output in the first period was mainly due to significant elevations in heart rate compared to stroke volume that only showed tendency to increase (Fig. 4C and D).

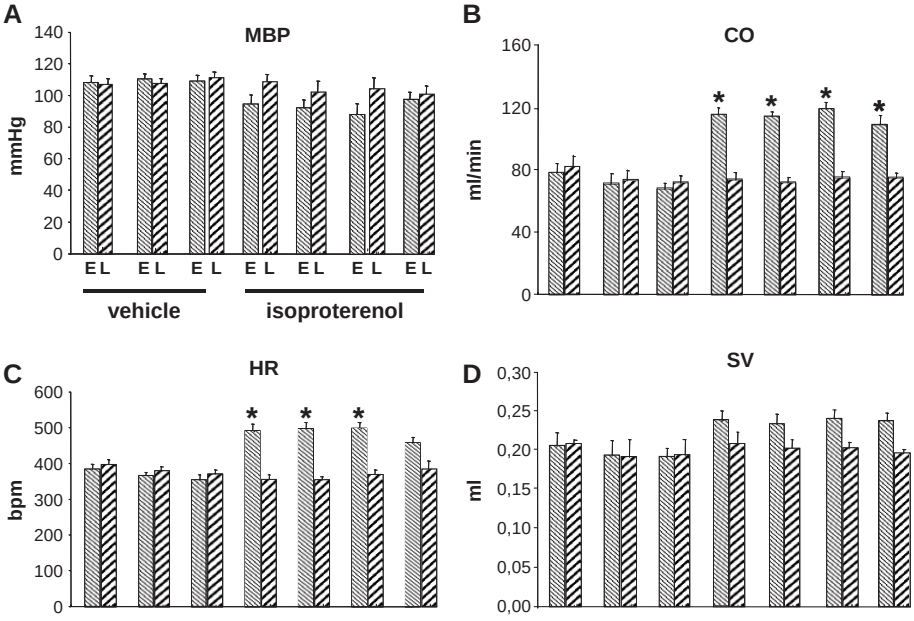

Fig. 4. Mean arterial blood pressure (MAP), cardiac output (CO), heart rate (HR), and stroke volume (SV) measured in control ($N=3$) versus isoproterenol (ISO) ($N=4$) treated groups. Recordings were performed both during the initial period (first 2 h after ISO injection, light bars) and final period (the day after, 2 h before the next injection, dark bars). Data are presented as mean±s.d. *$P<0.05$ compared to vehicle values.

4. Discussion

The main finding herein is that β-adrenoceptor stimulation by isoproterenol injection induces left ventricular hypertrophy associated with increased local angiotensin-converting enzyme activity/expression even when the systemic activation of renin is prevented by chronic salt loading the animals. The results also suggest that under the experimental conditions tested, isoproterenol treatment leads to daily transient hemodynamic overload, which may contribute to the cardiac hypertrophic response.

An array of stimuli determines the behavior of synthesis/secretion of the different components of the renin–angiotensin system. This complex interplay can be illustrated by a variety of stimuli including changes in renal sympathetic stimulation or blood flow and the sodium chloride delivered to the macula densa controlling renin secretion by the juxta-glomerular cells at the afferent arterioles of the renal glomeruli (Gomez et al., 1990; Vallotton, 1987). Renin secretion increases by β-adrenoceptor sympathetic stimulation, reduction of renal perfusion pressure or decreased NaCl delivery to the macula densa. The active and inactive forms of renin, once released, can trigger a cascade of reactions increasing plasma levels of angiotensins. These products can also reach distant sites including the heart and contribute to local generation of angiotensins. While there is little doubt regarding the existence of partial or complete local renin–angiotensin systems, its relative importance under different situations still remains unclear (Dzau, 1988a,b).

Previous results and the ones presented here have clearly demonstrated that isoproterenol treatment leads to cardiac hypertrophy, which is related to induction of local angiotensin-converting enzyme (Busatto et al., 1999; Grimm et al., 1998; Iwai et al., 1995). The positive correlation between the left ventricle hypertrophy index and angiotensin-converting enzyme expression/activity is consistent with a modulation of local angiotensin-converting enzyme activity by β-adrenoceptor stimulation. However, a close positive correlation of left ventricle angiotensin-converting enzyme activity and myocardial hypertrophy was demonstrated previously (Grimm et al., 1998) with a toxic dosage of isoproterenol (150 mg/kg body weight), which caused characteristic myocardial damage that subsequently resulted in mild heart failure. Similarly, Iwai et al. (1995) demonstrated, using a cardiac volume overload model by aortocaval shunt, a significant correlation between hypertrophy and the expression levels of angiotensin-converting enzyme mRNA in the rat left ventricle. These findings strongly suggest an association between angiotensin-converting enzyme activation and the development of cardiac hypertrophy and have lead to speculations regarding angiotensin-converting enzyme being a rate-limiting step in this process or at least having some effect on local generation of angiotensin II, which can cause cardiomyocyte hypertrophy and fibroblast proliferation (Baker and Aceto, 1990; Sadoshima et al., 1993). This cardiac hypertrophic response can be attributed at least in part to the enlargement of myocytes since their diameter increased in parallel with the left ventricle hypertrophy (data not shown).

Furthermore, we were able to demonstrate the association of cardiac hypertrophy and local activation of angiotensin-converting enzyme even when plasma renin activity was suppressed by chronic salt loading the animals. This maneuver leads to inhibition of the systemic renin–angiotensin system and prevented isoproterenol treatment to cause the expected induction of renin release. Despite that, isoproterenol produced similar left ventricle hypertrophy and enhancement of local angiotensin-converting enzyme activity in the heart and lung (Figs. 1B and 2B).

Other reports, using a similar approach, also suggested that the local renin–angiotensin system can be regulated independently from the systemic renin–angiotensin system and may contribute to the hypertrophic response (Nagano et al., 1992). Left ventricle hypertrophy, the rise of the plasma renin activity and angiotensin II concentrations in aorta and heart were all observed even after bilateral nephrectomy. These results reinforce our findings showing that even in the absence of systemic renin–angiotensin system activation, isoproterenol treatment induces local cardiac angiotensin-converting enzyme. In their study (Nagano et al., 1992), the use of angiotensin-converting enzyme inhibitor prevented the hypertrophic response while a similar reduction in blood pressure with hydralazine failed to produce comparable results pointing to a direct effect on local angiotensin-converting enzyme inhibition independent of hemodynamic influence. In contrast, other studies have failed to show that inhibiting components of the renin–angiotensin system can prevent the isoproterenol induced hypertrophic response (Golomb et al., 1994; Leenen et al., 2001). As indicated previously, even though we obtained similar results, we felt that the general health of the survival animals receiving a combination of angiotensin-converting enzyme inhibitor and isoproterenol was precarious that could jeopardize the interpretation of the results. Our results, however, add a new component to this debate indicating that daily injections of isoproterenol are associated with transient cardiac overloading secondary to elevations in heart rate and cardiac output (Fig. 4).

Pathological cardiac hypertrophy, induced by hemodynamic and/or activation of growth factors, is characterized by an increase in myocyte size and by the re-expression of fetal isoforms of contractile proteins and atrial natriuretic peptide (Childs et al., 1990; Izumo et al., 1988). Consistent with this, the isoproterenol treatment led to stimulation of atrial natriuretic peptide mRNA gene expression in the left ventricle (Fig. 3). Thus, the pattern of reprogramming of ventricular gene expression by isoproterenol appears to be similar to that observed in hemodynamic overload (Chassagne et al., 1993; Izumo et al., 1988). Thus, the present results add to previous evidence indicating that cardiac

hypertrophy ensues even when the systemic activation of renin–angiotensin system is prevented. Under this condition, the association of cardiac hypertrophy and local angiotensin-converting enzyme activation still remains but the relative role of the activation of angiotensin-converting enzyme for the development of the hypertrophic response remains to be determined.

A limitation of the present strategy to confirm the inhibition of the circulating renin–angiotensin system, in particular the release of kidney renin, is that plasma renin activity levels were determined following 24 h of the isoproterenol bolus injection and one may not exclude the possibility that a transient increase in renin may have occurred.

Taken together, the present data suggest that a) chronic β-adrenoceptor stimulation induces myocardial hypertrophy associated to local activation of angiotensin-converting enzyme even when the systemic activation of renin is prevented by chronic salt loading the animals. b) Isoproterenol treatment leads to daily transient cardiac hemodynamic overload which may contribute to the cardiac hypertrophic response. Therefore, the modulation of cardiac angiotensin-converting enzyme by isoproterenol can be both due to direct β-adrenoceptor stimulation in cardiac cells and also due to the secondary effects on the hemodynamics.

Acknowledgements

Research supported by grants from FAPESP 01/00009-0, CNPq 471219/01-0 and Fundação E.J. Zerbini. E.M. Oliveira was the recipient of a CNPq doctoral fellowship (No. 140621/93-8).

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