Isoproterenol-induced cardiac hypertrophy: role of circulatory versus cardiac renin-angiotensin system

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Isoproterenol-induced cardiac hypertrophy: role of circulatory versus cardiac renin-angiotensin system. Am J Physiol Heart Circ Physiol 281: H2410–H2416, 2001.—To assess the possible contribution of the circulatory and cardiac renin-angiotensin system (RAS) to the cardiac hypertrophy induced by a β-agonist, the present study evaluated the effects of isoproterenol, alone or combined with an angiotensin I-converting enzyme inhibitor or AT1 receptor blocker, on plasma and LV renin activity, ANG I, and ANG II, as well as left ventricular (LV) and right ventricular (RV) weight. Male Wistar rats received isoproterenol by osmotic minipump subcutaneously and quinapril or losartan once daily by gavage. Plasma and LV ANGs were measured by radioimmunoassay after separation by HPLC. Isoproterenol alone decreased blood pressure, more markedly when combined with losartan or quinapril. Isoproterenol significantly increased LV and RV weight and total collagen. Neither losartan nor quinapril inhibited the increase in LV or RV weight. Losartan prevented the increase in RV collagen but enhanced the increase in LV collagen. Isoproterenol increased plasma renin, ANG I, and ANG II three- to fourfold. Isoproterenol combined with losartan or quinapril, caused marked further increases except for a significant decrease in plasma ANG II with quinapril. Isoproterenol alone did not increase LV ANG II and, combined with losartan or quinapril, actually decreased LV ANG II. These results indicate that isoproterenol-induced cardiac hypertrophy is associated with clear increases in plasma ANG II, but not in LV ANG II. Both losartan and quinapril lower LV ANG II below control levels, but do not prevent the isoproterenol-induced cardiac hypertrophy. These findings do not support a role for the circulatory or cardiac RAS in the cardiac trophic responses to β-receptor stimulation.

β-agonist; angiotensin II; heart; angiotensin I-converting enzyme inhibitor; AT1-receptor blocker

It is well recognized that chronic increases in systolic or diastolic wall stress lead to cardiac hypertrophy. Both in vitro and in vivo studies suggest that trophic responses to stretch (increases in diastolic wall stress) involve ANG II (11). Cardiac trophic responses can also be induced by sympathetic stimulation. Whereas in vitro only α-receptor stimulation increases protein synthesis in cardiac myocytes (15), in vivo both α- and β-receptor stimulation induces cardiac hypertrophy (19). There is some evidence that trophic responses to cardiac sympathetic stimulation in vivo depend on the (cardiac) RAS. Chronic infusion of norepinephrine, even in subpressor doses, causes cardiac hypertrophy in rats and dogs (16, 19). In rats, this trophic response was partially blocked by either α1- or β-receptor blockade and fully by combined blockade (19). However, this trophic response was also blocked by the AT1-receptor blocker losartan (1), suggestive for the involvement of either the circulatory or cardiac RAS. Chronic administration of the β-agonist isoproterenol also increases cardiac weight (7). Nagano et al. (9) reported that isoproterenol for 7 days increased both left ventricular (LV) weight and LV ANG II, and that both of these increases were prevented by the ANG I-converting enzyme (ACE) inhibitor trandolapril but not by bilateral nephrectomy. These studies would suggest that cardiac sympathetic activity is one of the regulators of the cardiac RAS, and the latter may mediate the cardiac trophic responses to sympathetic stimulation. In contrast, the AT1-receptor blocker losartan did not prevent the cardiac hypertrophy induced by isoproterenol, and the authors of this study (4) concluded that ANG II does not mediate the induction of cardiac hypertrophy by isoproterenol but may contribute to the maintenance of the hypertrophy.

The present study was designed to address these opposite conclusions and had two objectives. The first objective was to assess effects of isoproterenol for 2 and 7 days on activity of the circulatory versus cardiac RAS. Both renin activity, as well as ANG I and II, were measured, whereas Nagano et al. (9) only measured ANG II. Both 2 and 7 days were chosen to assess changes early as well as later on. The second objective was to compare the effects of treatment with an ACE inhibitor versus AT1-receptor blocker on the changes in the RAS as well as on the cardiac hypertrophy induced by isoproterenol. This study was designed to assess whether effects of an ACE inhibitor (used by Nagano et al., Ref. 9) are not shared by an AT1-receptor blocker (used by Golomb et al., Ref. 4).
METHODS

Animals

Male Wistar rats weighing 200–250 g were obtained from Charles River Breeding Laboratories (Montreal, Canada). They were given food and water ad libitum and were kept on a 12:12-h light-dark cycle. The rats were allowed to acclimate for at least 4 days before being randomized into various groups for the experimental protocols, as outlined below. The rats were housed one per cage during the protocols. All surgical procedures were performed under halothane-oxygen inhalation anesthesia. All animals were treated according to the procedures recommended by the Canadian Council on Animal Care.

Experimental Protocols

Two-day study. On day 1 in the morning, an osmotic minipump (model 2001, Alzet) was surgically implanted subcutaneously into each rat. In the isoproterenol group (n = 10), isoproterenol (Sigma; St. Louis, MO) was delivered at a rate of 4.2 mg·kg⁻¹·day⁻¹. In the control group (n = 8), the pumps were filled with 0.9% saline. On day 2 in the afternoon, a polyethylene-50 (PE-50) catheter was placed in the left carotid artery. The next morning, resting blood pressure (BP) and heart rate (HR) were recorded, and blood was collected. The rats were then killed by injection of 2 M KCl (1 ml/kg) into the left carotid artery. The isoproterenol group was killed in the afternoons, a polyethylene-50 (PE-50) catheter was placed in the left carotid artery. The next morning, resting blood pressure (BP) and heart rate (HR) were recorded, and blood was collected. The rats were then killed by injection of 2 M KCl (1 ml/kg) through the carotid catheter and the hearts were removed.

Seven-day study. Rats were randomized into five groups. The first group received isoproterenol via minipump as described above. The second group was given 40 mg·kg⁻¹·day⁻¹ losartan once daily by gavage (1 ml·100 g body wt⁻¹·day⁻¹ of 4 mg/ml losartan in 0.9% saline). A third group received both isoproterenol and losartan. The last dose of losartan was given 12 h before the BP and HR assessment. A fourth group received isoproterenol as described above and 200 mg/l quinapril in the drinking water. The administration of losartan and quinapril started 2 days before the isoproterenol. Rats in the fifth, untreated group (n = 8) received 0.9% saline via minipump. Rats in the first and fifth groups were given 0.9% saline by gavage (1 ml·100 g body wt⁻¹·day⁻¹). Treatment lasted 6–8 days. The carotid artery was cannulated, BP and HR were recorded, and blood and tissues were collected as for the two-day study.

In an additional 7-day study, rats were randomized into four groups: isoproterenol alone, losartan alone, both isoproterenol and losartan, and control groups. Treatments were the same as above. After 7 days of treatment, under pentobarbital anesthesia, the hearts were removed for assessment of collagen content. The latter was measured by assessment of total hydroxyproline, as previously described in detail (6, 10).

Mortality Rate

In the 2-day study, the mortality rate was 0% for both the untreated and isoproterenol groups. In the 7-day study, the mortality rates were as follows: 0% for the untreated, control group and the losartan alone group, 25% in the isoproterenol alone group, 56% for the combined losartan and isoproterenol group, and 30% for the combined quinapril and isoproterenol group. Mortality appeared as “acute” and was not preceded by progressive weight loss or obvious illness of the animal. Number of rats in the tables refer to surviving rats.

Hemodynamic Measurements

BP measurements were recorded using the PE-50 catheter placed in the left carotid artery and a computerized system (model MP100, Bipac Systems; Gloeta, CA) in conscious, resting, unrestrained rats. HR was calculated from the BP curves.

Collection of Blood and Tissue Samples for Renin and Angiotensin Assays

Blood was collected from conscious, unrestrained rats, with care being taken to not unnecessarily disturb the rats in any way. For plasma ANG measurements, 2 ml of blood were collected into a prechilled microcentrifuge tube containing 1,10-phenanthroline and EDTA-Na₂ at final concentrations of 0.8 mM and 1.2 mM in blood, respectively. Subsequently, for plasma renin activity (PRA), 1.0 ml of blood was collected into a prechilled microtube containing 200 μl of 2.6 mM EDTA-Na₂. The blood was centrifuged for 5 min at 8,000 rpm and 4°C, using an Eppendorf model 5415 C microcentrifuge. The plasma for PRA was immediately frozen at −80°C until the actual assay. The plasma for ANG measurements was immediately applied to preconditioned Waters Sep-Pak C18 cartridges. The cartridges were washed with 10 ml of 0.1% trifluoroacetic acid (TFA), and then the ANG peptides were eluted with 2 ml methanol-water-TFA at a ratio of 50:19:9:0.1 vol/vol/vol. The eluates were evaporated to dryness using a Savant SpeedVac and then reconstituted with mobile phase before HPLC measurement.

To assess possible activation of the circulatory RAS by the 3 ml of blood sampling, in a separate group of rats (n = 7, body wt 275 ± 5 g), PRA was measured separately in the first 2 ml as well as the next 1 ml. BP and HR were measured before and after removal of 3 ml of blood, following the same procedures as above. Mean arterial pressure and HR did not change (100 ± 2 vs. 99 ± 2 mmHg and 392 ± 11 vs. 391 ± 12 beats/min). PRA showed a minor increase from 5.8 ± 1.5 to 8.4 ± 1.4 ng·ml⁻¹·h⁻¹ (P = 0.13 by one-tail paired t-test) in the first versus the second sample.

After blood sampling, the hearts were quickly removed and rinsed in ice-cold 0.9% saline. The ventricles were dissected on ice and blotted dry. A small piece was cut from the LV for tissue renin activity measurements, weighed, and frozen on dry ice. The remainder of the LV and the right ventricle (RV) were blotted dry and weighed, and the LV was immediately placed in boiling 1 M acetic acid for 15 min. The tissues were then homogenized using a Brinkmann Polytron, setting 8, for 25 s. After a 30-min centrifugation at 3,000 rpm and 4°C using a Sorvall RTC6000B centrifuge, the LV supernatants were applied to preconditioned Sep-Pak C18 cartridges, as described for the plasma samples.

Measurement of ANG I and II

Plasma and LV ANGs were measured as previously described in detail (12) by using a radioimmunoassay (RIA), after separation by HPLC, with some minor changes. Briefly, the ANGs were separated on a CSC (Montreal, Canada) Select 100A/ODS2 column, 15 × 0.46 cm, 5-μm particle size. Gradient elution was used, starting with 100:40% vol/vol methanol in 10 mM acetate buffer at time 0, changing to 100:80% vol/vol methanol in 10 mM acetate buffer at 38 min (slope = 4), flow rate 1.0 ml/min (models 501 and 510 HPLC pumps, Waters; controlled by Maxima 820 workstation software, Millipore). Eluate fractions were collected at 1-min intervals (with a Spectrum Spectra/Chrom CF-1 Fraction Collector) and evaporated to dryness in the SpeedVac. The
The homogenates were centrifuged for 5 min at 14,000 rpm and 4°C in an Eppendorf microfuge. Duplicate 200-μl aliquots of the supernatant were incubated for 1 h at 37°C with 250 μl of plasma from nephrectomized rats. RIA for ANG I was then performed using 200 μl of the incubation mixture, as described for PRA.

Data Analysis

Results are expressed as means ± SE. For the 2-day study, an unpaired t-test was used to compare the two rat groups. For the 7-day study, data were evaluated by analysis of variance, and Duncan's multiple-range test was used to locate differences between the experimental groups using SAS 6.02 software (Cary). Differences were considered statistically significant when P < 0.05.

RESULTS

Blood Pressure and Heart Rate

Treatment with isoproterenol significantly reduced systolic BP (106 ± 2 vs. 128 ± 2 mmHg) after 2 days but no longer after 7 days (Table 1). Losartan alone caused a modest fall in systolic BP. Combined isoproterenol and losartan or isoproterenol and quinapril caused a marked further reduction in systolic BP to 97 ± 4 and 91 ± 5 mmHg, respectively.

Isoproterenol significantly reduced diastolic BP after both 2 days (79 ± 2 vs. 94 ± 2 mmHg) and 7 days (82 ± 2 vs. 93 ± 3 mmHg). Losartan alone caused a similar fall in diastolic BP. A further reduction was noted with combined losartan and isoproterenol (61 ± 3 mmHg) and combined quinapril and isoproterenol (62 ± 5 mmHg).

Isoproterenol increased HR after both 2 and 7 days of treatment. Losartan alone did not change HR. Isoproterenol combined with losartan or quinapril caused similar increases in HR compared with isoproterenol alone.

Cardiac Weight and Collagen

LV and RV weight were both increased significantly after 2 days and after 7 days of treatment with isoproterenol (Table 2, Fig. 2). Losartan alone did not change LV or RV weight. Combined with losartan, isoproterenol still increased LV and RV weight and these increases were not significantly different from the effect with losartan or quinapril on BP and heart rate

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Results represent means ± SE; n = no. of surviving rats. BP, blood pressure. *P < 0.05 vs. control; †P < 0.05 vs. isoproterenol; ‡P < 0.05 vs. losartan.
isoproterenol alone or combined with losartan or quinapril on body weight and absolute LV weight and RV weight

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Results represent means ± SE; n = no. of surviving rats. Relative left ventricular (LV) and right ventricular (RV) weights (mg/100 g body wt) are reported in Fig. 2. *P < 0.05 vs. control; †P < 0.05 vs. isoproterenol; ‡P < 0.05 vs. losartan.

of isoproterenol alone (Fig. 2). Similarly, isoproterenol combined with quinapril increased LV and RV weight to the same extent as isoproterenol alone. Absolute increases in LV and RV weight were somewhat less in the combined treatment groups compared with isoproterenol alone (Table 2), e.g., an increase in LV weight of 147 mg with isoproterenol alone versus 115 mg with isoproterenol + losartan. However, the combined groups showed less gain in body weight (Fig. 2) and the relative increases in LV and RV weight (i.e., mg/100 g body wt) were similar in the groups treated with isoproterenol alone or combined with losartan or quinapril (Fig. 2).

Isoproterenol alone caused modest but significant increases in LV and RV total collagen with little change in the concentration (Table 3). Losartan alone caused minor, nonsignificant changes in the concentration and total amounts in the LV and RV. Isoproterenol combined with losartan caused more clear increases in the LV for both concentration and total collagen compared with isoproterenol or losartan alone and increases by twofold versus the control group (Table 3). In contrast, in the RV on combined treatment, the concentration decreased somewhat, resulting in an unchanged total amount of collagen compared with the control or losartan alone groups (Table 3).

**Plasma and Cardiac ANGs**

Three- to fourfold increases in plasma ANG I (149 ± 13 vs. 36 ± 7 pg/ml) and plasma ANG II (31 ± 4 vs. 7 ± 1 pg/ml) were noted after 2 days treatment with isoproterenol. Increases in plasma ANG I and plasma ANG II persisted after 7 days treatment. Losartan alone caused a marked increase in plasma ANG I and ANG II versus the untreated group. Isoproterenol combined with losartan caused significantly larger increases in both plasma ANG I and ANG II than either isoproterenol or losartan alone. In the isoproterenol + quinapril group, plasma ANG I showed a marked increase, whereas plasma ANG II was significantly decreased, compared with the group on isoproterenol alone (Fig. 3).

In the LV, ANG I was significantly increased but ANG II was not affected by isoproterenol for 2 days. After 7 days of treatment, neither LV ANG I nor LV ANG II was changed compared with the untreated group (Fig. 4). LV ANG I and LV ANG II were not affected by losartan alone. In contrast, combined isoproterenol and losartan did not change LV ANG I but caused a significant decrease in LV ANG II compared with the untreated or the losartan group. Similarly, combined isoproterenol and quinapril tended to increase LV ANG I, but significantly lowered LV ANG II, compared with the untreated or the isoproterenol groups (Fig. 4).

**Plasma and Cardiac Renin Activity**

Plasma renin activity was increased after both 2 and 7 days of isoproterenol compared with the untreated group. Losartan alone caused a clear increase in PRA. A further

Fig. 2. Effects of isoproterenol (Iso) alone or combined with losartan (Los) or quinapril (Qui) on left ventricular (LV; A) and right ventricular (RV; B) weight (mg/100 g body wt) of normotensive rats. Absolute LV and RV weights are reported in Table 2. Values represent means ± SE (for n of rats, see Table 2). *P < 0.05 vs. control; †P < 0.05 vs. Los.
marked increase in PRA was seen with isoproterenol combined with losartan or quinapril (Fig. 3).

In the LV, renin activity showed minor increases after 2 and 7 days of isoproterenol. Losartan alone also caused a minor increase in LV renin activity. In contrast, LV renin activity was markedly increased by isoproterenol combined with losartan or quinapril (Fig. 4).

The present study provides three major new findings: 1) isoproterenol-induced cardiac hypertrophy is associated with clear and persistent activation of the circulatory RAS, but only minor and short-lived activation of the cardiac RAS; 2) neither losartan nor quinapril prevents isoproterenol-induced cardiac hypertrophy despite lowering LV ANG II; and 3) both blockers markedly activate the circulatory RAS (except for lowering of plasma ANG II by quinapril) and increase LV renin activity, but neither changes LV ANG I, and both significantly lower LV ANG II.

The general cardiovascular effects of the β-agonist isoproterenol are well known. In rats, the potent arterial vasodilation results in decreases in diastolic as well as systolic BP, despite the positive inotropic and chronotropic effects on the heart. Renal renin release and resulting increases in PRA and plasma ANG I and II likely occur as a result of both renal β-receptor stimulation and the lower BP. The increased activity of the circulatory RAS appears to blunt the hypotensive effects of isoproterenol, because combined with losartan or quinapril, the BP further decreased significantly. A similar finding was also reported by Golomb.
et al. (4) with losartan, but not by Nagano et al. (9) with trandolapril. Combined treatment also led to a higher mortality rate, particularly with losartan [also noted by Golomb et al. (4)]. The effectiveness of the two blockers in the doses used is also apparent from their effects on the circulatory RAS: marked increases in PRA and plasma ANG I for both, further increase in plasma ANG II with losartan, and blockade of the increases in plasma ANG II by quinapril. These findings are consistent with interruption of the negative feedback by ANG II on renal renin release with either AT1-receptor blockade or ACE inhibition. In contrast, in the study by Nagano et al. (9), the same rate of infusion of isoproterenol increased plasma ANG II to a similar extent, but the ACE inhibitor trandolapril did not prevent the increase in plasma ANG II and did not enhance the hypertensive effect of isoproterenol. In the absence of measurements of plasma renin or ANG I, there was therefore no evidence for systemic ACE inhibition.

Cardiac hypertrophy induced by isoproterenol is a well-established phenomenon. Nagano et al. (9) reported that isoproterenol increased both plasma and LV ANG II and that trandolapril prevented isoproterenol-induced increases in LV ANG II and LV weight, but not the increase in plasma ANG II. They concluded that “cardiac tissue ANG II regulates myocyte growth in isoproterenol-induced LV hypertrophy.” In the present study, we confirm that isoproterenol increases LV and RV weight with clear activation of the circulatory RAS, but we did not find increases in LV ANG II at either 2 or 7 days. Moreover, both losartan and quinapril lower LV ANG II well below control levels, but do not prevent increases in LV or RV weight. From these findings, we conclude that isoproterenol does not cause a persistent activation of the cardiac RAS and that neither the circulatory nor the cardiac RAS contribute to isoproterenol-induced cardiac hypertrophy. It is possible that differences in handling of the LV tissue may explain the observed differences in LV ANG II. Nagano et al. (9) performed perfusion in vivo with heparinized saline, perhaps increasing LV ANG II only in isoproterenol-treated rats with higher circulatory renin and stored the LV tissue at −70°C until extraction. In the present study, no such perfusion was done, and the LV was immediately placed in boiling acetic acid followed by extraction of ANGs with Sep-Pak C18 cartridges. However, such differences cannot explain the different responses of LV weight. In the study by Golomb et al. (4), a large range of doses of isoproterenol was used and the AT1-receptor blocker losartan did not prevent the isoproterenol-induced increases in LV weight. In the present study, using the same infusion rate of isoproterenol as Nagano et al. (9), neither losartan nor quinapril prevented the increases in LV or RV weight despite lowering LV ANG II to below control levels. It is difficult to reconcile these opposite findings on LV weight in the study by Golomb et al. (4) and the present study versus the study by Nagano et al. (9). Golomb et al. (4) suggested that isoproterenol-induced cardiac hypertrophy may depend on a direct hypertrophic effect that may be enhanced by endogenous ANG II. The relative role of the latter may vary, depending on factors such as rat strains and diet.

In parallel with the increases in LV and RV weight, total collagen increased in both ventricles. The absence of a significant increase in the concentration of collagen was also reported by Golomb et al. (4) and suggests that increase in total collagen represents collagen laid down as part of the cardiac hypertrophy and does not represent fibrosis secondary to myocardial necrosis as induced by high doses of isoproterenol (5, 17). Concurrent treatment with losartan prevented the increase in RV total collagen, but unexpectedly potentiated the increase in LV total collagen by isoproterenol. Because the increases in LV and RV weight were not affected by losartan, these findings are consistent with a differential effect of the AT1-receptor blocker on effects of isoproterenol on myocyte versus collagen regulation, as well as on LV versus RV collagen. A discussion of the possible mechanisms leading to an increase in LV total collagen with combined isoproterenol-losartan treatment is beyond the scope of the present study. One possibility worth mentioning is stimulation of AT2-receptors on fibroblasts (14). The significant increase in LV collagen concentration by the combined treatment may reflect fibrosis predisposing to ventricular arrhythmias, possibly explaining the high mortality noted with combined treatment in the present study as well as the study by Golomb et al. (4). The surviving rats likely represent animals the least responsive for one or more of the drugs’ actions.

As reviewed recently (2, 11), the circulatory and cardiac RAS can show distinctly different patterns of changes. In the present study, as expected, isoproterenol and losartan alone increased plasma renin, ANG I, and ANG II, and the combination caused marked further increases in the three components in a parallel fashion. Quinapril caused similar increases in plasma renin and ANG I, but lowered plasma ANG II consistent with effective ACE inhibition. In contrast, isoproterenol caused only small, short-lived increases in LV renin activity and LV ANG I, but not with persistent treatment and without a parallel increase in LV ANG II. Moreover, losartan alone did not change any of the three cardiac RAS components. Losartan or quinapril combined with isoproterenol clearly increased LV renin activity, but not LV ANG I and actually lowered LV ANG II. The lowering of plasma and LV ANG II by quinapril is consistent with effective ACE inhibition, including ACE in cardiac tissue. However, ACE inhibition cannot explain the unchanged LV ANG I with a higher LV renin. The effects of losartan alone or combined with isoproterenol are also indicative of specific regulation of the cardiac RAS at different levels. Thus losartan or isoproterenol alone clearly increased the circulatory RAS, but not the LV RAS, consistent with previous studies (2, 13) indicating that passive uptake of circulatory renin, ANG I, or ANG II into cardiac tissue is not a significant determinant of LV renin, ANG I, or ANG II. However, it is possible that at the very high plasma renin associated with combined iso-
Isoproterenol and losartan or quinapril passive uptake of renin does lead to increases in LV renin. Alternatively, other factors, such as low LV ANG II, may enhance active uptake of renin or local production. However, these clear increases in LV renin activity with combined treatments were not associated with a parallel increase in LV ANG I and even a decrease in LV ANG II, despite the marked increases in plasma ANG I and II. Thus specific regulation appears to occur for formation of both ANG I and ANG II in the LV, independent of circulating levels. The latter is consistent with studies by e.g., Van Kats et al. (18), indicating that most cardiac tissue ANG I and II is locally produced and not derived from circulation. The dissociation of LV renin activity and LV ANG I suggests that in the heart in vivo substrate concentrations are so low that these are the rate-limiting step (2). In vitro, LV renin activity is measured by incubation with substrate-enriched plasma. Finally, LV ANG I and II did not change in parallel during combined isoproterenol-losartan treatment. Cardiac ANG II levels may be controlled by binding of ANG II to AT1 receptors and/or intracellular generation (2). During the development of isoproterenol-induced cardiac hypertrophy, operative AT1-receptors appear to become more important, because losartan only combined with isoproterenol lowered LV ANG II.

In conclusion, isoproterenol-induced cardiac hypertrophy is associated with clear increases in plasma ANG II, but not in LV ANG II. Both losartan and quinapril lower LV ANG II below control levels but do not prevent the isoproterenol-induced cardiac hypertrophy, and losartan actually enhances the increase in LV collagen. These findings indicate that neither circulatory nor cardiac RAS plays a major role in the cardiac trophic responses to β-receptor stimulation.

The authors express gratitude to Dr. Fred W. Keeley (Hospital for Sick Children, Toronto, Ontario, Canada) for the expert measurement of LV and RV collagen by the hydroxyproline assay. This work was supported by Operating Grant B-4087 from the Heart & Stroke Foundation of Ontario, Canada. Frans H. H. Leenen is a Career Investigator of the Heart & Stroke Foundation of Ontario, Canada. Losartan was a generous gift from Merek Frosst, Rahway, NJ, and quinapril was from Parke-Davis, Ann Arbor, Mich., USA. The antibody against ANG I was kindly provided by Dr. Fred W. Keeley (Hospital for Sick Children, Toronto, Ontario, Canada) for the expert measurement. Cardiac ANG II levels may be controlled by plasma. Finally, LV ANG I and II did not change in vivo substrate concentrations are so low that these are the rate-limiting step (2). In vitro, LV renin activity is measured by incubation with substrate-enriched plasma. Finally, LV ANG I and II did not change in parallel during combined isoproterenol-losartan treatment. Cardiac ANG II levels may be controlled by binding of ANG II to AT1 receptors and/or intracellular generation (2). During the development of isoproterenol-induced cardiac hypertrophy, operative AT1-receptors appear to become more important, because losartan only combined with isoproterenol lowered LV ANG II.

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