EFFECTS OF ANTIHYPERTENSIVE TREATMENT ON CARDIAC IGF-1 DURING PREVENTION OF VENTRICULAR HYPERTROPHY IN THE RAT

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Summary

There is some evidence that cardiac rather than circulating insulin-like growth factor-1 (IGF-1) levels contribute to the development of renovascular hypertensive left ventricular hypertrophy (LVH), remaining unknown the effects of antihypertensive drugs on IGF-1 levels. We have assessed here the preventive effects of enalapril, losartan, propanolol and α-methyldopa on left ventricle (LV) and circulating IGF-1 levels in a rat model of hypertension and LVH (Goldblatt, GB). Our results show that relative LV mass and the LV content of IGF-1 were significantly lower with all antihypertensive drugs in GB rats ($p<0.001$). Serum concentrations of IGF-1 were lower in GB rats treated with enalapril, α-methyldopa and propanolol ($p<0.01$), but not in those treated with losartan. These results support the hypothesis that local rather than seric IGF-1 contributes to the development of left ventricular hypertrophy induced by pressure overload in the rat.

Key Words: insulin-like growth factor-1, heart hypertrophy, hypertension, enalapril, losartan, α-methyldopa, propanolol

Both mechanical overload and neuroendocrine activation of the renin-angiotensin and adrenergic systems are recognized as major contributors to the development of left ventricular hypertrophy (LVH) (1,2). Cardiac growth factors which regulate the growth and differentiation of cardiac myocytes may also play a role in this process. Specifically, Insulin-like Growth Factor-1 (IGF-1), which is a mediator of the cardiovascular effects of growth hormone (3), may be significant in the initiation and development of LVH. In humans, increased myocardial mass and high blood IGF-1 levels accompany hypertension with LVH and exposure to excess of growth hormone (4,5); the

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relationship with IGF-1 content in heart muscle remains unknown. In animal models of LVH, increases in LV IGF-1 and the expression of IGF-1 mRNA are early events (6-9). We have recently observed that the LV content of IGF-1 and its mRNA were increased at 1 and 4 weeks of hypertension and hypertrophy, and that both returned to control values after 9 weeks during the development of hypertensive LVH (Goldblatt model) (10). These changes were unrelated to the seric concentration of IGF-1 in the blood. These results showed that local rather than circulating IGF-1 levels contributed to the development of renovascular hypertensive LVH (10).

In cultured rat cardiac myocytes, IGF-1 increases cell size by stimulating protein accumulation and the expression of early and late response cardiac genes (11-13). Changes at the level of IGF-1 binding proteins (IGF-BPs) have also been reported: there is an altered distribution of the molecular forms of serum IGF-BPs in patients with essential hypertension, and this is independent of IGF-1 concentrations (14, 15).

Regulation of the cardiac IGF-1 system is not well understood. Growth hormone is a major factor regulating cardiac expression of IGF-1 (16), although angiotensin II (Ang II) also stimulates IGF-1 expression in the heart (17). Contrary to its effect on vascular and cardiac IGF-1 expression, however, Ang II depresses circulating IGF-1 through a pressure-independent effect which also induces anorexia (18). After 6 months of therapy with angiotensin converting enzyme (ACE) inhibitors, plasma IGF-1 concentrations were decreased in hypertensive patients with LVH. (19, 20). To test the hypothesis that IGF-1 promotes the initial phase of LVH in spontaneously hypertensive rats, it has recently been shown that treatment with enalapril (an ACE inhibitor) or nifedipine (a calcium channel blocker) limit the development of LVH and also blunt induction of ventricular IGF-1 mRNA levels (21). Little is known, however, on the effects of enalapril and other common antihypertensive drugs (losartan, an Ang II type 1 receptor antagonist; propanolol, a non selective β-adrenergic antagonist; and α-methyldopa, a central level sympatholytic) on cardiac and circulating IGF-1 levels in hypertensive LVH. To address this issue and better characterize the role of IGF-1 on LVH, we have studied the effects of these commonly used antihypertensive agents, administered on a preventive basis, on cardiac and circulating levels of IGF-1 in a rat model of renovascular hypertension and LVH.

Methods

Experimental protocol. We followed the recommendations of the Guide for the Care and Use of Laboratory Animals (publication NIH N° 85-23, review 1985; approved by the Research Commission from our universities). Experimental hypertension (HT) and LVH were induced after 4 weeks using the Goldblatt (GB) model in the rat (2 kidneys -1 clipped) as previously described (22, 23). Briefly, male Sprague-Dawley rats (80-120 g) were anesthetized with ether. A small abdominal incision was made and a silver clip (0.2 mm thickness) was placed across the left renal artery to generate a uniform degree of constriction, leaving the right renal artery intact. The animals were kept under controlled conditions of light and dark, and were fed with a standard rat chow having unrestricted access to water.

Antihypertensive treatments. Pharmacological treatments (4 week duration) were started two days after surgery to prevent the appearance of hypertension and LVH (24, 25). GB rats were divided into 5 experimental groups. GB animals (GB group, n = 6) were controls and did not receive any drug. The remaining animals were divided in 4 experimental groups: GB-ENA (n = 6) received 10

mg/Kg/d enalapril (an ACE inhibitor from Laboratorio Saval, Santiago, Chile) (23). GB-LOS (n = 6) received 30 mg/kg per day of losartan (an angiotensin II type 1 receptor blocker from Merck Sharpe and Dohme, USA) (23). GB-MD (n = 6) were treated with 150 mg/Kg/day of α-methyldopa (a central level sympatholytic from Laboratorio Chile, Santiago, Chile). GB-PRO group (n = 6) received 90 mg/kg per day of propanolol (a non-selective β-adrenergic antagonist from Laboratorio Chile, Santiago, Chile). All drugs were given in the drinking water. Water intake was checked daily, and drug concentrations were adjusted to achieve the above daily doses. Sham group (n = 5) were sham-operated rats. All rats were housed in individual cages and were fed a standard rat chow and tap water *ad libitum*.

One day before killing, systolic blood pressure (SBP) was measured by the tail cuff method (22). Rats were weighed, anesthetized with ether and decapitated. The hearts were quickly extracted, atria removed, and the aorta, pulmonary arteries and the left ventricles were carefully separated and kept at -80°C. Hypertrophy was assessed from the relative left ventricular mass (mg left ventricle per 100 g body weight, RLVM) and the ratio of the weights of the two ventricles (22, 24). For the measurement of serum IGF-1 levels, 2 ml of blood were incubated for 30 min. at 37°C and then for 30 min. at 4°C. Blood was then centrifuged (1,000 g for 30 min. at 4°C), supernatants were removed, transferred to Eppendorf tubes and kept at –80°C until processing.

Radiolabeling of IGF-1. This growth factor was labeled with [^{125}I] using the iodogen method (26). Briefly, 10 μg iodogen (Sigma Chem Co., MO, USA) dissolved in chloroform were added to a glass tube. Thereafter 5 μg of recombinant human IGF-1 (donated by Dr. C. George-Nascimento, Chiron Corp., Emeryville, CA, USA), 20 μl PBS and 0.5 mCi Na^{125}I (Chilean Commission of Nuclear Energy, Santiago, Chile) were added. The mixture was incubated for 10 min. before addition of 100 μl tyrosine-saturated glycine (0.1M). After 5 min., 230 μl PBS-1% BSA (RIA grade, Sigma Chem. Co., MO, USA) was added and the reaction mixture was transferred to a G-25 Sephadex column previously equilibrated with PBS-1% BSA. Fractions (600 μl) were collected and kept at 4°C. The specific radioactivity was 10^7 cpm/μg. The stability of IGF-1 throughout this process was assessed by measurement of binding to its receptor (27).

IGF-1 radioimmunoassay. IGF-1 was separated from its plasma binding proteins using the Crawford's acid-ethanol method (21, 28). Forty microliters of ethanol/HCl 2N (87.5:12.5% v/v) were mixed with 100 μl of serum. After incubation for 30 min. at 37°C, samples were centrifuged at 4,000 g for 30 min. From each tube, 100 μl of supernatant was neutralized with 40 μl of Tris (0.855 M). After 2 h at 4°C, the samples were centrifuged at 4,000 g for 30 min. at 4°C; supernatants were then transferred to Eppendorf tubes and stored at –80°C. For measurement of IGF-1 in the LV, approximately 300 mg of the LV were cut into small pieces and transferred to a plastic tube with 1 ml of PBS containing triton X-100 (2% v/v). Tissues were homogenized with an Ultraturrax at 4°C and centrifuged at 4,000 g for 30 min. at 4°C, and the supernatants were kept at -80°C. IGF-1 in serum and left ventricles was measured as described by Crawford (28). In the first stage, 300 μl of IGF-1 (between 0.01-100 ng) or of the pretreated sample were mixed with 10 μl rabbit polyclonal antibody anti-IGF-1 (final dilution 1:18,000; donated by Dr. L Underwood, National Program of Hormone Distribution, NIH, USA). Tubes were incubated at 4°C for 24 h before addition of 10 μl [^{125}I]IGF-1 (30,000 cpm). After further incubation for 16 h at 4°C, 25 μl of normal rabbit serum (dilution 1:50) and 25 μl of goat anti-rabbit IgG (Sigma Chem. Co., MO, USA, dilution 1:5) were added to precipitate immune complexes. 600 μl polyethyleneglycol (6%

in 0.015% NaCl; Merck, Chile) were added to each tube. After centrifugation at 10,000 g for 15 min. at 4°C; the supernatants were discarded and the pellets washed 2 times with PBS-Triton X-100 (0.02% v/v) and the radioactivity associated with the immune complexes was determined by γ-counting. The sensitivity of the IGF-1 radioimmunoassay was 0.01 ng per tube; and intra- and inter-assay variation coefficients were 8% and 10%, respectively.

Statistical analysis. Results are shown as means ± SEM. Mean values were compared with one-way analysis of variance and subsequent intergroups comparisons with Student-Newman-Keuls test. Linear correlation (Pearson) was also used.

Results

Systolic blood pressure and left ventricular hypertrophy. As shown in Table I, four weeks after surgery, systolic blood pressure in GB group was increased to 194 ± 4 mm Hg and was significantly lower with all preventive drug treatments ($F=16.8$, $p<0.001$), being even lower in those GB rats treated with losartan. Although all antihypertensive drugs reduced body weight with regards to GB group, this reduction was not statistically significant. Left ventricular weight and cardiac weight were significantly reduced with all treatments ($F=15.2$, $p<0.001$ and $F=10.9$, $p<0.001$, respectively). The relative left ventricular mass (RLVM), the normalized LVH index most commonly used in rats, and the ratio of the weights of the two ventricles (data not shown) were significantly reduced with all the preventive antihypertensive treatments in comparison with the untreated GB rats ($F= 37.5$, $p<0.001$).

TABLE I

Effect of antihypertensive therapy on morphometric parameters and systolic blood pressure in a hypertensive left ventricular hypertrophy model.

	Sham	GB	GB-ENA	GB-LOS	GB-MD	GB-PRO
N° animals	5	6	6	6	6	6
BW (g)	330 ± 6	296 ± 13[c]	258 ± 10[a]	292 ± 9[c]	267 ± 22[a]	255 ± 13[a]
SBP (mm Hg)	137 ± 4[b,d]	194 ± 4[d]	128 ± 11[b,d]	86 ± 5[a,b]	153 ± 12[b,d]	149 ± 9[b,d]
HM (mg)	1136 ± 29	1300 ± 40	800 ± 50[a,b]	830 ± 30[a,b]	980 ± 80[b]	900 ± 70[b]
LVM (mg)	820 ± 29	1004 ± 23	559 ± 29[a,b]	546 ± 22[a,b]	748 ± 67[b]	668 ± 57[b]
RLVM	248 ± 6[b,d]	341 ± 12[d]	217 ± 6[b,d]	187 ± 6[a,b]	279 ± 4[b,d]	285 ± 14[b,d]

Mean ± SEM. BW: body weight, SBP: systolic blood pressure, HM: heart mass, LVM: left ventricular mass, RLVM: relative left ventricular mass (mg 100/BW). Symbols: a: $p<0.05$ vs. Sham; b: $p<0.05$ vs. GB; c: $p<0.05$ vs. GB-PRO; d: $p>0.05$ vs. GB-LOS after ANOVA, respectively.

Cardiac content of IGF-1. As shown in Fig. 1, left ventricular IGF-1 content was 36 ± 4 ng in the untreated GB rats and was significantly lower in sham operated rats (Sham group) as well as in GB rats treated preventively with antihypertensive drugs for 4 weeks ($F= 7.4$, $p<0.001$). LV content of IGF-1 and SBP were only correlated in the α-methyldopa treated group ($r= 0.98$, $p<0.01$).

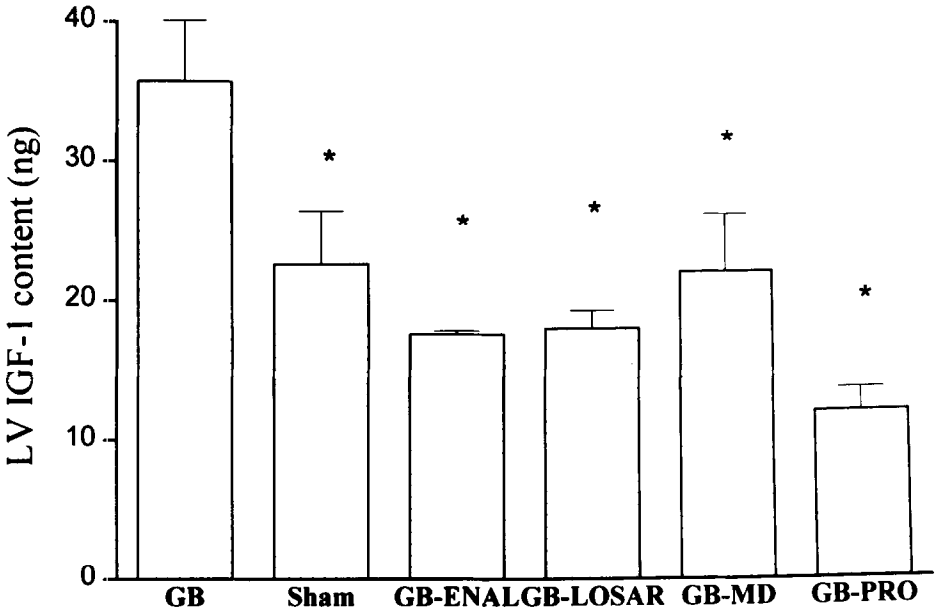

Fig. 1.

Effect of antihypertensive therapy on cardiac IGF-1 content in a hypertensive left ventricular hypertrophy model. IGF-1 content in left ventricle was determined by RIA as described in Materials & Methods. Results shown as mean ± SEM (n = 6). Abbreviations: GB: 4 week Goldblatt rats; Sham: sham-operated rats receiving *ad libitum* tap water; GB-ENA: GB rats treated with enalapril (50 mg/L); GB-LOS: GB rats treated with losartan (30 mg/kg/day); GB-MD: GB rats treated with α-methyldopa (150 mg/kg/day); GB-PRO: GB rats treated with propanolol (90 mg/kg/ day). All drugs were administered in the drinking water. Symbols: *$p < 0.05$ vs. GB group (after ANOVA).

Seric concentrations of IGF-1. Fig. 2 depicts that serum levels of IGF-1 were 345 ± 32 μg/L in untreated GB rats and 350 ± 18 μg/L in sham animals. These levels were significantly reduced in GB rats treated preventively with enalapril, α-methyldopa and propanolol, but not in those treated with losartan ($F= 14.8$, $p<0.001$). IGF-1 levels were not correlated with SBP neither with relative left ventricular mass.

Discussion

There is a growing interest to establish the role of different intrinsic cardiac systems (renin-angiotensin, adrenergic and growth factors) in normal and abnormal growth and differentiation of myocardial cells (29-31). It has been hypothesized that mechanical stimuli may be a primary event leading to the production of different hypertrophic agonists. Although *in vitro* studies in stretched cardiac myocytes have demonstrated that Ang II and endothelin-1 are produced by these cells (32), there is no evidence that the same is true for IGF–I. Increased IGF–I gene expression is, however, associated with the phenotypic adaptation and hypertrophy found in rabbit skeletal muscle subjected to stretch (33).

The contribution to the IGF-1 system (constituted by the IGFs, their receptors and binding proteins) to the LVH has not been completely studied. There are clinical and experimental evidences suggesting that IGF-1 induces cardiac hypertrophy *in vivo* and *in vitro*. However, some

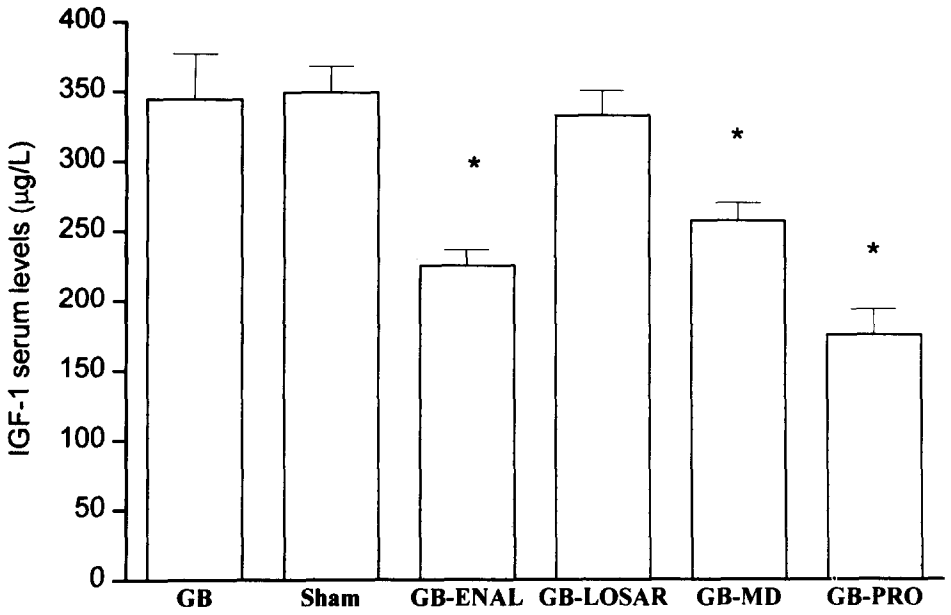

Fig. 2.

Effect of antihypertensive therapy on circulating IGF-1 levels in a hypertensive left ventricular hypertrophy model. Serum IGF-1 levels after 4 weeks of drug treatment were assayed by RIA as described in Material & Methods. Results shown as mean ± SEM (n = 5-6). Symbols: *p < 0.05 vs. GB group (after ANOVA). Abbreviations as in Fig. 1.

preliminary data in the mouse lacking this growth factor (induced by the gene knock-out procedure) indicate that IGF-1 deficit does not prevent the development of LVH induced by pressure overload suggesting that IGF-1 might need other factors to induce LVH (34). In different experimental models of hypertensive left ventricular hypertrophy (spontaneously hypertensive rats, aortic constriction above renal arteries or following DOCA administration) increased expression of cardiac IGF-1 mRNA has been observed without modification of IGF-1 receptor mRNA (7). Based on these findings, Donohue *et al.* have suggested an independence of cardiac IGF-1 expression from activation of the renin-angiotensin system (7). However, in rat vascular smooth muscle cells, IGF-1 mRNA is increased 7 and 21 days after banding of the abdominal aorta, a high renin model of HT (35). In the absence of HT, administration of IGF-1 induces LVH in normal rats as well as in rats with experimental myocardial infarction (36).

In the present work, at 4 weeks, no differences in serum IGF-1 but increased LV content of this growth factor in the GB group compared to Sham group were observed, which is similar to a previous study (10). Although during the first 4 weeks after surgery, our experimental model of hypertension and LVH is characterized by a sustained activation of the renin-angiotensin system, enhanced circulating levels of other peptides and hormones (prostaglandins, kallikreins and atrial natriuretic peptide) have also been detected (37). We show that prevention of hypertension and LVH in this model of renovascular HT using commonly antihypertensive drugs (enalapril, α-methyldopa, propanolol, and losartan) was accompanied by a reduction in LV IGF-1 content more than changes in IGF-1 circulating levels. Our results agree with a recent study in spontaneously hypertensive rats in which treatment with enalapril or nifedipine (a calcium channel blocker) limited development of LVH and blunted induction of ventricular IGF-1 mRNA levels (21). We did not determined here IGF-1 levels in the right ventricle, this normotensive and non-hypertrophied

cardiac tissue could provide further information in future studies to clarify the importance of hemodynamic versus circulating factors in the regulation of the IGF-1 expression and/or production in heart.

The presence and functionality of IGF-1 receptor has also been fairly documented in cardiac tissue as well as in cardiac myocytes (11, 12). In the GB model we have previously observed a progressive decrease of the cardiac IGF-1 receptor after 9 weeks of hypertension (38). The number of high affinity IGF-1 receptors in sham rats was 4.1 fmol/mg protein, and it was reduced by 60% in GB rats. Preventive treatment for 4 weeks with enalapril, propanolol and α-methyldopa to GB rats increased the number of high affinity IGF-1 receptors (unpublished data).

The present study does not explore the eventual changes in IGF-1 binding proteins (IGF-BPs) which are important in IGF-1 bioactivity. The regulation and effects of the IGF-BPs are complex. They serve a transport function, regulate the metabolic clearance of IGF-1, and may serve as a tissue specific reservoir of this growth factor (14). The IGF-BPs also modulate the interaction of IGF-1 with its receptor, and may have direct effects on cellular function (39). The specific role of IGF-BPs in modulating IGF-1 actions on the cardiac tissue has been partially explored (11, 15, 40). Both the regulation of plasma IGF-1 levels and its serum binding proteins are altered in patients with essential hypertension (15).

In GB rats treated with the ACE inhibitor enalapril, circulating IGF-1 levels changed in the same direction as those observed in humans with hypertensive LVH in whom circulating IGF-1 levels were reduced with an ACE inhibitor along with LVH regression (4). Interestingly, we did not notice the same effect on IGF-1 circulating levels upon inhibiting the AT1 receptor with losartan even in the latter group in which a greater antihypertensive effect was present. However, reduction of IGF-1 circulating levels using the central sympathetic blocker α-methyldopa and the β-blocker propanolol was also observed. Our data show that LV mass appears related to systolic arterial pressure and not with seric IGF-1 levels. The effect of losartan on IGF-1 in the cardiovascular system has been only evaluated in cultured vascular smooth muscle cells. In these cells, Ang II regulates transcription of the IGF-1 gene and moreover, IGF-1 is required for DNA synthesis induced by Ang II (41), suggesting a potential role of IGF-1 in mediating growth responses of vascular smooth muscle cells induced *in vivo* by activation of the renin angiotensin system.

Some studies have shown that Ang II plasma levels increases dose dependently after Ang II receptor antagonist administration (42, 43) while in one study the plasma clearance of Ang II was significantly greater in rats injected with losartan compared to those injected saline (44). On the other hand, Mizuno et al. also showed that losartan reduced the left ventricular Ang I and Ang II contents (43). Mizuno's results support the view that cardiac Ang II rather than circulating Ang II plays an important role in the pathophysiology of left ventricular hypertrophy. These changes on plasma Ang levels should not have effects on left ventricular IGF-1 because AT1 receptor is blocked with losartan.

Aside from the present study, there are no experimental or clinical studies evaluating the relationship between treatment with an AT1 receptor antagonist and IGF-1. In a recent study, Ang II administered with osmotic minipumps to normotensive rats, reduced plasma levels of IGF-1 and its binding protein-2 after 1 and 2 weeks (18). In the mentioned study a marked weight loss was observed, probably due to Ang II-induced anorexia. Both responses were blunted with losartan but not with hydralazine, demonstrating that these effects are due to Ang II and independent of blood pressure (18). Although antihypertensive drugs reduced body weight, this effect was not statistically

significant compared to untreated GB group. However, the use a pair-fed GB control group could better assess the effects of antihypertensives on food intake more precisely (18).

In relation to the stimulation of myocyte cardiac cells by the adrenergic system, there is an increase in the capacity for growth promotion of a conditioned culture medium (42). *In vitro* stimulation of α_1-receptors induces hypertrophy of cardiac myocytes (43) but the relationship between the blockade of the adrenergic system and IGF-1 has not been previously explored. In cultured rat aortic smooth muscle cells, insulin and IGF-1 regulate the expression of α1-receptors and may enhance the effects of catecholamines (44). Our data suggest that central inhibition of the adrenergic system (with α-methyldopa) as well as inhibition of the β-adrenergic receptor (propanolol) prevent LVH development by modulating the cardiac IGF-1 system.

In conclusion in this experimental model of HT and LVH, arterial blood pressure control, using antihypertensive treatment blocking different pathogenic mechanisms, prevented the development of LVH associated with a lower IGF-1 content in the LV. All antihypertensive treatments used in this study, excepting the AT1 receptor antagonist losartan, also reduced serum IGF-1 levels. Mechanisms controlling this growth factor as well as the therapeutic significance in the development, prevention and regression of different forms of LVH should be explored in future studies.

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