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Selective Increase in Cardiac IGF-1 in a Rat Model of Ventricular Hypertrophy

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There is evidence that insulin-like growth factor-1 (IGF-1) plays a role in the development of left ventricular hypertrophy, but it is uncertain whether cardiac IGF-1 changes before or after hypertension is established, and whether circulating IGF-1 are involved in cardiac hypertrophy. We have investigated changes in circulating and left ventricular IGF-1 and in the expression of the IGF-1 gene in the left ventricles of rats during the development of hypertensive left ventricular hypertrophy (Goldblatt model; 2 kidney-1 clamped). Our results show that the left ventricular contents of IGF-1 and its mRNA were increased at one and four weeks of hypertension and hypertrophy, and that both returned to control values after nine weeks. These changes were unrelated to the seric concentration of IGF-1 in the blood. These results show that local rather than circulating IGF-1 levels contributed to the development of renovascular hypertensive left ventricular hypertrophy. © 1998 Academic Press

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Left ventricular hypertrophy (LVH) is a consequence of the stimulation of the myocardium by a variety of mechanical and neurohumoral stimuli (1,2). It is primarily characterized by increases in both the mass and volume of the cardiomyocytes and the expression of specific genes with consequent changes in the amounts of certain proteins associated with the contractile mechanism (3). There are two main components in the regulation of the development of LVH, one of neuroendocrine origin involving the activation of the adrenergic and renin-angiotensin-aldosterone systems, the other of local origin involving peptides acting by autocrine or paracrine mechanisms (4). Among the locally-acting peptides implicated in LVH are insulin like growth factor-1 (IGF-1) (5–7), fibroblast growth factor (8,9) and transforming growth factor-β (10,11).

IGF-1 is a ubiquitous and highly conserved peptide which regulates proliferation, growth and differentiation in many types of cells and plays a crucial role in normal development (12–14). Most circulating IGF-1 is associated with high-affinity binding proteins (IGF-BPs), forming two complexes of 150 and 40-50 kDa (15). Both the regulation of plasma IGF-1 levels and its serum binding proteins are altered in patients with essential hypertension (16).

There is clear evidence for a role of IGF-1 in the initiation and development of LVH both in animal models and man (17–22). IGF-1 also acts directly on both adult and neonatal cardiomyocytes in culture to augment the synthesis and accumulation of contractile and associated proteins (7, 23, 24). Increased myocardial mass and raised blood IGF-1 concentrations are associated with the hypertension in experimental LVH and after exposure to growth hormone in clinical LVH (6,17,25,26). In experimental animals, it is uncertain whether cardiac IGF-1 changes before or after hypertension is established (6,27), and whether circulating IGF-1 concentrations are involved during the genesis and development of cardiac hypertrophy.

To resolve these issues and better characterize the role of IGF-1 in LVH, we now report studies on the expression of the IGF-1 gene in the heart and on IGF-1 itself in the left ventricles and plasma of rats during development of the Goldblatt model of LVH.

MATERIALS AND METHODS

The investigation conforms with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No 85-23, revised 1985).

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Animals. Sprague–Dawley rats obtained from our animal breeding facility (University of Chile, Santiago) were kept under controlled conditions of light and dark, and were allowed access to food and water *ad libitum*. They were kept in metabolic cages, and both body weight and caudal systolic arterial blood pressure were monitored periodically over the nine-week period.

Experimental model of cardiac hypertrophy. The Goldblatt model of ventricular hypertrophy (G; two kidneys with one clamped) was used as previously reported (28, 29). In brief, male animals, weighing 100–120 g, were anaesthetized with diethyl ether and a small incision was made on the left side of the abdomen to expose the kidney. A silver clamp (internal diameter 0.2 mm) was placed across the left renal artery to generate a uniform degree of constriction. Hypertension (blood pressure 150 mm Hg or greater) developed during the second week. Control animals were sham-operated (S). Hypertrophy was assessed from the relative left ventricular mass (mg left ventricle per 100 g body weight) and the ratio of the weights of the two ventricles (LV/RV).

Radioimmunoassay of IGF-1. IGF-1, labeled with ^{125}I (Comisión Chilena de Energía Nuclear) by the iodogen method (30) was used for assay of IGF-1. Human recombinant IGF-1 was a kind gift of Dr. C. George-Nascimento (Chiron Corp., CA, USA). The assay (31,32) involved competition between labeled and unlabeled IGF for binding to a specific polyclonal antibody (UB2-495) kindly provided by Drs. L. Underwood and J.J. Van Wyk (Hormone Distribution Program, NIDDK, Bethesda, MD). Before being killed, animals were anaesthetized with ether and weighed. Left ventricles were excised, washed in phosphate-buffered saline solution (PBS) and weighed. Unless stated otherwise, all subsequent procedures were performed at 4°C. Extracts for IGF-1 radioimmunoassay were prepared as follows: small pieces and representative samples of left ventricular tissue (300 mg) were homogenized (Ultraturrax, Janke and Kunkel AG, Germany) in 1 ml of ice-cold PBS (pH 7.4) containing 2% Triton X-100. Homogenates were centrifuged at 1000 × *g* for 30 min at 4°C ; the resulting supernatants were kept on ice.

Before assay, separation of IGF-1 from its binding proteins was performed by incubation of sera or supernatant samples (200 μl) with 800 μl of ethanol/2M HCl (7:1) for 30 min at room temperature. After centrifugation (2000 × *g*, 4°C, 30 min), portions of supernatants (500 μl) were neutralized with 200 μl of 0.855 M tris. Briefly, the assay involved mixing 300 μl of treated samples (or of standard solutions) with 100 μl of [^{125}I]IGF (3×10^4 cpm) and 100 μl of antibody (1:500), followed by incubation for 16 h at 4°C. The contents of each incubation were then mixed with 5 μl of goat anti-rabbit IgG antibody (Miles Lab Inc., Elkhart, IN, USA) and 5 μl of normal rabbit serum (diluted 1:10) and kept for 15 min at 4°C, before addition of 600 μl of polyethylene glycol (6%$^{w}/_{v}$ in NaCl, 0,15 g/l) and further incubation for 30 min at the same temperature. After tubes had been centrifuged and their contents washed in PBS containing 0.02% ($^{w}/_{v}$) Triton X-100, radioactivity in the pellets was measured by γ-counting. The recovery of purified IGF-1 was 80% and all samples (derived from at least three animals) were assayed in duplicate. The sensitivity of the IGF-1 assay was 0.01 ng/tube, inter- and intra-assay variation coefficients were 10% and 8% respectively and the recovery of IGF-1 was 85%.

Isolation of RNA. The method was as described by Chomczinsky and Sacchi (33) with minor modifications. The purity and quantity of the RNA was assessed from the ratio of the absorbancies at 260 and 280 nm.

Northern blot hybridization of IGF-1 mRNA. Ventricular RNA (15 μg per lane) was subjected to electrophoresis in a denaturing gel (1,25% agarose containing 6.6% $^{w}/_{v}$ formaldehyde) at 85 V for 5 h. The separated RNAs were transferred to nitrocellulose membranes (0.45 μm) by blotting for 16 h at room temperature. Membranes were then dried for 2 h at 80°C before being pre-incubated for 4 h at 65°C in hybridization solution (10× Denhardt's solution containing 2× SSC, 2.5% dextran sulfate, 0.1% SDS, 0.1 % $Na_4P_2O_7$, 2 mM EDTA and 100 μg/ml salmon DNA). They were then re-incubated in hybridization solution containing 10^7 dpm labeled probe) for 16 h at 65°C. Rat IGF-1 cDNA was kindly provided by Dr. D. LeRoith (NIDDK, NIH, Bethesda) and labeled using a random-primer labelling system (GIBCO-BRL, Gaithersburg, MD). The membrane was then washed twice in 2× SSC containing 0.1% SDS at 65°C for 30 min and once with 1× SSC–0.1% SDS for 30 min at 65°C. The membrane was then subjected to autoradiography at –80°C for 7 days. The sizes of mRNAs corresponding to IGF-1 were obtained by comparison with known RNA standards. Bands were quantified by densitometry and analysed using NIH Image software (NIH, Bethesda, MD). Ethidium bromide (0.5 μg/ml) was used as a stain for quantifying 18S RNA.

Statistical analysis. Results are means ± SEM. Mean differences were compared by ANOVA; p values less than 0.05 were regarded as significant.

RESULTS

Hypertension and Left Ventricular Hypertrophy

Figure 1A shows a significant and sustained increase in systolic blood pressure throughout the nine week period. One week after surgery, the mean systolic pressures were 149 and 125 mm Hg in Goldblatt and sham groups respectively ($p<0.001$); after nine weeks, these values were 200 and 138 mm Hg ($p<0.001$). Figure 1 (Panels B and C) illustrates the development of left ventricular hypertrophy after pressure overload. Both markers used to assess the progress of this condition—relative left ventricular mass (RLVM) and the ratio of the weights of the two ventricles (LV/RV)—also increased during the whole period, being about 62% ($p<0.001$) and 40% ($p<0.02$) higher than those observed for the control group after nine weeks, respectively.

Gene Expression of IGF-1 in the Left Ventricle

Northern blot analysis of tissues taken at weeks 1, 4 and 9 revealed three bands (0.9-1.2, 1.7 and 7.5 Kb) corresponding to IGF-1 mRNA in the left ventricles of rats from both groups. As shown in Fig. 2, left ventricles from hypertensive animals (1 and 4 weeks) had approximately 3-5 fold more IGF-1 mRNA than did those from controls. At nine weeks, ventricles from both groups had a decreased content of the specific IGF-1 mRNA.

Seric Concentrations and Left Ventricular Content of IGF-1

The seric concentrations of IGF-1 in control and experimental animals were approximately 230 and 350 μg/L respectively and did not change significantly between the groups or with time during the nine week period (Fig. 3A). In contrast, the left ventricular content of IGF-1 increased by approximately 75 and 55% respectively in the hypertrophied left ventricles of the experimental groups one and four weeks after surgery

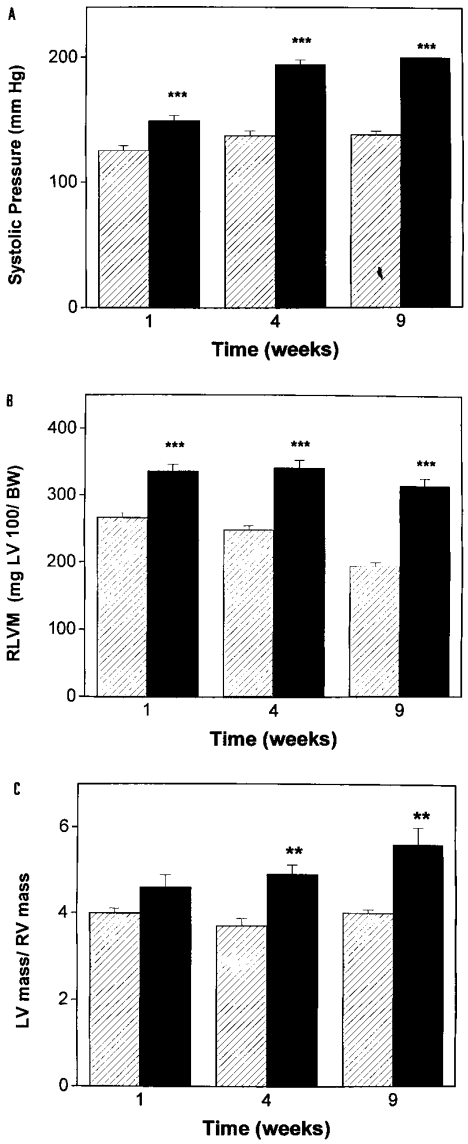

FIG. 1. Development of hypertension and ventricular hypertrophy in the Goldblatt model. Animals were treated as described under Materials and Methods. Systolic blood pressure (Panel A), relative left ventricular mass, RLVM (Panel B) and ratio left ventricle (LV) mass and right ventricle (RV) mass. Goldblatt (solid bar); sham (hatched bar). Values are means ± SEM (n = 5–8 for each group), **p<0.02 and ***p<0,001 vs sham.

(p<0.05); after nine weeks it was not different from that of the control group (Fig. 3B).

DISCUSSION

The primary source of seric IGF-1 is the liver after stimulation by growth hormone (34). IGF-1 is, however, also produced in cultures of fetal cells from several tissues including the heart (9, 12). Experimental and clinical evidence suggests that IGF-1 acts directly on the heart (17); increased IGF-1 concentration has been found in patients with essential hypertension and left ventricular hypertrophy together with an increase in the synthesis of type III collagen in hypertensive patients (35). This suggests a possible causal relationship between IGF-1 and these conditions and the interstitial fibrosis associated with hypertensive left ventricular hypertrophy. This is supported by the observation that administration of IGF-1 to normal rats causes cardiac hypertrophy (18).

Despite there being no change in circulating IGF-1 concentration in our rat hypertension model, there was a pronounced increase in the ventricular content of IGF-1 and its mRNA between the first and fourth weeks after surgery. This strongly suggests that increased local concentrations of IGF-1 could result from enhanced gene expression. Locally produced IGF-1 could act in an autocrine-paracrine fashion in heart hypertrophy and ventricular remodeling. We and others have documented the presence of functional IGF-1 receptors in rat cardiac myocytes (7,36). IGF-1 is synthesized by cardiomyocytes in the developing rat heart and in spontaneously hypertensive rat pups (5). The expression of IGF-1 mRNA is primarily localized in the myocytes. An increase in left ventricular IGF-1 mRNA and IGF-1 protein occurs in pressure-overload cardiac hypertrophy in various models of high, moderate and low renin hypertension, again suggesting that IGF-1 may be an important common mediator of an adaptive hypertrophic response (6;37). The heterogeneity of IGF-1 mRNA in the myocardium may be because of alternative splicing of the primary transcript in response to specific stimuli (38). This heterogeneity is associated with distinct receptors and binding proteins (activators and inhibitors);

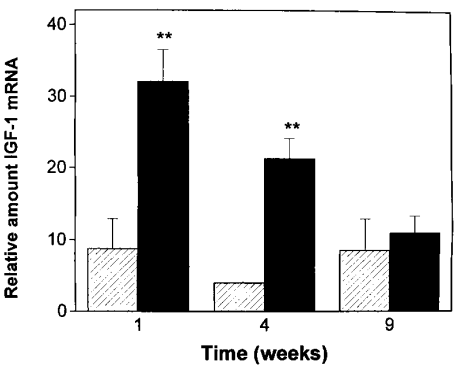

FIG. 2. Relative expression of IGF-I mRNA in the left ventricle during the development of ventricular hypertrophy induced by pressure overload. Northern blot analyses for IGF-1 mRNAs in left ventricles from Goldblatt (solid bars) and Sham (hatched bars) rats were performed as described under Materials and Methods. Autoradiograph of a representative experiment (n=3) in which the nitrocellulose membrane was incubated with ^{32}P-cDNA complementary to rat IGF-1. The IGF-1 mRNA corresponding (7.5 Kb) were normalized with 18S RNA, after bands were quantified by densitometry. *p<0.05 vs control.

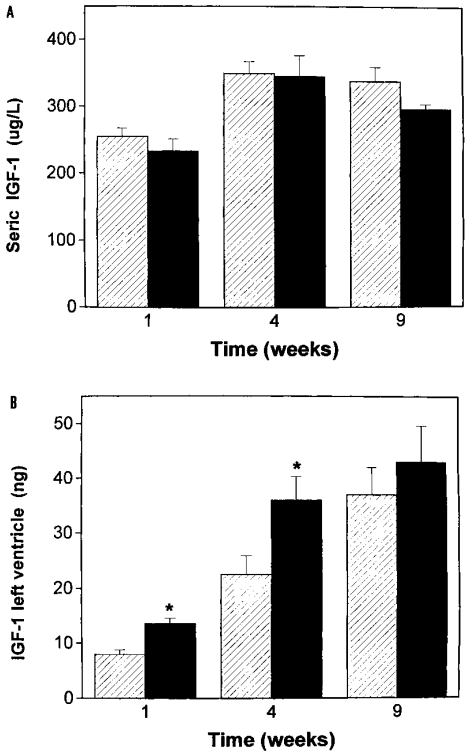

FIG. 3. Seric concentration and ventricular content of IGF-1 during the development of ventricular hypertrophy induced by pressure overload. Seric concentrations (Panel A) and the left ventricular contents of IGF-1 (Panel B) were determined by radioimmunoassay as described under Materials and Methods. Goldblatt (solid bar); sham (hatched bar). Values are means ± SEM (n = 5–8 for each group), *$p<0.05$ vs sham.

these may explain the pleiotropic actions of IGF-1 seen in various tissues and cells (14).

Our results confirm earlier findings that IGF-1 is a constituent of ventricular muscle in normal rats and that there are IGF-1 mRNAs in this tissue (5,6). Our results suggest that the left ventricle is a producer of IGF-1, whether stimulated by hormones or not. It is likely, however, that this IGF-1 has a physiological and pathological role because the tissue also contains high-affinity receptors for IGF-1, coupled through the phosphoinositide system (7, 36, 39).

It is most likely that the changes in ventricular IGF-1 probably result from changes in its local regulation, because the circulating levels of the growth factor remained comparatively unchanged during the experiment. The left ventricular content of the growth factor paralleled the level of its mRNA, either because IGF-1 may be not secreted by the tissue to the circulation or because there is regulation at the transcriptional level. The accumulation noted after one and four weeks may, however, reflect the pathological state of the tissue, with retention of IGF-1. This retention could, however, be responsible for control of the specific mRNA synthesis through a feedback mechanism during the genesis and establishment of hypertrophic process (1 and 4 weeks).

Left ventricular muscle from rats contains both high and low affinity IGF-1 receptors (39). In this same experimental rat model of renovascular hypertensive left ventricular hypertrophy, the number of high affinity IGF-1 receptors in left ventricular muscle is lower in the hypertrophic group than in the control group. This difference is apparent from week 4 onwards and is maximal at week 9; in this last period high affinity receptors for IGF-1 were undetectable. The retention of IGF-1 in heart muscle may thus down-regulate high affinity receptors.

It remains to be shown whether changes in IGF-1 correlate with modifications in the IGF-BPs. The regulation of the activity of the IGF-BPs is complex (15), affecting the interaction of IGF-1 with its receptor, and possibly having direct effects on cellular function (40).

The simultaneous nature of the changes in left ventricular size and IGF-1 content lend support to the suggestion that the hypertrophic response involves endogenous IGF-1, possibly as a result of a mechanical stimulus as has been proposed by Komuro and Yazaki (41) or as a result of neurohumoral modifications.

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