Induction of Myocardial Insulin-Like Growth Factor-I Gene Expression in Left Ventricular Hypertrophy

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Background Left ventricular hypertrophy is a generalized adaptation to increased afterload, but the growth factors mediating this response have not been identified. To explore whether the hypertrophic response was associated with changes in local insulin-like growth factor-I (IGF-I) gene regulation, we examined the induction of the cardiac IGF-I gene in three models of systolic hypertension and resultant hypertrophy.

Methods and Results The model systems were suprarenal aortic constriction, uninephrectomized spontaneously hypertensive rats (SHR), and uninephrectomized, deoxycorticosterone-treated, saline-fed rats (DOCA salt). Systolic blood pressure reached hypertensive levels at 3 to 4 weeks in all three systems. A differential increase in ventricular weight to body weight (hypertrophy) occurred at 3 weeks in the SHR and aortic constriction models and at 4 weeks in the DOCA salt model. Ventricular IGF-I mRNA was detected by solution hybridization/RNase protection assay. IGF-I mRNA levels increased in all three systems coincident with the onset of hypertension and the development of ventricular hypertrophy. Maximum induction was 10-fold over control at 5 weeks in the aortic constriction model, 8-fold at 3 weeks in the SHR, and 6-fold at 6 weeks in the DOCA salt model. IGF-I mRNA levels returned to control values by the end of the experimental period despite continued hypertension and hypertrophy in all three systems. In contrast, ventricular c-myc mRNA content increased twofold to threefold at 1 week and returned to control levels by 2 weeks. Ventricular IGF-I receptor mRNA levels were unchanged over the time course studied. The increased ventricular IGF-I mRNA content was reflected in an increased ventricular IGF-I protein content, as determined both by radioimmunoassay and immunofluorescence histochemistry.

Conclusions We conclude that (1) hypertension induces significant increases in cardiac IGF-I mRNA and protein that occur coordinately with its onset and early in the development of hypertrophy, (2) IGF-I mRNA levels normalize as the hypertrophic response is established, (3) in comparison to IGF-I, both c-myc and IGF-I receptor genes are differentially controlled in experimental hypertension. These findings suggest that IGF-I may participate in initiating ventricular hypertrophy in response to altered loading conditions. The consistency of these findings in models of high-, moderate-, and low-renin hypertension suggests that they occur independently of the systemic renin-angiotensin endocrine axis. (Circulation. 1994;89:799-809.)

Key Words • hypertension • pressure • myosin • peptides

Left ventricular hypertrophy is a generalized adaptive response to altered loading conditions. Previous studies of the hypertrophic response to supravalvular aortic constriction have demonstrated transient expression of proto-oncogene products thought to be involved in regulation of cardiac cell growth and differentiation as well as shifts in the major expressed isoforms of the contractile proteins myosin and actin.1-3 Myocardial growth factors that might participate in the establishment of ventricular hypertrophy have not been studied.

Insulin-like growth factor-I (IGF-I) is a polypeptide with marked structural similarity to proinsulin4 and functional similarity to insulin.5 It mediates many of the effects of growth hormone (GH) on peripheral tissues.5,6 IGF-I and insulin share the same spectrum of metabolic and growth-promoting activities, with IGF-I being less potent than insulin in its metabolic effects but more potent in its mitogenic effects.5 This homology extends to the receptors for these peptides, each being a tyrosine-specific protein kinase7,8 that binds its particular ligand with high affinity and the other ligand with 10- to 100-fold–lower affinity.9 For many years, it was thought that the liver was the sole site of synthesis of IGF-I and that this synthesis occurred only in response to GH stimulation.9 While the liver is indeed the main source of circulating IGF-I,10 both IGF-I protein11 and more recently IGF-I mRNA12,13 have been found in many organs, including heart. We and others have found that IGF-I gene expression may be regulated in an organ-specific manner by both GH-dependent14,16 and GH-independent mechanisms.17,24 These observations suggest an autocrine and/or paracrine role for IGF-I in the hypertrophic response of terminally differentiated tissue.
Myocardial tissue possesses both IGF-I and insulin receptors. An increased incidence of hypertrophic heart disease has been noted both in acromegaly (increased serum GH and IGF-I levels) and in clinical syndromes of marked peripheral hyperinsulinemia such as leprechaunism. The growth-promoting effects of high circulating levels of insulin in vivo appear to be mediated through interaction with the IGF-I receptor. These findings suggest that IGF-I may be important in the development of ventricular hypertrophy.

Working from this premise, we examined induction of myocardial IGF-I mRNA under conditions associated with the development of left ventricular hypertrophy. We used three model systems of systemic hypertension known to produce rapid and significant ventricular hypertrophy: suprarenal abdominal aortic constriction (SAC);32 animals uninephrectomized (UNX) spontaneously hypertensive rat (SHR);30 and the uninephrectomized, deoxycorticosterone acetate-treated, saline-fed rat (DOCA salt).33 These three models were chosen to differentiate the relative roles of hemodynamic versus humoral (ie, renin) stimuli in the induction of IGF-I mRNA.

Methods

Animals and Surgical Procedures

Aortic Constriction

Male Sprague-Dawley rats (7 to 8 weeks old) weighing 280 to 300 g were purchased from Taconic Farms (Germantown, NY) and used for the aortic constriction model. Animals were housed under a 12-hour light, 12-hour dark cycle and were cared for in accordance with the National Institutes of Health, Guide for the Care and Use of Laboratory Animals. All surgical procedures were carried out under pentobarbital anesthesia (50 mg/kg IP). Aortic constriction was performed by placing a silk ligature around the abdominal aorta in a suprarenal position. The ligature was tightened to create a 60 to 70 mg Hg gradient across the site of constriction as measured in a preliminary group of animals (n=12) with carotid and femoral arterial monitoring. Mortality was approximately 30% in this model, but all surviving animals developed significant hypertension and ventricular hypertrophy. Sham-operated animals (n=6) underwent an identical procedure that included isolation of the aorta, but no ligature was placed. The abdominal incision was closed, and the animals were allowed to recover. All animals were maintained on ad libitum tap water and standard rat chow. Experimental groups (n=5 or 6 in each group) were killed by carbon dioxide inhalation 1, 3, 5, and 6 weeks after the operative procedure, and the sham-operated animals were killed 3 weeks after surgery. The animals were anesthetized with pentobarbital, weighed, and then underwent placement of carotid arterial lines for blood pressure measurement before they were killed. After the animals were killed, the hearts were quickly excised, blotted dry, weighed, homogenized in 4 mol/L guanidine thiocyanate buffer, and stored at -70°C.

Uninephrectomized Spontaneously Hypertensive Rat

Male SHR (5 to 6 weeks old) weighing 130 to 140 g underwent right unilateral nephrectomy through a flank incision under pentobarbital anesthesia (50 mg/kg IP, n=20). The incision was closed, and the animals were allowed to recover. They were maintained on ad libitum tap water and standard rat chow, and systolic blood pressures were measured at 2-week intervals by a variation of the tail plethysmography method.32 Animals were killed 1, 3, and 5 weeks after surgery (n=6 or 7 in each group). Normotensive, uninephrectomized Wistar-Kyoto (WKY) rats (n=8) served as controls and were killed 3 weeks after surgery. All animals were killed by carbon dioxide inhalation. At the time the animals were killed, they were weighed and the hearts were rapidly excised, blotted dry, weighed, and handled as in the aortic constriction model.

Deoxycorticosterone-Treated, Saline-Fed Rat

Male Sprague-Dawley rats (n=48) underwent right unilateral nephrectomy at 8 weeks of age under pentobarbital anesthesia (50 mg/kg IP). Three days after surgery, each animal received a subcutaneous injection of 10 mg of deoxycorticosterone acetate in an oil suspension. These injections were repeated at weekly intervals until the time the animals were killed. During the experimental period, the animals were given 1% saline drinking water and standard rat chow. Groups of eight of these animals were killed at weekly intervals to 6 weeks. Control animals included unoperated, drug-free Sprague-Dawley rats (n=4) and uninephrectomized Sprague-Dawley rats treated with DOCA injections but maintained on standard rat chow and tap water with no additional salt (n=4). The two control groups were killed at 6 weeks. All were killed by carbon dioxide inhalation. At the time the animals were killed, they were weighed and the hearts were excised, blotted dry, weighed, and handled as described for the aortic constriction model.

Reagents

Phenol and sodium dodecyl sulfate were purchased from Boehringer Mannheim (Indianapolis, Ind). Tris (hydroxy-methyl) aminomethane, acrylamide, and N,N'-methylene-bisacrylamide were of electrophoresis purity and were purchased from Bio-Rad (Richmond, Calif). DNase I was obtained from Worthington (Freehold, NJ). Molecular biology grade RNase A, RNase T1, and urea were purchased from Sigma (St Louis, Mo). All other reagents were of reagent grade and were obtained from Sigma.

Molecular Biologicals

All four cold ribonucleotide and deoxyribonucleotide triphosphates, RNAsin, dithiothreitol, transcription buffer, SP6 and T7 polymerases, and pSP71 and pGEM4 were obtained from Promega (Madison, Wis). Restriction enzymes used in cloning and plasmid linearizations were purchased from New England Biolabs (Beverly, Mass) or Boehringer Mannheim (Indianapolis, Ind). The Msp I digest of pBR322 and large fragment of DNA polymerase I (Klenow) were from New England Biolabs, and [32P]-UTP and [32P]-CTP were provided by Du Pont NEN (Boston, Mass).

Preparation of RNA

The atria and great vessels were removed by sharp dissection from each heart before weighing. Ventricular tissue was then homogenized in 4 mol/L guanidine isothiocyanate buffer with a Polytron homogenizer (Brinkman Instruments, Westbury, NY). Total cellular RNA was extracted by a modification33 of the acid-phenol-chloroform method34 and recovered by isopropanol precipitation. RNA was quantitated by UV absorbance measurements at 260 nm, its purity documented by comparison of UV absorbance measurements at 260 and 280 nm, and its integrity checked by demonstration of intact 28S and 18S ribosomal bands visualized by ethidium bromide staining after agarose gel electrophoresis.

Riboprobe Preparation

To detect the specific mRNA for IGF-I, a 500-bp rat kidney IGF-I cDNA encoding the sequence of mature IGF-I prepropeptide from amino acid -3 to +105 as well as 176 bp of 3'-untranslated sequence was used.35 Antisense RNA probes transcribed from this cDNA will detect IGF-I transcripts with any of the alternative 5'-untranslated sequences.36 This cDNA
was cloned into the EcoRI polylinker site of pSP71, which contains both SP6 and T7 promoters and allows transcription of high–specific activity, single-stranded RNA probes corresponding to either the coding or noncoding strand of the ligated cDNA. Template DNA is digested with DNase I, and the RNA transcription product is purified by phenol-chloroform-isooamyl alcohol extraction and ethanol precipitation.

To detect c-myc mRNA, a cDNA corresponding to exon 3 of the v-myc gene23 was cloned into pGEM4. The IGF-I receptor cDNA used in these studies was a clone containing sequences encoding 15 bases of the 5'-untranslated region, the signal peptide, and the first 53 amino acids of the α-subunit of the rat granulosa cell IGF-I receptor24 ligated into pGEM3.

Solution Hybridization/RNAse Protection Assay

This assay was performed using a modification of described methods.13-39 Twenty-five-microgram samples of total RNA were precipitated from 300 mmol/L sodium acetate, pH 6.0, with 2.5 vol ethanol, and recovered by centrifugation. Samples were resuspended in hybridization buffer containing 20 mmol/L Tris HCl, pH 7.6, 0.5 mmol/L EDTA, 400 mmol/L NaCl, 0.1% SDS, and 75% formamide. Before addition of 150 000 to 200 000 cpm 32P-labeled antisense RNA probe, UV absorbance at 260 nm was again checked on an aliquot of each sample to ensure inclusion of equal amounts of RNA in each assay. After heating to 85°C for 5 minutes, the samples were incubated for 16 hours at 45°C. Single-stranded RNA was digested by addition of 270 μL of digestion buffer containing 10 mmol/L Tris HCl, pH 7.6, 5 mol/L EDTA, 300 mmol/L NaCl, 40 μg/mL RNase A, and 2 μg/mL RNase T1 in a 1-hour incubation at 37°C. After treatment with 0.6% SDS and 50 μg proteinase K for 15 minutes at 37°C, the RNA was purified by PCI extraction and precipitated with 2.5 vol ethanol using 8 μg of tRNA as carrier. After washing with 95% ethanol, the samples were resuspended in loading buffer (80% formamide, 0.2% xylene cyanol, 0.2% bromphenol blue), denatured by heating for 3 minutes at 95°C, and chilled, and the “protected” fragments were separated by electrophoresis on 4% polyacrylamide/8 mol/L urea denaturing gels. The dried gels were subjected to autoradiography, and band signal intensity was quantitated by scanning densitometry of autoradiograms obtained from several exposures of original gels.

Each solution hybridization assay was performed at least twice, and each experimental protocol was repeated in its entirety once (in the UNX SHR and DOCA-salt models) or twice (in the SAC model) with identical results.

Molecular size markers were prepared by 3'-end labeling40 of an Msp I digest of pBR322 with the large (Klenow) fragment of DNA polymerase I in the presence of [α-32P]-dCTP.

IGF-I Radioimmunooassay

After retrograde flushing of the heart chambers with normal saline, the atria and great vessels were removed from each specimen by sharp dissection. Ventricular tissue was homogenized in 62 mmol/L Tris, pH 6.8, 0.1% Triton, and an aliquot acidified in 0.5 mol/L hydrochloric acid to dissociate IGF-I from its binding proteins. Triton-insoluble material was separated by low-speed centrifugation, and the resultant supernatant was passed through a Sep-Pak C18 solid-phase extraction cartridge (Millipore, Bedford, Mass.). IGF-I buffer was eluted with methanol, and the eluant was demonstrated to be free of binding proteins by iodinated ligand blotting techniques.41 The eluant was dried and reconstituted in radioimmunooassay buffer containing 20 mmol/L sodium phosphate, pH 7.5, 150 mmol/L sodium chloride, 1% bovine serum albumin, 0.1% sodium azide, and 1 U/mL heparin.42 The radioimmunooassay was performed using a polyclonal IGF-I antibody (kindly provided by Dr Richard Furlanetto) at a final dilution of 1:12 500.

Detection of IGF-I Protein by Immunofluorescence Histochemistry

Ventricles were bisected longitudinally and snap-frozen in liquid nitrogen, and serial midpoint sections were obtained by microtome. Acetone-fixed, phosphate-buffered saline–washed slides were stained first with polyclonal IGF-I antibody at a 1:50 dilution for 30 minutes, then with a fluorescein isothiocyanate goat anti-rabbit conjugate (Sigma) at the same dilution for an additional 30 minutes. After washing with fresh phosphate-buffered saline, the primary and secondary antibody incubations were repeated, and the slides were washed again and then prepared for mounting.

Results

Aortic Constriction

The blood pressure and growth characteristics of rats subjected to suprarenal aortic constriction and the sham-operated control animals are shown in Fig 1. In the left panel, increases in body weight and heart weight are shown as a function of time after aortic constriction. All animals continued to gain weight throughout the experimental period. Heart weights increased more dramatically than did body weights, with heart weights reaching a 43±2.6% (mean±SEM) increase by 6 weeks as compared with a 29±1.7% increase in body weights over the same time course. In contrast, the heart weight of the sham-operated animals that were killed at 3 weeks increased in parallel with body weight, as shown in the hatched box. In the right panel, systolic blood pressure at different times after aortic constriction or at 3 weeks after sham surgery is shown. Rats subjected to suprarenal abdominal aortic ligation became hypertensive by 3 weeks after surgery, with blood pressures reaching approximately 205 mm Hg at 6 weeks. Sham-operated animals remained normotensive during the 3 weeks after surgery.

The time course of induction of ventricular IGF-I mRNA in response to suprarenal aortic constriction is shown in Fig 2A. A fourfold to fivefold induction of IGF-I mRNA was noted at 3 weeks, and a 10-fold induction was observed at 5 weeks after constriction when compared with levels of ventricular IGF-I mRNA in sham-operated animals. These increases were coincident both with the onset of hypertension and the development of ventricular hypertrophy in these animals (Fig 1). Ventricular IGF-I mRNA content returned toward baseline levels by 6 weeks after constriction despite continued increases in blood pressure and ventricular weight to body weight ratio.

The time course of induction of ventricular c-myc mRNA in response to suprarenal abdominal aortic constriction is shown in Fig 2B. Previous studies have demonstrated early, transient c-myc mRNA induction in response to supravalvular aortic constriction.2 In our model system, c-myc mRNA levels increased twofold to threefold at 1 week after constriction and returned to control levels by 2 weeks. Time points earlier than 1 week were not examined in this study. The transient, early, increased expression of c-myc mRNA occurs before the onset of significant hypertension and clearly differs in time course from that noted for IGF-I mRNA. This indicates that c-myc and IGF-I gene expression are differentially controlled during the onset of hypertension and the development of ventricular hypertrophy.
Uninephrectomized, Spontaneously Hypertensive Rat

We next investigated the induction of myocardial IGF-I mRNA in a genetic form of hypertension, the SHR. These animals develop significant hypertension and ventricular hypertrophy by 3 months of age,30 but the time course of development of these changes may be accelerated by unilateral nephrectomy (UNX). We therefore studied UNX SHRs for 5 weeks after surgery and compared these with uninephrectomized, normotensive WKY control rats. The hemodynamic and growth characteristics of these animals are illustrated in Fig 3. As shown in the left panel, heart weight and body weight in the UNX SHRs increased in parallel until week 5, when heart weight began to increase disproportionately to body weight. This differential growth pattern was not noted in the uninephrectomized WKY group. The more rapid growth of the SHR animals over the experimental period reflected their lower average body weights at the beginning of the study when compared with their age-matched WKY counterparts. As shown on the right, the UNX SHRs became hypertensive by week 3 after nephrectomy (ie, 8 to 9 weeks of age) and eventually reached systolic blood pressures of approximately 205 mm Hg. UNX WKY control animals remained normotensive during the 3-week period after surgery.

The time course of induction of ventricular IGF-I mRNA in the UNX SHRs is shown in Fig 4. These animals were still normotensive at 1 week after nephrectomy (Fig 3), and ventricular IGF-I mRNA levels were similar to those in the normotensive UNX WKY rats. At 3 weeks, however, there was a sixfold to eightfold induction coincident with the onset of hypertension and measurable ventricular hypertrophy. IGF-I mRNA returned to baseline levels at 5 weeks despite the continued increases in systolic blood pressure and ventricular weight.

DOCA Salt Rat

The aortic constriction and UNX SHR models are representative of high- and moderate-renin states, respectively. We therefore examined induction of the myocardial IGF-I gene in a model of hypertension and hypertrophy that is completely renin independent, ie, the uninephrectomized, deoxycorticosterone-treated, salt-loaded rat (DOCA salt).43 These animals develop significant systolic hypertension and left ventricular hypertrophy under conditions that completely inhibit renin production.43 The body and heart weights of the DOCA salt animals over the 6-week period after nephrectomy are shown in Fig 5A. The growth characteristics of the two control groups (unoperated animals receiving neither DOCA nor saline [control]) and uninephrectomized, DOCA-treated animals given tap water to drink (DOCA NS [no salt]) are depicted in Fig 5B. Only the DOCA salt animals developed significant increases in heart weight versus body weight. In both control groups, the percent increase in heart weight did not exceed that in body weight over the 6-week experimental period.

Systolic blood pressures over the experimental period in the three groups of animals are illustrated in Fig 6. The DOCA salt group developed significant hypertension at 4 to 5 weeks, whereas both control groups remained normotensive. Hypertension developed more gradually in the DOCA salt animals than it did in the
Fig 2. A, Induction of cardiac insulin-like growth factor-I (IGF-I) mRNA in the weeks after aortic constriction. Total cellular RNA was isolated from ventricular myocardium at the times indicated (in weeks). Twenty-five-microgram aliquots of RNA obtained from pooled samples from 5 to 6 animals were hybridized with antisense (32P)-labeled cRNA probes, treated with single strand-specific RNases, and electrophoresed as described in “Methods.” The band represents the 500-bp “protected” fragment corresponding to IGF-I mRNA. STD indicates molecular size markers; S, sham-operated rats killed at 3 weeks. B, Time course of induction of cardiac c-myc mRNA in the weeks after aortic constriction. Each sample was obtained from pooled total cellular RNA preparations from 4 animals. The 420-bp doublet band represents c-myc mRNA detected using this approximately 850-bp v-myc-derived probe. C indicates control unoperated rats at 1 week. Each solution hybridization assay in both panels was performed in duplicate, and the entire experimental protocol was repeated once in identical fashion to validate the results presented here.

SAC and UNX SHR models. These animals also did not achieve the same level of hypertension, with peak systolic blood pressures reaching only 190 mm Hg. Once again, however, the increased rate of ventricular growth in the DOCA salt group is manifested between 4 and 5 weeks (Fig 5A), which is coincident with the onset of hypertensive blood pressures. Fig 7 illustrates the time course of induction of ventricular IGF-I mRNA in the DOCA salt rats. IGF-I mRNA content was identical to that of controls at 4 weeks (lane 4) as well as at 1, 2, and 3 weeks (data not shown). A fourfold to fivefold induction of IGF-I mRNA was noted at 5 weeks followed by a sixfold induction at 6 weeks. In a single experiment carried out to 7 weeks, IGF-I mRNA content returned to basal levels (data not shown). This suggests that the increased IGF-I mRNA levels are transient in this model as well as in the SAC and UNX SHR models, with the more protracted time course probably reflecting the more gradual onset of hypertension and/or lower peak systolic pressures in the DOCA-salt animals. Again, the onset of induction was coincident with the onset of hypertensive blood pressures and the development of ventricular hypertrophy. Ventricular IGF-I mRNA content in DOCA NS animals killed at 6 weeks remained 300% to 400% below that of DOCA salt animals studied at the same time point.

IGF-I Receptor

The IGF-I receptor is expressed at low levels in the basal state in the rat ventricle. Presumably, it can bind both locally produced IGF-I (paracrine/autocrine effect) and circulating IGF-I (endocrine effect). To determine whether expression of the myocardial IGF-I receptor gene is also affected by pressure overload, we studied ventricular IGF-I receptor mRNA content in the DOCA salt model of hypertension and hypertrophy. Fig 8 shows that in marked contrast to what was seen for IGF-I mRNA, the low basal level of IGF-I receptor mRNA was unchanged throughout the course of development of hypertension and hypertrophy. Thus, the myocardial IGF-I receptor mRNA levels appear to be
unresponsive, at least over this time course, to local changes in pressure and ventricular growth in the adult animal. Our data do not address the possibility that subcellular localization of IGF-I receptors is altered under these conditions. These data do support, however, the specificity of the changes in IGF-I gene expression that accompany hypertension and the early phases of hypertrophy.

**Ventricular IGF-I Protein Content**

To determine whether these changes in ventricular IGF-I mRNA content are reflected in changes in pro-

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**Fig 3.** Left, Plot shows time course of percent increase in heart weight and body weight in the weeks after unilateral nephrectomy in spontaneously hypertensive rats (SHR) and in uninephrectomized Wistar-Kyoto rats (WKY). Error bars represent SEM, with n=6 or 7 for the SHR groups and n=8 for the WKY group. Right, Bar graph shows time course of changes in systolic blood pressure (mm Hg) in the weeks after unilateral nephrectomy in the SHR and WKY animals. Error bars indicate SEM, with n=6 or 7 for each group of SHR animals and n=4 for each group of WKY rats.

**Fig 4.** Induction of cardiac insulin-like growth factor-I mRNA in the weeks after unilateral nephrectomy in spontaneously hypertensive rats. Each sample shown was obtained from pooled RNA from 6 to 7 uninephrectomized spontaneously hypertensive rats killed 1, 3, or 5 weeks after surgery (lanes 1, 3, and 5) or from 8 uninephrectomized Wistar-Kyoto rats (C, control) killed 3 weeks after surgery. The band represents the 500-bp "protected" fragment corresponding to insulin-like growth factor-I mRNA. STD indicates molecular size standards. Each solution hybridization assay was performed in duplicate, and the entire experimental protocol was repeated once in identical fashion to validate the results presented here.
tein content, we assessed ventricular IGF-I protein both by tissue radioimmunoassay and by immunofluorescence histochemistry in animals subjected to SAC or to sham surgery. The ventricular IGF-I protein content of sham-operated rats (n=3) with systolic blood pressures <130 mm Hg was 1270±194 ng per gram of tissue. In contrast, ventricular IGF-I protein content of SAC animals (n=6) with systolic blood pressures ≥165 mm Hg was 2250±311 ng per gram of tissue (P<.05 compared with sham-operated, normotensive animals). Each sample was run in triplicate, and the entire assay was repeated in duplicate, resulting in 180% and 171% increases in experimental above control levels, respectively.

A representative ventricular section incubated first with polyclonal IGF-I antibody and second with fluorescein-conjugated, antispecies antibody from a SAC animal (panel A) and a sham-operated animal (panel B) is shown in Fig 9. Each animal was killed 5 weeks after surgery; systolic blood pressure was 170 mm Hg in the experimental animal and 100 mm Hg in the sham animal. The increased staining of the ventricular tissue of the SAC animal compared with that of the sham animal is evident and appears to be localized to myocyte cytoplasm.

Both methods for detection of protein document a significant increase in ventricular IGF-I content in hypertensive SAC animals compared with sham-oper-
ated, normotensive controls. The time point chosen for these studies corresponds to that at which IGF-I mRNA induction was maximal in this model system. As a result, although we may conclude that expression of the IGF-I gene (both mRNA and protein) is increased in this system, our data do not address the time course of the increase in protein content or the stoichiometry of the relation between the altered mRNA and protein contents. Therefore, several possible explanations exist for the 8- to 10-fold increase in ventricular IGF-I mRNA versus the twofold increase in ventricular IGF-I protein noted in these experiments. These include relative sensitivity of the techniques for measurement of the IGF-I mRNA and protein, choice of time point for measurement of protein, half-life or relative translational potential of the various IGF-I mRNA species, tissue half-life of the IGF-I protein, and the relation of the tissue IGF-I to local IGF binding proteins (see “Discussion”).

Discussion

Both quantitative and qualitative changes characterize the adaptation of the heart to pressure overload. The quantitative changes include increased muscle mass by the addition of sarcomeric units and dramatic increases in the number of interstitial cells and collagen content. Some of the qualitative changes that have been described include the reversion to fetal isoforms of the myosin heavy chain isozyme, reexpression of skeletal α-actin mRNA, and ventricular expression of the atrial natriuretic factor gene. Early, transient induction of various proto-oncogenes that function as transcriptional regulators such as c-myc and c-fos also has been well studied. On the other hand, the growth factors that may mediate these structural and functional changes have not been extensively investigated. Although transforming growth factor-β (TGF-β) and the acidic and basic fibroblast growth factors (FGFs) have been localized to cardiac tissue, changes in expression of their genes in response to altered loading conditions have not been demonstrated.

IGF-I influences both growth and differentiation in fetal and neonatal rat hearts. Both IGF-I and IGF-II are expressed in the developing rat heart during the stage of maturation (postcytokinetic stage with an increase in cell size, i.e., hypertrophy) and cellular binucleation. In this same study, IGF-I mRNA was also found to be more abundant in the neonatal prehypertensive SHR ventricle than in control WKY rat ventricles. This finding may be relevant to the accelerated maturation of the cardiomyocyte in the SHR, culminating in larger though fewer cells. The myocyte growth-promoting properties of IGF-I in neonatal rats suggest a possible role for this peptide in promoting hypertrophy of the
adult ventricle. Consistent with this hypothesis, IGF-I mRNA accumulates in skeletal muscle induced to hyppertrophy by an increased load, suggesting that IGF-I may participate in dynamic remodeling processes in all striated muscle tissues.

In the present study, we have used cDNA probes to IGF-I, IGF-I receptor, and c-myc to identify and quantify their corresponding mRNAs in rat ventricular muscle undergoing hypertrophy in response to pressure overload. In all three models of hypertension studied, ventricular IGF-I mRNA content increased significantly, coincident with the onset of systolic hypertension and at an early stage of accelerated ventricular growth. The induction was clearly present but appeared to be transient, with IGF-I mRNA levels returning to baseline levels despite continued pressure overload and ongoing hypertrophy. These data represent the first demonstration of alteration of expression of a cardiac growth factor mRNA in response to hemodynamic perturbations. In addition, we have shown a significant increase in ventricular IGF-I protein content by two independent methods in one of the three model systems, demonstrating that these changes in mRNA are indeed reflected in changes in protein.

The pattern of induction of IGF-I mRNA in response to pressure loading of the ventricle suggests a role for IGF-I in initiation of the hypertrophic process. The increase in ventricular IGF-I mRNA content coincident with the onset of hypertension and the return to baseline values despite ongoing hypertension and ventricular hypertrophy suggest that continued high local expression of IGF-I may not be necessary for the maintenance of ventricular hypertrophy. In vitro, IGF-I has been shown to be necessary for the full biological effect of other growth factors such as platelet-derived growth factor. This suggests that IGF-I may participate in initiating the process and then may relinquish growth control to other factors. The observation of early, high levels of IGF-I mRNA in response to a growth stimulus followed by a fall toward control levels once the growth process is established has been described previously both in vascular smooth muscle cells and in endothelial cells and most recently in left ventricular tissue of young WKY rats with acute onset of hypertension after creation of two-kidney, one-clip Goldblatt renal artery stenosis.

The time course of IGF-I mRNA induction was markedly similar in all three model systems of pressure-overload hypertrophy. IGF-I mRNA levels in the DOCA salt model, however, did not decline to baseline levels at 6 weeks, as was observed in the other two models. This may be explained by the more gradual onset of hypertension and lower peak systolic blood pressures in these animals compared with those in the SAC and UNX SHR models. Aortic constriction and DOCA salt are, respectively, high and extremely low circulating renin forms of hypertension. SHR animals demonstrate a bimodal pattern with respect to circulating renin levels but are thought not to be a renin-dependent form of hypertension in the adult animal. The consistency of IGF-I mRNA induction in these three models suggests that it is independent of the systemic renin-angiotensin endocrine axis. Possible autocrine and/or paracrine roles of locally produced components of this system have not been addressed in this study.

The stability of ventricular IGF-I receptor mRNA content in the DOCA salt studies and the different time course of c-myc mRNA induction in the SAC studies demonstrate discordant, differential control of the IGF-I, IGF-I receptor, and c-myc genes in myocardium subjected to variations in load. Although we have provided evidence that discoordinate local regulation of IGF-I gene expression may occur at the pretranslational level (ie, transcription, mRNA stability, etc), we emphasize that translational and posttranslational factors must be considered when evaluating a role for IGF-I in tissue growth. For example, the sequence of the 5′ and 3′-untranslated regions of one rat IGF-I cDNA suggests the capability for formation of a stable duplex structure that could hinder ribosomal access to translation initiation codons upstream of the coding region for the pre-pro-IGF-I peptide. Thus, one of the species of IGF-I mRNA detected by our coding region IGF-I cRNA probe may not be as readily translated as another. In addition, it is also possible that both transcriptional and translational or posttranslational control occur and that the relative roles of each of these may...
differ in the initiation and/or maintenance phases of the hypertrophic response.

Other investigators have demonstrated that measurement of actual organ or tissue IGF-I protein content is difficult because of the extreme abundance of IGF-I in serum compared with its level in any organ, including liver.11 This reflects the high proportion of circulating IGF-I bound to specific serum binding proteins.39,60 Our tissue radioimmunoassay methodology incorporates saline flushing through the great vessels to minimize the potential effects of blood trapping on subsequent IGF-I measurements as well as acid extraction of homogenized tissue to separate IGF-I from its binding proteins. It should be remembered, however, that increased organ IGF-I protein content may be due not only to increased IGF-I gene expression at the pretranslational level as demonstrated here but also to increased myocardial IGF-I binding or decreased clearance. In addition, IGF-I binding proteins are powerful modulators of the biological actions of both IGF-I and IGF-II.62 The relative abundance of the various binding proteins in tissues differs from that found in body fluids, suggesting local production of these proteins, which, unlike the major circulating form of binding protein, may potentiate rather than limit the biological effects of IGF.61

Future studies of the role of local IGF-I in the hypertrophic response in terminally differentiated tissue, therefore, must involve evaluation of IGF-I ligand, receptor, and binding protein expression. All three probably play important roles in an autocrine/paracrine growth system.

Conclusions

We have demonstrated induction of IGF-I mRNA in renin-dependent and renin-independent models of pressure-induced ventricular hypertrophy in the rat. We have also demonstrated differential control of c-myc, IGF-I, and IGF-I receptor mRNAs. These findings suggest that IGF-I may be important in the early response of the ventricle to pressure overload and in the development of ventricular hypertrophy. Further studies of IGF-I gene expression in other forms of hypertrophy (ie, volume overload or after myocardial infarction) as well as the effect of drugs that control hypertension with differential effects on the development or resolution of ventricular hypertrophy will be important.

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