Increased Expression of Growth Hormone Receptor mRNA and Insulin-like Growth Factor-I mRNA in Volume-Overloaded Hearts

Jörgen Isgaard, Håkan Wåhlander, Michael A. Adams, Peter Friberg

Abstract Recent results suggest that insulin-like growth factor-I (IGF-I) may be involved in the transition of a hemodynamic load into cardiac hypertrophy and that the expression of IGF-I seems to be coupled to increased wall stress. The present study investigated the role of growth hormone (GH) and IGF-I in myocardial hypertrophy induced by volume overload. An aortocaval fistula (ACF) was created in male Wistar rats, and experiments were performed 2, 4, and 7 days after the onset of volume overload. Right and left ventricular (RV and LV, respectively) myocardial expression of GH receptor mRNA and IGF-I mRNA were quantitated by a solution hybridization RNase protection assay. RV GH receptor mRNA content was elevated on the fourth and seventh days after the induction of the shunt, with peak levels (0.63±0.16 versus 0.14±0.03 amol/μg DNA for the sham-operated animals; P<.01) after 4 days. Similarly, IGF-I mRNA was significantly increased in the RV of shunted animals (1.26±0.13 versus 0.56±0.05 amol/μg DNA; P<.01) 7 days after surgery. In the left ventricle, where systolic pressure was reduced in ACF rats, no differences could be detected in GH receptor and IGF-I mRNA content between ACF and sham-operated rats on any of the experimental days. There was no difference in the ratio of RV to LV weight during the experimental period. We have shown that the thin-walled right ventricle responds to volume overload with an increase of GH receptor mRNA content followed by elevated expression of IGF-I mRNA. The present results suggest that the heart regionally produces trophically acting factors, such as IGF-I, which may be of importance for the initiation of the hypertrophic process, at least when a ventricle is being challenged, such as the right ventricle in volume overload. (Hypertension. 1994;23[part 2]: 884-888.)

Key Words • rats • cardiac volume • insulin-like growth factor I • growth hormone • fistula • wall stress

The heart responds to an increased systemic pressure load with an adaptive hypertrophy of the left ventricle. Moreover, volume overload (ie, increased venous return resulting in increased cardiac output) induces cardiac hypertrophy characterized by a proportional increase in internal chamber volume and wall thickness. However, the mechanisms involved in the conversion of the mechanical stimuli into an increased tissue mass are less known, although several factors, including proto-oncogenes and growth factors, have been proposed to be involved in this process.

The presence of insulin-like growth factor-I (IGF-I) mRNA in the rat heart has been shown and it was recently demonstrated that IGF-I mRNA and protein were induced specifically in the pressure-overloaded left ventricle of two-kidney, one clip rats. Presumably, IGF-I gene expression was coupled to the increased left ventricular (LV) workload, and IGF-I thus constitutes a possible mediator of cardiac hypertrophy in response to hemodynamic load. This adds further support to the fact that IGF-I is synthesized in peripheral tissues and exerts paracrine-autocrine effects during tissue growth and that it can be produced in specific regions within the heart. The primary regulator of local IGF-I mRNA content is growth hormone (GH), and GH enhances IGF-I transcription as well as the abundance of IGF-I mRNA in most rodent tissues. The effects of GH are evoked by the interaction of the ligand with its cellular receptor, and recent studies suggest that the tissue response is also dependent on the expression of the GH receptor. GH receptor mRNA is present in most rat tissues, including heart. Few studies have so far addressed the transcriptional regulation of the GH receptor gene, although there are indications that GH regulates GH receptor gene expression in chondrocytes and in adipose tissue.

The surgically induced fistula between the abdominal aorta and the inferior caval vein (ACF) is an experimental model for volume overload; a detailed hemodynamic characterization of a very similar model used in the present study was presented in 1991 by Liu and coworkers. In accordance with the hypothesis that wall stress is important for the induction of growth factors and adaptive cardiac hypertrophy, we used this model in the present study to investigate the right ventricular (RV) and LV expression of IGF-I mRNA. Moreover, the expression of GH receptor was studied to explore a possible functional role for GH and IGF-I during development of cardiac hypertrophy. Myocardial GH receptor mRNA and IGF-I mRNA was detected by Northern blot assays and quantitated in a solution hybridization RNase protection assay.

Methods

General Procedures

Volume overload was induced in male normotensive Wistar rats (Møllegaard Breeding Center Ltd, Ejby, Denmark) weigh-
Effects of Volume Overload by Means of an Aortocaval Fistula on Body Weight at Time of Surgery, Body Weight at Time of Experimentation, Blood Pressure, and Left and Right Ventricular Weight per Body Weight

<table>
<thead>
<tr>
<th>Group</th>
<th>BW_{op}, g</th>
<th>BW_{exp}, g</th>
<th>BP, mm Hg</th>
<th>RV Weight/BW_{exp}, mg/g</th>
<th>LV Weight/BW_{exp}, mg/g</th>
<th>RV-LV Weight Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACF</td>
<td>190±1</td>
<td>179±2</td>
<td>118±7</td>
<td>0.703±0.021</td>
<td>2.15±0.05</td>
<td>0.33±0.0088</td>
</tr>
<tr>
<td>Sham</td>
<td>188±4</td>
<td>179±3</td>
<td>132±6</td>
<td>0.701±0.014</td>
<td>2.10±0.048</td>
<td>0.33±0.0073</td>
</tr>
<tr>
<td>4 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACF</td>
<td>203±2</td>
<td>199±3</td>
<td>119±5</td>
<td>0.710±0.038</td>
<td>1.98±0.055</td>
<td>0.36±0.014</td>
</tr>
<tr>
<td>Sham</td>
<td>202±2</td>
<td>197±6</td>
<td>137±4</td>
<td>0.618±0.015</td>
<td>1.84±0.047</td>
<td>0.34±0.0081</td>
</tr>
<tr>
<td>7 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACF</td>
<td>188±2</td>
<td>206±4</td>
<td>115±5</td>
<td>0.860±0.045*</td>
<td>2.51±0.093*</td>
<td>0.34±0.0088</td>
</tr>
<tr>
<td>Sham</td>
<td>186±2</td>
<td>217±4</td>
<td>134±5</td>
<td>0.698±0.021</td>
<td>2.12±0.047</td>
<td>0.33±0.0080</td>
</tr>
</tbody>
</table>

BW_{op} indicates body weight at time of surgery; BW_{exp}, body weight at time of experimentation; BP, blood pressure; RV, right ventricular; LV, left ventricular; and ACF, aortocaval fistula. Values are mean±SEM.

*P<.01.

During 180 to 200 g by means of an ACF according to Garcia and Diebold.21 In brief, during a short-lasting barbiturate methohexital sodium anesthesia (Brietial, Eli Lilly and Co) (75 mg•kg⁻¹ body wt IP), the aorta and inferior caval vein were exposed via a midline incision, and the aorta was punctured between the renal arteries and the aortic bifurcation with a disposable needle (diameter, 1.2 mm) connected to a syringe. The needle was then advanced into the inferior caval vein. After clamping the aorta, the needle was withdrawn, and the puncture site was sealed with cyanoacrylate glue. A sham operation merely exposing the aorta and inferior caval vein was performed in control rats. Experiments were performed on groups of six to eight ACF and sham-operated animals 2, 4, and 7 days postoperatively. The degree of shunt was moderate, and no cardiac failure was noticed as measured by wet to dry weight ratios of lungs and liver.

Probes

Antisense GH receptor (digoxigenin [DIG]- or ³²P-uridine triphosphate [UTP]-labeled) RNA was synthesized according to the manufacturer's instructions (Boehringer Mannheim) with T3 RNA polymerase (Pharmacia) using EcoRI linearized plasmid pT7T3 18U as a template.² The pT7T3 18U plasmid contains a 560-bp BamHI fragment of the rat GH receptor cDNA, which encodes a part of the extracellular domain of the GH receptor. The probe would allow the detection of both the GH receptor and GH binding protein. A 153-bp SmaI fragment of a genomic subclone of mouse IGF-I (corresponding to exon 3 by analogy to human IGF-I) subcloned into a pSP64 plasmid was used as a template for probe synthesis.² The plasmid was linearized with EcoRI and used as a template for synthesis of ³²P- or DIG-labeled RNA probes by in vitro transcription (Promega).

Northern Blot Analysis

Total RNA was prepared essentially according to Chomczynski and Sacchi.² Twenty micrograms of total RNA was electrophoresed in an agarose (1%) formaldehyde (0.7 mol/L) gel with ethidium bromide. The RNA was transferred to Hybond-N membranes (Amersham) with a vacuum transfer system (LKB). Before hybridization with DIG-labeled, antisense GH receptor RNA, the membranes were stratalinked for 55 seconds and prehybridized in 20 mL of hybridization buffer per 100 cm² of filter containing the following: formamid 50% (vol/vol), 5x SSC; binding reagent, 2% (wt/vol) added from 10% sterile blocking stock solution; N-lauroylsarcosine, 0.1% (wt/vol); and sodium dodecyl sulfate (SDS), 0.02% (wt/vol) at 68°C (Boehringer Mannheim). The prehybridization buffer was then replaced with 2.5 mL/100 cm² filter hybridization buffer containing 500 ng RNA probe and incubated overnight at 68°C. Filters were then washed twice for 5 minutes each at room temperature with 50 mL of 2x SSC and SDS 0.1% (wt/vol) per 100 cm² filter and washed three times for 15 minutes each at 68°C with 0.1x SSC and SDS 0.1% (wt/vol). DIG-labeled RNA probes were detected after hybridization by anti-DIG alkaline phosphatase conjugate (Boehringer Mannheim). For IGF-I mRNA detection, total RNA was prepared, electrophoresed, and transferred as described above. The membranes were prehybridized, hybridized, washed, and RNase treated as previously described.²³

Solution Hybridization

Frozen tissue was homogenized and treated with proteinase-K, and total nucleic acids were extracted with phenol-chloroform, as previously described.²⁴ A solution hybridization assay was used to quantify IGF-I mRNA and GH receptor mRNA.²⁴ Results are expressed as IGF-I mRNA/DNA (attomoles per microgram), and the DNA content was assayed according to Labarca and Paigen.²⁵

Statistical Analysis

Values are given as mean±SEM. The statistical differences between groups were analyzed by one- or two-way ANOVA followed by the Student-Newman-Keuls multiple test between individual groups.

Results

The Table shows a comparable weight loss 2 and 4 days after surgery in both sham-operated and ACF animals. In 7-day ACF animals the recovery of body weight was slightly delayed compared with 7-day sham animals (P<0.01), indicating a reduced body weight gain due to the shunt. However, body weight was restored within another 7 days (H. Wählander, unpublished data, 1993). A significant increase in both RV and LV weight...
FIG 1. Effect of volume overload on growth hormone (GH) mRNA levels analyzed with Northern blot. An aortocaval fistula (ACF) was created in male normotensive Wistar rats weighing 180 to 200 g. In control rats, a sham operation was performed. Experiments were then performed 7 days after surgery when the rats were weighed, hearts were excised, and total RNA was extracted (see "Methods"). Total RNA (20 μg) was electrophoresed, transferred, and hybridized to a digoxigenin-labeled GH receptor mRNA probe, as described in "Methods." Top panel shows hybridized mRNAs from ACF (A) and sham (B) rats. Bottom panel shows ethidium bromide staining of ribosomal RNA.

occurred in 7-day ACF rats (F=8.2, P<.005 and F=10.6, P<.002, respectively); there were no statistically significant differences in heart weights in 2- and 4-day shunted animals (Table). The degrees of weight increase between 2 and 7 days were 22% and 17% for the right and left ventricles, respectively, although there was no significant difference in RV-LV weight ratio on any of the experimental days (Table). Systemic blood pressure was significantly reduced in all shunted animals (F=11.6, P<.001; Table) 2 days after surgery and remained so throughout the experimental period.

RNA extracted from the right ventricle of sham and ACF rats 7 days postoperatively was analyzed with Northern blot with a DIG-labeled GH receptor probe corresponding to part of the extracellular domain and thus recognizing both the GH receptor and the GH binding protein transcript. Transcripts were found with estimated sizes of 4.0 kb in both ACF and sham-operated animals, corresponding to the full-length GH receptor mRNA (Fig 1). Using a mouse 32P-labeled IGF-I cRNA probe, one major transcript was revealed with an estimated size of 7.0 kb in both ACF and sham-operated animals (Fig 2). Moreover, a minor transcript of approximately 0.9 to 1.2 kb was detected in the liver.

The quantity of GH receptor mRNA and IGF-I mRNA was studied in a solution hybridization RNase protection assay using the same probes as for the Northern blot. There was a statistically significant fourfold increase of GH receptor mRNA in the right ventricle of ACF rats, which peaked 4 days after shunting (F=21.4, P<.001 versus sham-operated rats and F=10.8, P<.01 versus 2-day ACF rats) and remained elevated at 7 days (F=15.7, P<.016 versus sham-operated rats and F=13.6, P<.01 versus 2-day ACF rats) (Fig 3). No differences with respect to GH receptor mRNA levels could be detected in the left ventricle on any of the experimental days (Fig 3). RV IGF-I mRNA levels were elevated in ACF rats 7 days after surgery (F=31, P<.0003 versus sham-operated rats and F=22.2, P<.01 versus 2-day ACF rats) (Fig 4). In analogy with GH receptor mRNA, there were no differences in IGF-I mRNA levels in the left ventricle of ACF rats during the experimental period (Fig 4).

There were no differences in serum IGF-I levels between the groups measured by radioimmunoassay (data not shown).
Values are mean±SEM. n=6 to 8 rats in each group. **P<.01.

were excised and processed (see “Methods”). Levels of IGF-I mRNA are expressed as 10^-18 moles per microgram DNA.

FIG 4. Bar graphs show effect of volume overload on insulin-like growth factor-I (IGF-I) mRNA content analyzed with a solution hybridization RNase protection assay. An aortocaval fistula (ACF) was created in male normotensive Wistar rats weighing 180 to 200 g (striped bars). In control rats, a sham operation was performed (open bars). Experiments were then performed 2, 4, or 7 days after surgery when the rats were weighed and hearts were excised and processed (see “Methods”). Levels of GH receptor mRNA are expressed as 10^-18 moles per microgram DNA. Values are mean±SEM. n=6 to 8 rats in each group. **P<.05, ***P<.01.

Discussion

The main findings of the present study are that experimentally induced cardiac volume overload results in increased levels of GH receptor mRNA and IGF-I mRNA in the right but not left ventricle. This suggests (1) that the combined increase in both diastolic and systolic RV wall stress in the ACF animals may elicit a growth response with increased gene expression of the GH receptor and IGF-I and that these gene expressions are associated but not mandatory with myocardial hypertrophy and (2) that a temporal relation exists between the expression of GH receptor and IGF-I.

During pressure overload in two-kidney, one clip renal hypertension, we recently demonstrated increased content of IGF-I at mRNA and protein levels in the left ventricle specifically, suggesting regional production of IGF-I in the heart that is most likely due to augmented LV systolic wall stress. This was further supported by the finding that IGF-I was more abundant in the subendocardial layers of the left ventricle, where wall stress is highest. Activation of the IGF-I gene appeared to be specific because LV levels of β-actin mRNA were unchanged in hypertensive rats compared with sham-operated rats. **To further study the possible influence of wall stress on the expression of IGF-I we modified a model in which volume overload was induced by means of an ACF. In contrast to renal hypertension, in which only the left ventricle is exposed to the increased pressure load, the ACF model allows both ventricles to be subjected to the increased venous return. However, as demonstrated by Liu et al.,9 systolic pressure was enhanced in the right but not the left ventricle in the ACF group. This was due to low vascular resistance in the pulmonary circulation before shunting, which could not be reduced to the extent needed to keep RV systolic pressure constant. Thus, RV systolic (but not necessarily diastolic) wall stress would be enhanced to a greater extent than in the left ventricle in the ACF group. Consequently, the restricted localization of the increased IGF-I expression to the right ventricle suggests that increased systolic wall stress serves as a stimulus for local IGF-I production. It has also been demonstrated that RV myocytes displayed a greater increase in volume, length, and cross-sectional area than did LV myocytes. Thus, the increased IGF-I mRNA levels in the right ventricle may be a reflection of the combined pressure and volume overload of the right chamber. Moreover, the thinner wall of the right compared with the left ventricle may also be of importance for the wall stress response. It is reasonable to assume that the relatively thinner RV wall was exposed to a much more rapid and abrupt increase in filling pattern and consequently subjected to higher wall stress compared with the left ventricle, and it was therefore compelled to alter its structure and function accordingly. In contrast, the left ventricle was initially exposed to an actual lowering of diastolic pressure until a new hemodynamic state prevailed. Thus, the left ventricle would undergo a more graded mechanical stimulus change compared with the right ventricle, and this could affect the gene expression of IGF-I and GH receptors. In this context, it is noteworthy to stress that the left ventricle actually underwent a considerable hypertrophic response without a corresponding increase of IGF-I or GH receptor mRNA. Thus, a hypertrophic response may not necessarily be associated with increased expression of IGF-I.

It is generally recognized that both GH and IGF-I have important roles for somatic growth, and IGF-I has recently been shown to increase myocardial protein synthesis,26 as well as gene expression of the β-myosin heavy chain.27 Moreover, IGF-I has been suggested to be directly involved in cardiac hypertrophy by increasing cardiomyocyte cell size in vitro and by increasing gene expression of myosin light chain-2 and troponin I.28 In the present study as well as in our previous study on renal hypertensive rats,7 the left and right ventricles were exposed to identical plasma concentrations of GH, which would imply that the increased myocardial IGF-I expression in response to wall stress was independent of changes in circulating GH levels. Therefore, the finding that RV GH receptor mRNA increased before the expression of IGF-I mRNA is particularly intriguing. The similar pattern of GH receptor and IGF-I mRNA expression thus suggests that local production of IGF-I could be under GH regulation independent of systemic GH concentrations. GH has been demonstrated previously to be of importance for the development of LV hypertrophy in response to pressure overload and for...
the associated structural changes of vessels and kidneys. In a recent study using in situ hybridization, it was also demonstrated that GH receptor mRNA was expressed in rat skeletal muscle during regeneration after ischemic injury. Furthermore, IGF-I mRNA expression was increased during muscle regeneration, suggesting that IGF-I may be regulated by GH without GH plasma concentrations necessarily being altered. Stimulation of local and regional IGF-I synthesis constitutes a possible mechanism for the action of GH during development of myocardial hypertrophy.

In conclusion, the present in vivo study demonstrates that a mechanical stimulus, probably systolic wall stress, can induce myocardial expression of the GH receptor, followed by induction of local IGF-I gene expression and increased synthesis of IGF-I, which at least to some extent may possess direct trophic effects in the myocardium and may be part of the development of RV hypertrophy.

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References
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