Cardiac and Renal Effects of Growth Hormone in Volume Overload–Induced Heart Failure

Role of NO

Ines Pagel, Thomas Langenickel, Klaus Höhnel, Sebastian Philipp, Andreas K. Nüssler, Werner F. Blum, Michel L. Aubert, Rainer Dietz, Roland Willenbrock

Abstract—Growth hormone (GH) application is a new strategy in the treatment of heart failure. However, clinical and experimental investigations have shown contradictory effects of GH on cardiac performance. We tested the hypothesis that GH could improve cardiac and renal function in volume overload–induced heart failure. The effect of 4 weeks of GH treatment (2 mg/kg daily) was investigated in Wistar rats with aortocaval shunt. GH application did not influence left ventricular contractility and end-diastolic pressure in rats with aortocaval shunt. In contrast, GH treatment normalized impaired diuresis (vehicle 10.8±0.6 mL/d, GH 15.8±0.7 mL/d; P<0.05) and sodium excretion (vehicle 1.5±0.1 mmol/d, GH 2.2±0.1 mmol/d; P<0.001) in shunt-operated rats, with a similar increase of fractional sodium excretion. The urinary excretion of cGMP, the second messenger of atrial natriuretic peptide and NO, was higher in animals with shunts than in sham-operated animals and was further increased by GH (vehicle 293±38 nmol/d, GH 463±57 nmol/d; P<0.01). Although the atrial natriuretic peptide plasma levels were unchanged after GH, the excretion of NO metabolites (nitrate/nitrite) was elevated (vehicle 2020±264 nmol/d, GH 2993±375 nmol/d; P<0.05) in parallel with increased renal mRNA levels of inducible NO synthase 2. The changes of renal function after GH and the increased excretion of NO metabolites and cGMP were abolished by simultaneous treatment with the NO synthase inhibitor N^G-nitro-L-arginine methyl ester. GH treatment did not influence cardiac function in rats with aortocaval shunts. However, GH improved renal function by increasing diuresis and sodium excretion. The responsible mechanism might be the enhanced activity of the renal NO system. (Hypertension. 2002;39:57-62.)

Key Words: heart failure  growth substances  kidney  nitric oxide  nitric oxide synthase  cyclic GMP

Growth hormone (GH) administration has been shown to be useful in the treatment of heart failure because of its salutary cardiac effects.1 However, cardiac actions of GH in heart failure have been investigated with contradicting results, which could be due to differences in species or in the type of heart failure. GH had beneficial effects in experimental heart failure induced by myocardial infarction in rats2 but did not improve cardiac performance in dogs with pacing-induced heart failure3 or in cardiomyopathic hamsters with severe heart failure.4 Similarly, observations in humans are ambiguous. GH therapy could improve cardiac performance in an uncontrolled study in patients with dilated cardiomyopathy,1 and acute intravenous GH application increased the cardiac index and reduced the elevated pulmonary pressure in patients with severe heart failure.5 In contrast, after 3 months of GH therapy, cardiac function remained impaired in patients with dilated cardiomyopathy, despite an increase of left ventricular mass.6 Along with its cardiac effects, GH application enhances glomerular filtration rate and renal plasma flow in normal humans and animals.7 Whether GH treatment induces beneficial renal effects in experimental heart failure and whether renal and cardiac effects are correlated is not known. No data are available on the effects of GH on volume overload–induced heart failure, which is characterized by biventricular dilation and impaired water and sodium handling. Therefore, the present study was designed to determine the effects of GH treatment on cardiac and renal function in rats with aortocaval shunt, a model of volume overload–induced heart failure.

Methods

Animals and GH Treatment

Male Wistar rats (230 to 250 g) were used for all experiments. An infrarenal aortocaval shunt was induced under ether anesthesia by

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Influence of 4-wk GH Treatment on Body and Organ Weight, Plasma Levels of Rat GH, IGF-1, Angiotensin II, ANP, Nitrate/Nitrite, and cGMP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n=11)</th>
<th>Shunt/Vehicle (n=9)</th>
<th>Shunt/GH (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>331±6</td>
<td>306±7</td>
<td>336±9</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.05±0.02</td>
<td>1.76±0.02†</td>
<td>1.99±0.09†‡</td>
</tr>
<tr>
<td>Relative heart weight, g/100 g BW</td>
<td>0.32±0.01</td>
<td>0.57±0.02†</td>
<td>0.60±0.03†</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>2.25±0.05</td>
<td>2.08±0.05*</td>
<td>2.20±0.08</td>
</tr>
<tr>
<td>Relative kidney weight, g/100 g BW</td>
<td>0.68±0.01</td>
<td>0.68±0.02</td>
<td>0.67±0.04</td>
</tr>
<tr>
<td>GH, pmol/L</td>
<td>130±23</td>
<td>177±33</td>
<td>202±72</td>
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<tr>
<td>IGF-1, nmol/L</td>
<td>151±8</td>
<td>155±9</td>
<td>151±12</td>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td>153±15</td>
<td>467±79†</td>
<td>517±119†</td>
</tr>
<tr>
<td>ANP, fmol/L</td>
<td>178±52</td>
<td>2046±422†</td>
<td>862±434*</td>
</tr>
<tr>
<td>Nitrate/nitrite, μmol/L</td>
<td>50±11</td>
<td>58±9</td>
<td>60±11</td>
</tr>
<tr>
<td>cGMP, nmol/L</td>
<td>43±8</td>
<td>98±10†</td>
<td>147±37*</td>
</tr>
<tr>
<td>LV dP/dtmax, mm Hg</td>
<td>9.2±1.8</td>
<td>16.5±2.1*</td>
<td>19.1±2.4*</td>
</tr>
<tr>
<td>LV dP/dtmax, mm Hg/s</td>
<td>4927±242</td>
<td>4019±281*</td>
<td>3568±377*</td>
</tr>
</tbody>
</table>

BW indicates body weight; LVdP/dtmax, left ventricular end-diastolic pressure; and LV dP/dtmax, left ventricular maximal pressure development. Values are mean±SEM.

*P<0.05 and †P<0.01 vs sham; ‡P<0.05 vs shunt/vehicle.

Measurement of the NO Metabolites, NOS Activity, and Renal NOS mRNA Expression

Oral concentrations of stable NO metabolites (nitrate/nitrite) were estimated by the Griess reaction as described previously. Renal NOS activity was measured as a production of the nitrate/nitrite and S-nitrosothiol (RSNO) in cytosolic extracts by Griess reaction in the presence or absence of 1 mmol/L L-EGTA, an inhibitor of constitutive NOS isoforms, or 1 mmol/L aminoguanidine, an inhibitor of inducible NOS isoforms. Total RNA was isolated from kidney, liver, and cardiac left ventricular tissues by using the RNasey Kit (Qiagen GmbH) according to the manufacturer’s protocol. The expressions of NOS and GAPDH mRNA were analyzed by TaqMan–polymerase chain reaction (TaqMan–EZ RT-PCR Kit, Perkin–Elmer) by using the following primers: NOS1 forward (5'-CTGGAGGATGTCAAGGCTTC-3'), NOS1 reverse (5'-ACAGTAGTCAGGACGCCG-3'), NOS2 forward (5'-AGGACTAAACATGCTGGCTTTG-3'), NOS2 reverse (5'-GTCCTG CTGACTGCTGCC-3'), NOS3 forward (5'-CAACTCTGTTCA CGCC-3'), NOS3 reverse (5'-TCACATGAGGCGTGGTCCC-3'), GAPDH forward (5'-GATGCACTACCGGCAAGT-3'), and GAPDH reverse (5'-GATGCGTCTGCCATTGATGAC-3'), and specific probes. RNA expression data were calculated from the cycle threshold (Ct) value and normalized to the corresponding GAPDH mRNA expression.

Statistical Analysis

Differences between groups were evaluated by ANOVA with a posteriori comparison (Fisher protected least significant difference). All data are expressed as mean±SEM.

Results

Effect of GH Treatment on Body Weight, Organ Weight, GH, and IGF-1 Plasma Levels

The Table shows the effects of GH treatment. GH treatment for 4 weeks increased the body weight in shunt-operated rats compared with vehicle-treated rats. Absolute heart weight was higher in GH-treated rats, whereas the relative heart weight in GH-treated rats was not different from that in vehicle-treated rats. The absolute kidney weight was significantly reduced 4 weeks after shunt induction and was normalized by GH application. Plasma levels of rat GH were
measured to test whether GH secretion was impaired in rats with shunts. Rat GH plasma levels did not significantly differ between vehicle-treated shunt-operated rats and sham-operated rats and were not influenced by treatment with human GH, indicating that the endogenous GH secretion was not modified by exogenous human GH. Similarly, plasma levels of IGF-1 were not different between shunt- and sham-operated rats and were not influenced by treatment with human GH.

**Hemodynamic Effects**

To determine the influence of GH on cardiac performance in this model of heart failure, we analyzed the left ventricular function. In vehicle-treated animals with aortocaval shunt, heart failure was characterized by elevated central venous pressure (data not shown), increased left ventricular end-diastolic pressure, and decreased left ventricular contractility (Table). GH treatment did not modify left ventricular end-diastolic pressures or left ventricular contractility in shunt-operated animals.

**Renal Function After GH Treatment**

Water and sodium excretion were significantly reduced 4 weeks after the shunt operation compared with the sham operation (Figure 1a and 1b). After GH treatment, urinary output was enhanced (shunt/vehicle 10.8±0.6 mL/d, shunt/GH 15.8±0.7 mL/d; P<0.01), and sodium excretion was increased (shunt/vehicle 1.5±0.1 mmol/d, shunt/GH 2.2±0.1 mmol/d; P<0.01). To assess the mechanism for the improvement of renal function, glomerular filtration rate (GFR) was measured (Figure 2a). The GFR was reduced in shunt-operated animals compared with sham-operated control animals (sham 2.6±0.2 mL/min, shunt/vehicle 1.6±0.1 mL/min; P<0.001) and was not significantly changed after GH treatment (1.8±0.1 mL/min). However, fractional sodium excretion (Figure 2b) was significantly increased in the GH-treated group compared with the sham-operated group and with the vehicle-treated shunt-operated group (sham 0.33±0.04%, shunt/vehicle 0.40±0.04%, and shunt/GH 0.52±0.04%; P<0.05 versus shunt/vehicle), indicating a reduced tubular reabsorption of sodium after GH treatment.

**Hormonal Changes and cGMP Formation After GH Administration**

Because the renin-angiotensin and natriuretic peptide systems are activated in heart failure and are important contributors to fluid homeostasis, we measured plasma levels of angiotensin II and ANP (Table). Angiotensin II plasma concentration was elevated in shunt-operated rats receiving vehicle and did not significantly change after GH treatment. ANP plasma concentrations were significantly elevated in shunt-operated animals receiving vehicle compared with sham-operated control animals and showed a tendency toward lower levels after GH application. Plasma cGMP levels and urinary cGMP excretion were elevated in shunt-operated rats compared with sham-operated control animals and showed a tendency toward lower levels after GH administration. These data indicate that elevated cGMP formation after GH may not be explained by changes of the circulating ANP.

**Influence of Chronic GH Treatment on NO Production and Excretion of NO Metabolites**

NO is known to induce cGMP synthesis via activation of soluble guanylate cyclase. The plasma concentrations of nitrate/nitrite were not changed in shunt-operated animals compared with sham-operated control animals, and these concentrations were not influenced by GH application (Table). The excretion of NO metabolites was diminished in shunt-operated animals receiving vehicle compared with sham-operated control animals and showed a tendency toward lower levels after GH application. NO metabolites were not significantly changed after GH treatment. ANP plasma concentrations were significantly elevated in shunt-operated animals receiving vehicle compared with sham-operated control animals and showed a tendency toward lower levels after GH administration. These data indicate that elevated cGMP formation after GH may not be explained by changes of the circulating ANP.
inhibition of constitutive NOS isoforms by EGTA, renal RSNO generation was higher in GH-treated animals than in vehicle-treated animals. After the addition of aminoguanidine, the RSNO formation did not differ between the groups. These data indicate that the activation of the inducible NOS predominantly contributes to elevated NO and RSNO generation after GH.

mRNA Expression of NOS Isoforms

The expression of NOS1 and NOS3 mRNA in the kidney was not changed by either shunt operation or GH treatment (for NOS1, shunt/vehicle 64±7% of sham, shunt/GH 72±17% of sham; for NOS3, shunt/vehicle 97±22% of sham, shunt/GH 97±27% of sham). NOS2 mRNA expression was elevated in shunt-operated rats and showed a further, 3-fold, activation on GH administration (Figure 3b). NOS2 mRNA expression was investigated in liver and cardiac tissue of vehicle- and GH-treated animals. NOS2 mRNA was downregulated in the liver after shunt operation (33±7% of sham, *P<0.05) and was not modified by GH application (54±21% of sham). In contrast, cardiac NOS2 mRNA expression was not different in sham- and shunt-operated (78±6% of sham) rats but decreased after GH administration (24±5% of sham, **P<0.01 versus sham and shunt/vehicle).

Effect of Chronic NOS Inhibition With L-NAME

L-NAME treatment did not influence mean arterial blood pressure in either shunt group (shunt/vehicle 74±4 mm Hg, shunt+L-NAME 72±2 mm Hg, shunt/GH 67±3 mm Hg, and shunt/GH+L-NAME 67±2 mm Hg). Although L-NAME alone had no effect on urine volume and sodium excretion in shunt-operated rats receiving vehicle, the increases of diuresis and sodium excretion on day 28 after GH were partially reversed by L-NAME (Figure 1). Similarly, the elevation of fractional sodium excretion after GH treatment was reversed in rats receiving L-NAME, whereas the GFR was not influenced by either treatment (Figure 2a and 2b). As demonstrated in Figure 2c, urinary cGMP excretion in shunt-

$$\text{Figure 2. GFR (a) and fractional sodium excretion (FE}_\text{Na} \text{(b), urinary excretion of cGMP (c), and excretion of nitrate/nitrite (d) are shown for sham-operated rats and for shunt-operated rats with vehicle, with L-NAME, with GH, or with GH+L-NAME treatment. }^* \text{P}<0.05, \quad \text{**P}<0.01, \quad \text{***P}<0.001 \text{ vs sham; } ^# \text{P}<0.05 \text{ and } ^## \text{P}<0.01 \text{ vs shunt/vehicle; and } ^\$ \text{P}<0.05 \text{ vs shunt/GH.}}$$

$$\text{Figure 3. Renal RSNO formation (a) and NOS2 mRNA expression (normalized to GAPDH mRNA) (b) in sham-operated rats and in shunt-operated rats with vehicle or with GH. }\Delta \text{P}<0.05 \text{ vs total RSNO; } ^* \text{P}<0.05 \text{ and } ^** \text{P}<0.01 \text{ vs sham; } ^# \text{P}<0.05 \text{ vs shunt/vehicle; and } ^\$ \text{P}<0.05 \text{ vs shunt/vehicle+EGTA.}$$
operated rats was not influenced by L-NAME alone, whereas the increase of cGMP excretion after GH application was abolished by L-NAME administration (shunt/GH 463±57 nmol/d, shunt/GH+L-NAME 347±42 nmol/d; \( P<0.05 \)). In parallel, urinary nitrate/nitrite excretion was significantly reduced after L-NAME treatment (shunt/GH 2993±375 nmol/d, shunt/GH+L-NAME 2111±247 nmol/d; \( P<0.05 \) versus shunt/GH; Figure 2d).

Discussion
The present study demonstrates that treatment with human GH does not improve cardiac function in rats with heart failure induced by chronic volume overload. In this model, heart failure was demonstrated by elevated left ventricular end-diastolic pressure and depressed left ventricular contractility. After GH treatment for 4 weeks, these hemodynamic parameters were unchanged. In contrast to the failure of improving cardiac function, favorable effects on the renal function were observed after chronic GH treatment. Fazio et al\(^1\) have demonstrated hemodynamic improvement by chronic GH treatment in 7 patients with dilated cardiomyopathy,\(^1\) and the beneficial cardiac effects of GH have been confirmed in patients with ischemic heart disease\(^12\) and in rats with myocardial infarction.\(^2\)\(^,\)\(^3\)

In contrast to the salutary GH effects observed in ischemic cardiomyopathy, the present study failed to show an improvement of cardiac performance in volume overload–induced heart failure. This is in agreement with the findings of Shen et al\(^3\) who reported no effect of GH on impaired cardiac function due to rapid ventricular pacing in dogs. When patients with heart failure of different etiologies were analyzed, no effect of chronic GH administration on either cardiac function or neuroendocrine activation was observed.\(^14\) One reason for these contradictory findings might be the heterogeneous origin of heart failure. An alternative explanation is the possible involvement of cardiomyocyte apoptosis occurring after myocardial infarction,\(^15\) which might have been suppressed by GH and IGF-1,\(^16\) suggesting a possible specific effect of GH in ischemic heart failure. Because the dose of 2 mg/kg GH has been shown to improve cardiac function after 2 weeks in rats with myocardial infarction,\(^2\) we applied this dose, but we cannot exclude beneficial effects on the cardiac performance after a higher dose and/or prolonged GH treatment in the model of volume overload–induced heart failure.

IGF-1 is known to mediate many of the physiological actions of GH.\(^17\) Low levels of IGF-1 have been observed in patients with dilated cardiomyopathy,\(^18\) suggesting a relative GH resistance in severe human heart failure. In the present study, no differences in plasma levels of either GH or IGF-1 could be detected between sham- and shunt-operated rats, suggesting that GH resistance might be dependent on the species investigated. The possibility that the salutary renal effects observed in the present study are independent of IGF-1 is supported by the lack of downregulation of endogenous GH, whose secretion is known to be depressed by high levels of IGF-1.\(^19\)

To explore the potential effects of chronic GH treatment on fluid and sodium retention in congestive heart failure, we assessed renal excretory function. Although shunt-operated animals showed a progressive water and sodium retention, GH treatment enhanced diuresis and total and fractional sodium excretion after 4 weeks. To our knowledge, this is the first report demonstrating an improvement of renal excretory function by GH in congestive heart failure. When renal function was assessed in dogs with pacing-induced heart failure, GH treatment did not influence the glomerular filtration rate, diuresis, or sodium excretion in these animals.\(^3\) However, renal function was preserved in this model of heart failure. Although fluid retention during GH administration has been observed and has been associated with a temporary activation of the renin-angiotensin system,\(^20\) these effects are inconsistent. GH administration for 6 months did not impair water and sodium homeostasis in normal rats.\(^21\) We did not observe any increase in plasma angiotensin II concentration after 4 weeks of treatment, supporting the notion that the activation of the renin-angiotensin system might be a transient phenomenon.

The beneficial effect on renal function in the present study was not induced by an increase in GFR. When GH was administered in rats with reduced renal mass, compensatory renal hypertrophy occurred without enhancement of GFR.\(^22\) cGMP, the mediator of the actions of NO and of the natriuretic peptides, was excreted more in shunt-operated rats receiving GH. Plasma concentrations of ANP, whose actions are mediated by the particulate guanylate cyclase, although elevated in rats with heart failure, were not influenced by GH treatment, suggesting that ANP is not involved in the enhanced renal cGMP excretion. Because the soluble guanylate cyclase is activated by NO, we investigated this pathway and observed an elevated urinary excretion of nitrate and nitrite, without changes of the corresponding plasma levels, reflecting enhanced renal NO generation.

Several in vivo and in vitro studies have demonstrated that NO induces natriuresis.\(^23\) The influence of NO on renal transport processes is indicated by reduced sodium reabsorption in the proximal tubule\(^24\) and the cortical collecting duct,\(^25\) in parallel with cGMP accumulation in these nephron segments. Acute inhibition of NO synthesis by L-NAME has been shown to decrease fractional sodium excretion and also to decrease urinary and plasmatic cGMP concentrations in healthy humans.\(^26\) These observations indicate that NO plays an important physiological role in the regulation of sodium and water transport in the kidney. In the present study, shunt-operated animals had lower nitrate and nitrite excretion compared with control animals, indicating a reduced activity of the NO system in heart failure. GH treatment improved diuresis and natriuresis in parallel with the enhanced excretion of the NO metabolites nitrate and nitrite. Thus, the improved renal NO generation might have induced beneficial effects in our model of heart failure.

The administration of L-NAME abolished the GH-induced enhancement of diuresis, sodium excretion, and fractional sodium excretion along with a reduction of nitrate/nitrite and cGMP excretion. The inhibition of NOS by L-NAME supports the concept of NO as a mediator of the renal effects of GH via an activation of the renal NO and cGMP pathway. Recently, NOS expression has been shown to be induced by...
GH overexpression in mesangial cells,27 and enhanced NO excretion after GH administration has been described after chronic replacement therapy in GH-deficient patients.28

The exact mechanism for the activation of the NO pathway by GH is not known. The measurement of renal NOS mRNA in the present study identified an increase of NOS2 mRNA expression after GH treatment, suggesting an involvement of renal inducible NOS synthesis. In accordance with the mRNA data, the present data reflected an enhanced activity of the renal inducible NOS. Interestingly, we found a different regulation of the NOS2 isoform mRNA in the liver and heart, suggesting that the regulation of NOS mRNA expression is tissue specific.

In summary, GH treatment does not influence cardiac function in rats with aorto-caval shunts. However, GH improved renal function, as demonstrated by increased diuresis and sodium excretion along with enhanced fractional sodium excretion. The responsible mechanism might be the specific induction of renal inducible NOS and subsequent enhanced activity of the renal NO system. Even though the present study demonstrates beneficial effects on renal excretory function, it is presently uncertain whether GH might be a useful adjunctive treatment in some forms of heart failure.

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References

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