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Reference

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Cardiac and Renal Effects of Growth Hormone in Volume Overload–Induced Heart Failure
Role of NO

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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 mmol/d, GH 463±57 mmol/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 mmol/d, GH 2993±375 mmol/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 Nω-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. 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 rats but did not improve cardiac performance in dogs with pacing-induced heart failure or in cardiomyopathic hamsters with severe heart failure. Similarly, observations in humans are ambiguous. GH therapy could improve cardiac performance in an uncontrolled study in patients with dilated cardiomyopathy, and acute intravenous GH application increased the cardiac index and reduced the elevated pulmonary pressure in patients with severe heart failure. In contrast, after 3 months of GH therapy, cardiac function remained impaired in patients with dilated cardiomyopathy, despite an increase of left ventricular mass. Along with its cardiac effects, GH application enhances glomerular filtration rate and renal plasma flow in normal humans and animals. 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
using a needle with a 1.8-mm outer diameter. Sham-operated control rats were treated identically, except that no puncture of the vessels was performed. Each group consisted of 9 to 14 animals. Starting the first day after surgery, shunt-operated animals received either human recombinant GH (2 mg/kg body wt per day, SQ, Lilly Deutschland GmbH) or isotonic saline for 4 weeks. Sham-operated control rats were treated identically, except that no puncture of the aorta on day 28. Plasma concentrations of the peptides and of cGMP were measured by radioimmunoassay as described previously. ANP and cGMP antibodies were generously donated by Dr J. Gutkowska and Dr P. Hamet, Montreal, Canada.

### Hemodynamic Measurements
Under chloral hydrate anesthesia, a PE-50 tubing catheter was inserted via the right carotid artery into the left ventricle for measurement of mean arterial and end-diastolic pressures. Central venous pressure was measured by cannulating the left jugular vein. Left ventricular contractility (dP/dtmax) was obtained from the ventricular pressure curves.

### Renal Excretory Function
Animals were kept in individual metabolic cages 1 day before surgery and during postoperative days 1 to 5, 15 to 17, and 26 to 28. Urine was collected daily, and water intake and urine volume were measured. Plasma and urine creatinine concentrations were measured for analysis of the glomerular filtration rate on day 28. Daily diuresis and sodium excretion were calculated by averaging the values for 3 consecutive days.

### Chronic NOS Inhibition
To investigate the role of NO for renal GH effects, 2 additional groups treated with either vehicle or GH received a subpressor dose of the NO synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 50 mg/L) in their drinking water, as described previously.

### Determination of IGF-1, GH, ANP, cGMP, and Angiotensin II
Blood samples for insulin-like growth factor (IGF)-1, rat GH, atrial natriuretic peptide (ANP), cGMP, and angiotensin II were taken via puncture of the aorta on day 28. Plasma concentrations of the peptides and of cGMP were measured by radioimmunoassay as described previously. ANP and cGMP antibodies were generously donated by Dr J. Gutkowska and Dr P. Hamet, Montreal, Canada.

### Measurement of the NO Metabolites, NOS Activity, and Renal NOS mRNA Expression
Urinary 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 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 RNeasy 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'-CTGGAGTTCAGGCCTGTC-3'), NOS1 reverse (5'-ACAGTAGTCCAG GACGCCG-3'), NOS2 forward (5'-AGCCCTAAGC ACGTG GCTTGTGTT-3'), NOS2 reverse (5'-GTCTCTGT GCTGCTAGTGTCCC-3'), NOS3 forward (5'-CAATCTTGGT CAGCCACAT-3'), NOS3 reverse (5'-TCCA GATCCGAAGTCTCC-3'), GAPDH forward (5'-TGATTT ACCCA GCCAACAGTT-3'), GAPDH reverse (5'-GATGGGT TCTCCATGATGAC-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
water and sodium excretion were significantly reduced 4 weeks after the shunt operation 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 rats (Table, Figure 2c) and were further increased 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 animals (sham 2.6±0.2 mmol/d, shunt/vehicle 1.6±0.1 mmol/d; *P<0.01) and was not significantly changed after GH treatment (1.8±0.1 mmol/d). 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.

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.

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-systolic pressure, and decreased left ventricular contractility (Table). GH treatment did not modify left ventricular end-systolic pressures or left ventricular contractility in shunt-operated animals.

Renal Function After GH Treatment

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Hemodynamic Effects

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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<0.05 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-
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 contrac-
tility. 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 have demonstrated hemodynamic improvement by
chronic GH treatment in 7 patients with dilated cardiomyop-
athy, and the beneficial cardiac effects of GH have been
confirmed in patients with ischemic heart disease and in rats
with myocardial infarction.

In contrast to the salutary GH effects observed in ischemic
cardiomyopathy, the present study failed to show an improve-
ment of cardiac performance in volume overload--induced
heart failure. This is in agreement with the findings of Shen
et al, 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 anal-
yzed, no effect of chronic GH administration on either
cardiac function or neuroendocrine activation was observed.
One reason for these contradictory findings might be the
heterogeneous origin of heart failure. An alternative explana-
tion is the possible involvement of cardiomyocyte apoptosis
occurring after myocardial infarction, which might have
been suppressed by GH and IGF-1, 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, 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. Low levels of IGF-1 have been observed in
patients with dilated cardiomyopathy, 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 endog-
enous GH, whose secretion is known to be depressed by high
levels of IGF-1.

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 filtra-
tion rate, diuresis, or sodium excretion in these animals.
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, these effects are
inconsistent. GH administration for 6 months did not impair
water and sodium homeostasis in normal rats. 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 tran-
sient 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.
Cyclic GMP, 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 en-
hanced 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, reflect-
ing enhanced renal NO generation.

Several in vivo and in vitro studies have demonstrated that
NO induces natriuresis. The influence of NO on renal
transport processes is indicated by reduced sodium reabso-
ption in the proximal tubule and the cortical collecting duct,
in parallel with cGMP accumulation in these nephron seg-
ments. 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. 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 excre-
tion 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 sup-
ports 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, and enhanced NO excretion after GH administration has been described after chronic replacement therapy in GH-deficient patients.

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 aortocaval 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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