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Effects of rhGH and rhIGF-1 on renal growth and morphology

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Effects of rhGH and rhIGF-1 on renal growth and morphology. It is known that in rodents recombinant human growth hormone (rhGH) and recombinant human insulin-like growth factor (rhIGF-1) increase renal mass. It is uncertain, however, whether renal mass increases in proportion to body growth, or whether renal growth is stimulated selectively. In 120 to 150 g female Sprague-Dawley rats, we measured the effects of rhGH and rhIGF-1 and their combination by the following parameters: kidney weight/body weight ratio, DNA/protein ratio, mRNA of GH receptor and of IGF-1, mitosis index and PCNA (by immunohistology), zonal architecture and glomerular diameter by micromorphometry. Both rhGH and rhIGF-1 dose-dependently increased renal weight and body weight over vehicle treated controls. With rhGH, liver dry weight/body weight ratio increased, but kidney dry weight/body weight ratio remained unchanged ($0.99 \pm 0.06 \times 10^{-3}$ vs. 1.02 ± 0.07 in vehicle controls). In contrast, a significant increase of kidney dry weight/body weight ratio was seen in rats treated with rhIGF-1 ($1.3 \pm 0.21 \times 10^{-3}$). Addition of high doses of rhGH to high doses of rhIGF-1 caused no further increase of the ratio despite a significant further increase of body weight. rhGH increased the abundance of renal GH receptor mRNA (0.46 ± 0.32 amol/μg DNA vs. 0.08 ± 0.07 in controls) and of IGF-1 mRNA (1.35 ± 0.5 pg/μg DNA vs. 0.35 ± 0.17), whereas no change was seen with IGF-1 treatment. rhGH and rhIGF-1 increased kidney DNA/protein ratio, mitoses and PCNA expression in various renal structures. Further stimulation of mitoses by rhGH was seen even after subtotal nephrectomy, which was associated with markedly stimulated baseline proliferation of renal cells. The results document that rhGH increases renal weight in proportion to body weight while rhIGF-1 increases it out of proportion to body weight. Both peptides increase renal glomerular and tubular cell proliferation and renal DNA/protein ratio. This observation points to a major role of hyperplasia in renal weight gain of young female animals treated with the peptides. The size of glomeruli increased, but the increment was in proportion to body weight. Glomerulosclerosis was not detected even after 60 days of treatment with rhGH or with rhIGF-1.

Acromegaly is associated with reversible nephromegaly and increased glomerular filtration rate (GFR) [1–3]. Furthermore, it is known that extractive growth hormone (GH) [4] or recombinant GH [5] and insulin-like growth factor (IGF)-1 [6] raise GFR.

Recombinant human (rh) GH promotes growth in uremic animals [7–9] and uremic children [10], and is therefore widely used for the treatment of growth retardation in children with renal failure. Concern has been raised that stimulation of GFR and renal growth might accelerate progression of renal failure, since both hyperfiltration [11] and glomerular enlargement [12] are important determinants of progression of renal failure. Although renal failure is not a known consequence of acromegaly [13], this concern is well founded since glomerulosclerosis occurs in transgenic mice expressing the GH gene [14, 15].

It has been shown that renal weight is increased in transgenic mice expressing GH [14, 16], in acromegalic rats bearing GH producing hypophyseal tumors [17], and rats treated with rhGH [18–20]. It is currently unknown, however, whether rhGH and rhIGF-1 have specific renotropic effects, that is, whether renal growth outstrips body growth, or whether they stimulate renal growth in proportion to body growth. The present study was carried out to address this issue.

Methods

Animals

Female Sprague-Dawley (SD) rats (Ivanovas Co., Kisslegg/Allgäu, Germany), weighing 120 to 150 g, were used for the experiments 1 to 8. Female rats were chosen because a better response to growth hormone in female rats had previously been documented by others [8]. One week prior to the study, the animals were transferred to single cages at constant room temperature (24°C) and humidity (70%) on a 12 hour on/12 hour off light cycle. The diet contained 13800 kJ/kg, 0.95% calcium, 0.8% phosphorus, 500 IU/kg vitamin D_3, and 18% protein (wt/wt). Experimental animals and controls had free access to food (Altromin C 1000 diet, Altromin Co., Lage/Lippe, Germany) and deionized water.

The experiments 1 to 8 were not contemporaneous. In each experiment, animals were assigned to the experimental or control groups using random numbers.

In experiment 8, the animals were subjected to two-stage subtotal nephrectomy (NX) or sham operation as described previously [7, 21]. Subtotal nephrectomy of the left kidney was performed one week prior to surgical removal of the right kidney. At this moment, the animals had a mean body weight of 136 g. Control animals (Co) were sham-operated (renal decapsulation). The control group was pair fed as previously described [7].

Recombinant peptides

rhGH and rhIGF-1 were administered subcutaneously twice daily in doses indicated under *Protocols*. rhGH (Genotropin)

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Table 1. Dose dependent effect of rhGH for seven days on growth in SD rats

rhGH *IU/kg/day*	Weight gain *g*	Length gain *cm*	Cumulative food intake *g/7 days*	Weight gain *g* / food intake *g*	Kidney dry weight *mg*	Kidney dry weight *g* / body weight *g* $\times 10^{-3}$
Vehicle	16.4 ± 7.1	1.93 ± 0.29	91 ± 10	0.17 ± 0.07	168 ± 16	1.0 ± 0.06
2.5	32.1 ± 4.9[b]	2.30 ± 0.29[a]	97 ± 13	0.33 ± 0.03[b]	179 ± 14	0.97 ± 0.07
5.0	36.0 ± 6.1[b]	2.54 ± 0.10[b]	98 ± 8	0.36 ± 0.04[b]	179 ± 3	0.96 ± 0.03
10.0	35.4 ± 6.1[b]	2.62 ± 0.39[b]	96 ± 11	0.36 ± 0.05[b]	186 ± 10	1.0 ± 0.04
20.0	41.0 ± 6.1[b]	2.61 ± 0.29[b]	106 ± 9	0.39 ± 0.05[b]	188 ± 7	0.97 ± 0.03

$N = 6$ animals per group.
Significant difference between vehicle group and experimental group ([a] $P < 0.05$, [b] $P < 0.01$)

Table 2. Organ weight/body weight ratio under rhGH treatment with increasing duration of treatment

Treatment duration *days*	Kidney dry weight *g* / body weight *g* $\times 10^{-3}$		Heart dry weight *g* / body weight *g* $\times 10^{-3}$	
	10 IU rhGH/kg/day	Vehicle	10 IU rhGH/kg/day	Vehicle
0		1.19 ± 0.05		0.98 ± 0.04
4	1.11 ± 0.04	1.14 ± 0.05	0.98 ± 0.05	0.95 ± 0.08
8	1.03 ± 0.05	1.11 ± 0.04	0.90 ± 0.05	1.02 ± 0.13
11	1.10 ± 0.20	1.10 ± 0.08	0.94 ± 0.03	0.94 ± 0.04
21	1.25 ± 0.12	1.24 ± 0.06	0.95 ± 0.06	0.95 ± 0.05
60	0.88 ± 0.06	0.88 ± 0.06	0.70 ± 0.04	0.77 ± 0.07

$N = 7$ animals per group.

was provided by Kabi Pharmacia Peptide Hormones (Stockholm, Sweden), rhIGF-1 by Ciba-Geigy Co. (Basel, Switzerland; CGP 35'126), and by Kabi Pharmacia Peptide Hormones (Stockholm, Sweden; CH/B/60229–51).

Protocols

Experiment 1: Dose dependent effects of rhGH for seven days on growth in SD rats (Table 1). Five groups of healthy animals ($N = 6$ animals per group) were treated with increasing doses of rhGH ranging from 0 to 20 IU/kg/day given in two divided doses s.c. for a period of seven days.

Experiment 2: Time course of the effect of 10 IU rhGH/kg/day on growth in SD rats (Table 2). Five groups of healthy animals ($N = 7$ animals per group) were treated with 10 IU rhGH/kg day s.c. in two divided doses for various time intervals ranging from four to 60 days.

Experiment 3: Dose- and time-dependent effects of rhIGF-1 on growth in SD rats (Table 3). Six groups of healthy animals ($N = 7$ animals per group) were treated with increasing doses rhIGF-1 ranging from 0 to 16 mg/kg/day in two divided doses s.c. for a period of seven days. Five animals were treated with 3 mg rhIGF-1/kg/day for 60 days.

Experiment 4: Effects of concomitant treatment with rhGH (20 IU/kg/day) and rhIGF-1 (8 mg/kg/day) for 14 days on growth in SD rats (Table 4). The number of animals was 10 for the three treatment groups (20 IU rhGH/kg/day; 8 mg rhIGF-1/kg/day; 20 IU rhGH + 8 mg rhIGF-1/kg/day) and for the control (vehicle) group. Peptides and vehicle were injected in two divided doses twice daily s.c.

Experiment 5: Effect of rhGH (10 IU/kg/day) and of rhIGF-1 (3 mg/kg/day) for five days on GHR mRNA and IGF-1 mRNA in kidney and liver tissue (Table 5). The number of animals was seven for the vehicle and treatment groups. Peptides and vehicle were injected in two divided doses twice daily s.c.

Experiment 6: Effects of 10 IU rhGH/kg/day for 14 days on renal morphology in SD rats (Table 6). Nine animals were studied in the treatment and control (vehicle) groups, respectively. The animals were injected with 10 IU rhGH/kg/day s.c. in two divided doses twice daily. At the end of the experiment, the kidneys were fixed by perfusion technique for analysis of renal architecture and glomerular stereology.

Experiment 7: Effect of treatment with rhGH (10 IU/kg/day) or rhIGF-1 (4 mg/kg/day) for five days on renal DNA/protein ratio in SD rats (Table 7). The number of animals was seven for the treatment and for the control (vehicle) groups. The animals were injected with 10 IU rhGH/kg/day or 4 mg rhIGF-1/kg/day, respectively. The peptides were administered s.c. in two divided doses twice daily.

Experiment 8: Effect of treatment with rhGH (10 IU/kg/day) or rhIGF-1 (4 mg/kg/day) for 4 days on mitoses and PCNA expression of tubular and glomerular cells (Table 8). The number of animals was seven for the treatment groups [renal intact animals and subtotally nephrectomize (NX) animals treated with rhGH or with rhIGF-1] and the control groups (renal intact animals and subtotally NX animals treated with vehicle). All groups had free access to food. The renal intact animals were pair fed to subtotally NX animals. The animals received 5 i.p. injections of colchicine (Demecolcin, Serva Co., Heidelberg, Germany) or vehicle at 30 minute intervals prior to the end of the experiment in the morning hours (yielding a total colchicine dose of 800 μg). Kidneys were excised under anesthesia and fixed in 5% buffered formalin. A second set of animals was not pretreated with colchicine prior to death. Renal tissue was fixed in methyl Carnoy's solution and processed for detection of PCNA.

Analytical techniques

In vivo *measurements*. Body weight was measured during the afternoon in non-fasting animals. Food intake was measured daily and food conversion ratio (weight gain per food intake) was calculated [8]. Nose to tail tip distances were measured in anaesthesized animals under complete muscle relaxation as described previously [21]. Blood pressure was measured by tail plethysmography as described previously [21].

Organ weight. The animals were sacrificed by aortic puncture under intramuscular anesthesia (30 mg/kg Ketanest, Park Davis Co., Berlin, Germany and 0.3 mg/kg Valium, Hoffmann La Roche, Grenzach-Wyhlen, Germany). Organs were weighed before and after desiccation (24 hr; 80°C in the presence of desiccant). Kidney volume was quantitated by measuring the volume of water displaced after submersion.

Biochemistry and hormonal measurements. Serum biochemistry was analyzed using a multichannel Technicon Autoanalyzer (Technicon Instruments, Tarrytown, New York, USA).

Table 3. Dose dependent effect of rhIGF-1 for seven days on growth in SD rats

rhIGF-1 *mg/kg/day*	Weight gain *g*	Length gain *cm*	Cumulative food intake *g*	Weight gain *g* / food intake *g*	Kidney dry weight *mg*	Kidney dry weight *g* / body weight *g* $\times 10^{-3}$
Vehicle	25.6 ± 3.0	2.81 ± 0.30	117 ± 9	0.21 ± 0.03	158 ± 6	1.06 ± 0.06
1	33.0 ± 4.1[b]	3.33 ± 0.28[a]	109 ± 9	0.25 ± 0.04[a]	170 ± 8	1.09 ± 0.04
2	34.3 ± 5.0[b]	3.80 ± 0.70[b]	132 ± 13	0.26 ± 0.04[a]	175 ± 12	1.10 ± 0.04
4	35.4 ± 5.3[b]	3.84 ± 0.40[b]	104 ± 10	0.34 ± 0.05[b]	180 ± 6	1.13 ± 0.03[a]
8	39.6 ± 6.9[b]	4.04 ± 0.16[b]	113 ± 12	0.35 ± 0.05[b]	189 ± 14	1.15 ± 0.08[b]
16	40.2 ± 3.3[b]	4.20 ± 0.2[b]	112 ± 9	0.36 ± 0.03[b]	193 ± 16	1.18 ± 0.08[b]

$N = 7$ animals per group
Significant difference between vehicle group and experimental group ([a] $P < 0.05$; [b] $P < 0.01$)

Table 4. Effect of concomitant treatment with rhGH (20 IU/kg/day) and rhIGF-1 (8 mg/kg/day) for 14 days on growth in SD rats

	Vehicle ($N = 10$)	rhGH ($N = 10$)	rhIGF-1 ($N = 10$)	rhGH + rhIGF-1 ($N = 10$)
Initial weight *g*	135.9 ± 5.6	136.0 ± 5.6	135.3 ± 6.0	135.8 ± 5.9
Final weight *g*	178.9 ± 9.9	219.3 ± 8.2[b]	202.1 ± 10.3[c]	236.6 ± 7.4[e]
Δ Weight *g*	43.0 ± 5.6	83.3 ± 7.6[b]	66.8 ± 7.2[c]	100.8 ± 6.5[e]
Initial length *cm*	33.1 ± 0.9	33.1 ± 0.8	33.2 ± 0.9	33.2 ± 0.6
Final length *cm*	36.8 ± 0.7	39.1 ± 0.7[b]	39.3 ± 0.8[c]	39.9 ± 0.5
Δ Length *cm*	3.7 ± 0.7	6.0 ± 0.9[b]	6.1 ± 0.7[c]	6.7 ± 0.7
Kidney dry weight *mg*	182 ± 15	216 ± 18[b]	269 ± 42[c]	266 ± 34[e]
Liver dry weight *mg*	2,121 ± 160	3,195 ± 210[b]	2,955 ± 172[c]	3,695 ± 320[e]
Spleen dry weight *mg*	109 ± 16	141 ± 15[b]	153 ± 19[c]	164 ± 18[e]
Heart dry weight *mg*	160 ± 8	195 ± 13[b]	185 ± 17[c]	212 ± 19
Muscle dry weight *mg*	342 ± 40	392 ± 44[a]	367 ± 37	399 ± 22
Kidney dry weight / body weight $\times 10^{-3}$	1.02 ± 0.07	0.99 ± 0.06	1.30 ± 0.21[c]	1.12 ± 0.13[d]

[a] $P < 0.05$, [b] $P < 0.01$, rhGH vs. vehicle
[c] $P < 0.01$, rhIGF-1 vs. vehicle
[d] $P < 0.05$; [e] $P < 0.01$, rhGH + rhIGF-1 vs. rhGH

Table 5. Effect of 3 mg rhIGF-1/kg/day or 10 IU rhGH/kg/day for five days on growth hormone receptor (GHR) mRNA and IGF-1 mRNA in kidney and liver

	Vehicle	rhGH	rhIGF-1
Kidney			
IGF-1 mRNA *pg/μg DNA*	0.35 ± 0.17	1.35 ± 0.5[a]	0.6 ± 0.33
GHR mRNA *amol/μg DNA*	0.08 ± 0.07	0.46 ± 0.32[a]	0.16 ± 0.14
Liver			
IGF-1 mRNA *pg/μg DNA*	30.7 ± 12.5	43.0 ± 11.05	30.9 ± 4.94
GHR mRNA *amol/μg DNA*	3.28 ± 0.55	2.69 ± 0.43	3.3 ± 0.43

$N = 7$ animals per group.
[a] $P < 0.001$, rhGH vs. vehicle and rhIGF-1 (ANOVA)

Table 6. Effect of 10 IU rhGH/kg/day for 14 days on renal morphology in SD rats

	Vehicle ($N = 18$)	rhGH ($N = 18$)
Body weight *g*	176 ± 8.2	199 ± 7.9
Kidney volume[a] cm^3	1.04 ± 0.06	1.18 ± 0.08[b]
Cortex volume *% of total kidney volume*	78.4 ± 2.6	78.4 ± 2.4
Width of cortex + medulla *mm*	8.60 ± 0.75	8.80 ± 0.54
Width of outer + inner medulla *mm*	6.10 ± 0.71	6.30 ± 0.56
Width of inner medulla *mm*	4.20 ± 0.50	4.40 ± 0.47
Glomerular area μm^2	8481 ± 430	8951 ± 321[b]
Glomerular (3D) diameter *μm*	137.2 ± 3.5	140.5 ± 2.8[b]

[a] After perfusion fixation
[b] $P < 0.05$ vehicle vs. rhGH

Creatinine was determined by a kinetic method according to Jaffé without deproteinization; the within assay coefficient of variation was <3%. Measurements of rhGH, rhIGHF-1 and of rat GH were done by RIA techniques as described earlier [10, 18].

Chemical analysis of renal tissue. Renal tissue was frozen in liquid nitrogen and powdered. Total protein was measured according to Lowry. DNA was measured using a modified method according to Blin and Stafford [22]. In brief, tissue frozen in liquid nitrogen was powdered, 100 mg were mixed with the lysis buffer (0.075 M NaCl, 0.024 M ETDA, 0.02 M SDS, 1 M $NaHPO_4$) and extracted with 1:1 phenol-chloroform and 24:1 chloroform-isoamylalcohol (vol/vol). After precipitation with isopropanolol, DNA was quantitated photometrically.

Solution hybridization for GH receptor mRNA and IGF-1 mRNA in renal and liver tissue. (a) GH-receptor mRNA probe. A pT7T3 18 U vector carrying a 560 base pair BamHI fragment [23] was linearized with EcoRI cleaving in the cloning box of the vector, and labeled ([^{32}P]UTP or [^{35}S]UTP) cRNA was generated with T_3 polymerase (Promega, Madison, Wisconsin, USA) under the conditions indicated by the manufacturer.

(b) IGF-1 mRNA probe. The radioactive probe was prepared as described by Melton et al [24]. In brief, the DNA clone used was

Table 7. DNA/protein ratio of kidney in rhGH and IGF-1 treated SD rats

	Vehicle	rhGH *10 IU/kg/day*	rhIGF-1 *4 mg/kg/day*
Body weight *g*	141 ± 6	159 ± 6	143 ± 4
Δ Weight/5 days *g*	22.9 ± 2.6	40.4 ± 5.9	25.6 ± 2.5
Kidney dry weight *mg* left kidney	136 ± 8	143 ± 8	144 ± 15
Protein content *mg* right kidney	47.2 ± 4.6	53.9 ± 8.0	58.5 ± 4.9[a]
DNA content *μg*	1344 ± 395	3031 ± 923[a]	3432 ± 692[a]
DNA/protein ratio *μg/mg*	30.1 ± 10.6	58.8 ± 23.4[a]	59.0 ± 12.6[a]

$N = 7$ animals per group; duration of treatment 5 days.
[a] $P < 0.05$ difference treatment group vs. vehicle

a 153 bp genomic subclone of pSP64 in both orientations of mouse IGF-1 corresponding to exon 3 (by analogy with human IGF-1). Analyses of cDNA clones for IGF-1 indicate that two forms of IGF-1 mRNA could exist [25]. The structure of the probe used in this study would allow detection of both forms of IGF-1 mRNA.

GH receptor (GHR) mRNA was quantified by solution hybridization as previously described [26]. In brief, the tissue was homogenized in a buffer containing 1% $NaDodSO_4$, 20 mM Tris-HCl (pH 7.5), and 4 mM EDTA and digested with proteinase-K (200 μg/ml) at 35°C for 35 minutes. Total nucleic acids (TNA) were then extracted with phenol-chloroform. The TNA samples were prepared from liver and kidney tissue from individual rats.

TNA samples were hybridized at 70°C for 24 hours in 0.06 mM NaCl, 20 mM Tris-HCl (pH 7.5), 4 mM EDTA, 0.1% $NaDodSO_4$, 10 mM dithiothrol, and 25% formamide (vol/vol) with a ^{35}S-labeled GH-receptor cRNA probe in a volume of 40 μl. The samples were then treated with 40 μg RNase A and 2 μg RNase T_1 in the presence of 100 μg herring sperm DNA for 45 minutes at 37°C in a volume of 1 ml. Protected probe was precipitated with 100 μl trichloracetic acid (6 mol/liter), collected on glass-fiber filters (GF/C, Whatman International Ltd., Maidstone, UK) and counted in a scitillation counter. A tissue TNA preparation, originally compared with an *in vitro* transcribed GH-receptor mRNA, was used to generate a standard curve. The standard curve was linear between 0.1 and 16 amol. The DNA content of the samples was analyzed [27], and 10 to 45 μg DNA in samples were assayed. Within this range, the hybridization signal paralleled the standard curve. In all assays, the lowest concentration of GH receptor mRNA measured was well above the lowest point of the standard curve. Each TNA sample was analyzed in duplicate. The results are expressed as the amount of GHR mRNA per DNA (amol/μg). The coefficient of variation was 6% within and between experiments.

The solution hybridization assay for quantification of IGF-1 mRNA was described earlier [28]. It contained principally the same steps as the assay for quantification of GHR mRNA. The hybridization signal was compared with that of a tissue standard curve originally compared with known amounts of the synthetic 153 nucleotide mRNA strand, as described previously [29]. Results are expressed as the amount of IGF-1 mRNA/DNA (pg/μg). Each sample was analyzed in duplicate. The within-assay coefficient of variation (C.V.) was less than 20% in the range of 40 to 400 amol of the RNA standard. The between-assay C.V. was estimated by repeated analysis of the same TNA preparation and was 23%.

Mitosis counts in renal tissue and immunoperoxidase staining. (a) Counting of tubular cell mitoses in renal tissue. HE stained 8 μ sections of paraffin embedded tissue were used as described before [30]. Mitoses were counted at 400 × magnification using a Zeiss Co. Integrationsplatte II 100/25. Mitoses were counted in 50 fields each in the cortex, outer stripe, inner stripe and inner medulla using a systematic random sampling technique.

(b) Immunoperoxidase staining for counting glomerular cell proliferation. Four micrometer sections of methyl Carnoy's fixed renal tissue were processed by an indirect immunoperoxidase technique as previously described [31]. The proliferating nuclear antigen (PCNA), which is expressed by actively proliferating cells [32], was detected using 19A2 (Coulter Electronics, Hialeah, Florida, USA), a murine IgM monoclonal antibody against human PCNA. For all tissues, negative controls consisted of substitution of the primary antibody with equivalent concentrations of an irrelevant murine monoclonal antibody. In each kidney, over 40 cross sections (range 40 to 80) of consecutive cortical glomeruli containing more than 20 discrete capillary segments each were evaluated by a person who was unaware of the origin of the slides. Mean values per kidney were then calculated for the number of proliferating (PCNA+) cells per glomerular cross section.

Renal histology and renal morphometry. For histological evaluation of glomerulosclerosis and tubulointerstitial lesions in experiment 2 and experiment 3, the kidneys were fixed in formalin stained with hematoxylin/eosin and analyzed by light microscopy.

For morphometric studies, the viscera were fixed by retrograde vascular perfusion at a pressure of 110 mm Hg after catheterization of the distal abdominal aorta. Before fixation, the vascular system was flushed, with a dextran solution (Rheomacrodex^R; Schiwa Co., Glandorf, Germany) containing 0.5 g/liter procaine HCl. The vena cava inferior was incised to drain the blood.

The kidney was dissected into 1 mm thick slices cut perpendicularly to the interpolar axis. The specimen was embedded in paraffin and cut into 4 μm thick slices and then stained with hematoxylin/eosin. For area measurements, a semiautomatic image analyzing system (Videoplan, Kontron Co., Eching, Germany) was used. At a magnification of × 10, the area fraction of the cortex and medulla was determined and volume density and total volume were calculated as described [33]. At a magnification of × 400, the mean area of glomerular profiles was measured. Data are given as mean cross sectional area of glomeruli. The diameters were determined by identifying the inner edges of the thin parietal layer of cells forming Bowman capsule in every sixth consecutive glomerular section. Assuming a spherical size of glomeruli, the true three-dimensional diameter (stereological diameter) was obtained from the size distribution of diameters according to Weibel [33].

Statistics

Data are given as X ± SD or as mean (range). Results were evaluated using Wilcoxon's test for random samples or paired differences and by ANOVA.

Table 8. Effect of rhGH and of rhIGF-1 on mitoses in tubular cells of SD rats

Treatment	Renal status	Number of mitoses/mm²		
		Distal tubule	Proximal tubule	Total tubules
Vehicle	intact	8.3 ± 3.9	10.0 ± 5.4	18.3 ± 8.0
10 IU rhGH/kg/day	intact	12.2 ± 5.0[a]	17.7 ± 6.9[b]	29.9 ± 6.0[b]
Vehicle	subtotal NX	140.9 ± 29.7	209.8 ± 45.6	350.7 ± 67.8
10 IU rhGH/kg/day	subtotal NX	168.0 ± 22.2[a]	325.6 ± 30.3[b]	493.6 ± 38.6[a]
Vehicle	intact	8.3 ± 1.9	12.8 ± 2.5	21.1 ± 4.2
4 mg rhIGF-1/kg/day	intact	13.0 ± 2.2[b]	20.3 ± 4.4[a]	33.3 ± 5.5[b]
Vehicle	subtotal NX	130.3 ± 13.7	267.3 ± 14.8	397.6 ± 25.8
4 mg rhIGF-1/kg/day	subtotal NX	163.3 ± 15.3[b]	349.8 ± 33.8[b]	513.4 ± 43.7[b]

Duration of treatment was 4 days.
$N = 7$ animals per group.
[a] $P < 0.05$ and [b] $P < 0.01$ rhGH vs. vehicle or rhIGF-1 vs. vehicle

Results

Dose-dependent effects of rhGH for seven days on growth in SD rats

Short term s.c. administration of 2.5 to 20.0 IU of rhGH/kg/day in two divided doses caused significant increments in weight and length with inconsistent effects on cumulative food intake, but caused a significant increase of the food conversion ratio, that is, weight gain per food intake (Table 1). There were no significant effects on systolic blood pressure (vehicle 110 ± 5 mm Hg, 20 IU/kg/day 112 ± 4 mm Hg), serum creatinine (0.31 ± 0.06 vs. 0.32 ± 0.06 mg/dl) or serum phosphate (2.41 ± 0.15 vs. 2.46 ± 0.12 mmol/liter). Serum concentration of rhGH (by human GH RIA) 12 hours after the last injection of 5 IU rhGH/kg (which had been administered every 12 hr) was 13.9 ± 5.2 ng/ml. (The assay measured < 0.17 ng/ml in rats without rhGH injection). Concomitant measurements of rat GH showed that endogenous production of GH was suppressed (by specific rat assay 0.9 ± 0.6 ng/ml vs. 10.2 ± 7.3 ng/ml without rhGH treatment). There was some increase of IGF-1 serum concentration measured with RIA adapted for rats [18]: 479 ± 64 ng/ml without versus 654 ± 87 ng/ml with 10 IU rhGH/kg/day. Kidney dry weight increased with increasing doses of rhGH, but the kidney/body weight ratio was not significantly changed by rhGH.

Time course of the effect of rhGH on growth in SD rats

Ten international units of rhGH/kg/day by twice daily s.c. injections caused a progressive increment of cumulative weight gain and length gain above the vehicle treated controls (Table 2). As in experiment 1, there was a consistent increase in the food conversion ratio, but no significant increase of the kidney dry weight or heart dry weight/body weight ratio despite treatment for up to 60 days. The organ weight/body weight ratios changed with time both in the experimental and in the control groups. Renal histology did not demonstrate the development of glomerulosclerosis or tubulointerstitial lesions, even after 60 days.

Dose- and time-dependent effect of rhIGF-1 on growth in SD rats

rhIGF-1 had similar actions on weight gain, length gain, and food conversion ratio as rhGH (Table 3). There were no effects on systolic blood pressure, serum creatinine and serum phosphate (data not given). Serum concentration of (exogenous) rhIGF-1 measured with human RIA [10] 12 hours after the last injection of 4 mg rhIGF-1/kg (given every 12 hours) was 932 ± 102 ng/ml versus 208 ± 29 ng/ml in vehicle injected animals. rhIGF-1 not only caused a significant increase of kidney dry weight, but, in contrast to rhGH, also caused a dose dependent increase of the kidney dry weight/body weight ratio.

After 60 days of treatment with rhIGF-1, kidney weight/body weight ratio was 0.91 ± 0.05 in rhIGF-1 treated rats and 0.88 ± 0.05 in controls ($N = 5$ per group). Renal histology did not demonstrate the development of glomerulosclerosis or tubulointerstitial lesions, even after 60 days of rhIGF-1 treatment.

Effect of concomitant treatment with rhGH and rhIGF-1 for 14 days on growth in SD rats

To test whether rhGH and rhIGF-1 have additive effects, we used the doses of rhGH (20 IU/kg/day) and of rhIGF-1 (8 mg/kg/day) which had caused maximal effects on weight gain in the previous experiments 1 and 2 (Table 4). In comparison to treatment with rhGH, the combined administration of the two peptides caused a significantly greater weight gain and also a small but significant ($P < 0.05$) further increase in length gain. The combined administration caused a further significant rise of liver, spleen and of kidney dry weight, but no further increment of heart and muscle dry weight. While the dry weight/body weight ratio was not further increased for heart and muscle, spleen dry weight/body weight ratio ($\times 10^{-3}$) tended to increase with rhGH + rhIGF-1 (0.69 ± 0.06) versus rhGH alone (0.64 ± 0.05). The same was noted for liver dry weight/body weight ratio ($\times 10^{-3}$) (15.6 ± 0.82 vs. 14.6 ± 0.9).

Consistent with the observations in Tables 1 and 3, rhIGF-1, but not rhGH, caused a significant increase in kidney dry weight/body weight ratio. Administration of rhGH together with IGF-1 caused no further increase.

Development of glomerulosclerosis again was not seen in any of the treatment groups.

Effects of rhGH and rhIGF-1 on GHR mRNA and IGF-1 mRNA in kidney and liver tissue

Treatment with 10 IU rhGH/kg/day significantly increased the GHR mRNA in the kidney, but not in the liver (Table 5). At the same time, an increase of IGF-1 mRNA was noted which again was only significant within the kidney. Treatment with 3 μg rhIGF-1/kg/day did not cause a significant increase of GHR mRNA or IGF-1 mRNA in kidney and liver.

Effects of rhGH on renal morphology

After treatment of female SD rats with 10 IU rhGH/kg/day by twice daily s.c. injections for 14 days, kidney volume by Archimedian principle was significantly greater in rhGH-treated animals (Table 6). In contrast, the kidney volume/body weight ratio was not significantly different (vehicle 0.99 ± 0.06 cm^3/g; rhGH 0.97 ± 0.07 cm^3/g), but the ratio is based on perfusion fixed renal tissue. The proportion of kidney volume comprised of cortex was unchanged. An increase of zonal width and of glomerular area was noted in rhGH-treated animals. The percent increase of the respective mean values was comparable to the mean percent increase of kidney volume (+ 10%) and of body weight (+ 12%).

Effect of treatment with rhGH or rhIGF-1 for five days on renal DNA/protein ratio in SD rats

Treatment of female SD rats with submaximal doses of rhGH or rhIGF-1 respectively for five days caused an (insignificant) increase of kidney dry weight, while protein content increased significantly (but marginally) together with a striking increase in DNA content (Table 7). In parallel, an increase of DNA/protein ratio was noted for rhGH as well as for rhIGF-1 treated animals. The results suggest that renal growth was accounted for more by hyperplasia than by hypertrophy.

Effect of treatment with rhGH or rhIGF-1 for five days on mitosis and PCNA expression of tubular and glomerular cells in SD rats

In renal intact animals, 10 IU rhGH/kg for four days caused a significant increase in the number of mitoses demonstrable in SD rats treated with colchicine for three hours prior to the end of the experiment (Table 8). A significant increase was seen both in distal and proximal tubular epithelial cells.

When the baseline number of mitoses was raised by prior subtotal nephrectomy before administration of rhGH, a further increment of mitoses was still noted after rhGH. The results observed with 4 mg rhIGF-1/kg/day were comparable.

To validate the colchicine method with an independent approach, in a second set of animals the expression of proliferating nuclear antigen (PCNA) was determined using a murine IgM monoclonal antibody against human PCNA. The number of PCNA positive nuclei per glomerular cross section was significantly increased ($P = 0.008$; ANOVA) both after rhGH (3.08 ± 0.53) and rhIGF-1 (2.95 ± 0.41) compared to vehicle controls (2.2 ± 0.55).

Discussion

The salient features of the present study are the observation that both rhGH and rhIGF-1 increase renal weight. The increase of renal weight following administration of rhGH was in proportion to the increase in body weight. In contrast, administration of rhIGF-1 either alone or in combination with rhGH increased the renal weight/body weight ratio, suggesting some selective stimulation of renal growth. After administration of rhGH, the glomerular volume increased in proportion to the increase in body weight. Administration of rhGH and of rhIGF-1 up to 60 days did not cause glomerulosclerosis or tubulointerstitial changes in female animals.

This systematic analysis extends previous observations of Guler et al in hypophysectomized rats, which documented that rhGH increased renal weight in proportion to body weight [34]. Our study shows that rhGH, in doses which are close to those maximally stimulating body growth, does not significantly affect the renal and other organ weight/body weight ratio in hypophysis intact young female SD rats on *ad libitum* intake of food. Protein intake has a known stimulatory effect on renal growth and GFR [12]. The possibility that the effect of rhGH was mediated by changes in protein intake was not formally excluded by a pair feeding experiment, but we found no significant increase of food intake. This is in agreement with previous data of other [20] and our own groups [8, 18]. In contrast to previous studies [35], the mortality in our animals was zero. Most of our experiments lasted for one to two weeks. For longer experiments, one important potential limitation of the efficacy of (foreign) rhGH or rhIGF-1 to rats is antibody production. For rhGH this is known to occur in female SD rats (A. Skottner-Lundin, Kabi Pharmacia, Peptide Hormones, Stockholm, personal communication) after two to three weeks of administration. In accordance with this, after three weeks the body weight increment in human peptide hormone treated animals became identical with that in solvent treated animals (experiments 2 and 3) and growth curves ran parallel, but the initial growth advantage was preserved throughout the experiment.

It is not excluded that more prolonged effective treatment with rhGH would progressively increase the kidney weight/body weight ratio in parallel to what has been reported for transgenic mice expressing GH [15, 36, 37]. But even in transgenic mice expressing GH this finding has not been reported uniformly [16, 38]. One difficulty of measuring the kidney weight/body weight ratio in transgenic mice is the fact that body weight increases rapidly during the first weeks of life, but decreases continuously during the last weeks before death. Therefore, different results will be obtained at different stages of the experiment. This has not been worked out systematically. It can also not be excluded that a vastly greater amount of GH produced by the transgenic animals accounts for the different results. In transgenic mice expressing human GH, serum hGH levels up to 900,000 ng/ml have been reported [15]. In this respect, it is interesting that the serum concentration of rhGH in SD rats treated with 10 IU rhGH/kg/day ranged between 3.3 and 20 ng/ml 12 hours after the last injection. This is comparable to the serum concentrations in rhGH treated children with chronic renal failure (mean 20 ± 3.4 ng/ml 12 hr after injection of 4 IU rhGH/m^2; [13]).

In striking contrast to rhGH, rhIGF-1 dose-dependently increased the renal weight/body weight ratio above the ratio in vehicle controls. This result extends the previous findings of Guler et al [34] in hypophysectomized rats and demonstrates greater proportional stimulation of renal growth even in hypophysis-intact rats. This result is remarkable since rhIGF-1 increased heart and muscle weight without increasing heart and muscle weight/body weight ratio. In contrast, spleen and liver weight/body weight ratios were slightly increased, illustrating considerable target organ selectivity.

The present finding of a selective renotrophic effect of IGF-1 is in agreement with previous observations in nitrogen restricted rats [39] and in subtotally nephrectomized rats [40]. We did not specifically address the issue concerning the effects of

rhIGF-1 on renal growth after subtotal nephrectomy. In pilot experiments it had proven extremely difficult to ablate a standardized amount of renal tissue. Our results show, however, that the effect of IGF-1 on renal growth does not require prestimulation of renal growth by ablation.

The kidney is a known target organ for both GH and IGF-1. GH receptors [41] and IGF-1 receptors [20, 42–44] have been demonstrated and IGF-1 is locally produced in the kidney, particularly when renal growth is stimulated, such as after ablation [43, 45, 46], during diabetes mellitus [43] and in hypersomatotrophic states [47]. The reasons why rhGH and rhIGF-1 act differently on renal growth are unknown. It is of note, however, that addition of rhGH to rhIGF-1 (Table 2) failed to increase the kidney weight/body weight ratio above that seen in animals given rhIGF-1 alone. A significant further increment in weight gain was noted, however. Interestingly enough, rhGH increased the GHR mRNA and IGF-1 mRNA in the kidney. These findings would be compatible with the notion that the effects of IGF-1 are mediated, at least in part, by pathways different from those mediating the effects of rhGH.

The finding that rhGH differently stimulated GHR mRNA and IGF-1 mRNA in the kidney and in the liver points to organ selectivity, and deserves further investigation. It has been reported that rhGH stimulates GHR mRNA in the liver of hypophysectomized rats [48]. However, studies in hypophysis-intact rats have not been reported to date.

We considered that an unchanged renal weight/body weight ratio after rhGH does not necessarily exclude potential changes of renal architecture, such as the redistribution of the relative width of different zones as seen after glucagon or vasopressin which causes a disproportional increase of medullary width [49]. The present study, however, showed no significant change of the relative widths of the different renal zones in rhGH treated animals. Glomeruli are enlarged in acromegalic patients [3] and transgenic mice expressing GH [14], IGF-1- [14, 38] or GH-releasing factor [14, 50]. Potential effects on glomerular size are of interest, since an increase in glomerular size may be permissive for the development of glomerulosclerosis. Indeed, glomerulosclerosis was seen in transgenic mice expressing GH, but this was not seen in transgenic mice expressing IGF-1 [14]. In our animals on short-term rhGH treatment, glomerular size increased, but in proportion to the body weight gain, so that the ratio glomerular volume/body weight did not change. We emphasize that glomerulosclerosis or tubulointerstitial lesions were not seen in our study in *female* rats, even after prolonged (60 days) administration of rhGH or rhIGF-1 in doses per body weight which were considerably in excess of what is administered to uremic children [10]. The interpretation of these findings must be cautious in view of antibody formation against rhGH and rhIGF-1 as discussed above. On the other hand, the development of glomerulosclerosis has been reported in *male* SD rats treated with rhGH for 28 weeks [35] in similar doses. It is known that male rats are more prone to the development of glomerulosclerosis than female rats. Whether this explains the difference of results requires further studies.

Renal growth after ablation involves mainly hypertrophy [51], although in younger animals hyperplasia does also contribute [52, 53]. To address the relative contribution of hypertrophy and hyperplasia to the renal growth response to rhGH and rhIGF-1, respectively, we examined the renal DNA/protein ratio and the proportion of renal cells undergoing proliferation and mitosis. Both methods suggested a major contribution of renal cell proliferation, indicating that in these young female animals the increment in renal weight mainly involves hyperplasia at least within the time frame of the study. The results in the young animals cannot necessarily be extrapolated to older animals.

With respect to rhGH treatment of children with chronic renal failure, concern has been raised that such treatment accelerates progression of renal failure by promoting the genesis of glomerulosclerosis. Although our experiments do not directly bear on this issue, the results are reassuring in that they indicate that rhGH (i) fails to stimulate renal growth and glomerular volume out of proportion to the increase in body weight, and (ii) does not cause glomerulosclerosis in experiments of two months duration. Different effects on kidney growth should be expected if one considers the use of rhIGF-1 alone or in combination with rhGH for the treatment of stunting and GH insensitivity [54] in chronic renal failure.

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