Kidney International, Vol. 49 (1996), pp. 1413–1421

Growth promoting effects of growth hormone and IGF-I are additive in experimental uremia

GÁBOR T. KOVÁCS, JUN OH, JÓZSEF KOVÁCS, BURKHARD TÖNSHOFF, ERNST B. HUNZIKER, JÜRGEN ZAPF, and OTTO MEHLS

Second Children's Hospital, Semmelweis University Budapest, Hungary; Department of Pediatrics, University of Heidelberg, Germany; Albert Szent-Györgyi Medical University, Pediatric Department, Szeged, Hungary; M.E. Müller-Institute for Biomechanics, University of Bern, Bern, and Department of Internal Medicine, University Hospital, Zürich, Switzerland

Growth promoting effects of growth hormone and IGF-I are additive in experimental uremia. Exogenous growth hormone (GH) stimulates the endogenous production of IGF-I and improves growth in uremia. We investigated whether exogenous IGF-I is also able to improve uremic growth failure in rats and whether the growth promoting effects of GH and IGF-I are additive. In female 150 g uremic (subtotal nephrectomy, NX) Sprague-Dawley rats, both rhGH in doses from 2 × 1.25 to 2 × 10 IU/kg bid s.c. and rhIGF-I in doses from 2 × 0.5 to 2 × 4.0 mg/kg bid s.c. caused a dose-dependent increase in weight gain and length gain. However, endogenous production of GH was suppressed by both agents. Peptide hormone treatment did not affect cumulative food intake, but significantly increased food efficiency ratio (weight gain/food intake). Concomitant s.c. treatment with maximally effective doses of rhGH (12 × 5 IU/kg bid) and of rhIGF-I (2 × 2 mg/kg bid) resulted in additive growth promoting effects in NX and pair-fed control (CO) animals during the observation period of 12 days. Cumulative length gain was 3.2 ± 0.5 cm in solvent-treated NX-animals, 4.1 ± 0.5 cm with rhGH (+ 28% above solvent), 4.2 ± 0.6 cm with rhIGF-I (+ 31%) and 4.9 ± 0.5 cm with both peptides (+ 53%). The food efficiency ratio was 0.16 ± 0.05 in solvent NX, 0.33 ± 0.04 with rhGH (+ 106% above solvent), 0.23 ± 0.02 with rhIGF-I (+ 44%), and 0.38 ± 0.02 with both peptides (+ 138%). Histomorphometric analysis and measurements of length gain by fluorescence microscopy in the upper tibial metaphysis confirmed the growth promoting effects of both peptide hormones. The serum concentrations of IGF binding protein (BP)-4 (Western ligand blotting analysis) and of IGFBP-2 (immunoblot) were increased in uremic animals whereas IGFBP-3 was unchanged. Treatment with IGF-I and/or rhGH increased serum concentration of IGF-I but did not change the IGFBP pattern. rhIGF-I lowered blood glucose levels within one to two hours after injection. The effect was most pronounced during the first treatment day and declined thereafter. Concomitant treatment with rhGH attenuated the glucose lowering effect of rhIGF-I (glucose serum concentration at day one: 120 ± 11 mg% in solvent NX, 50 ± 21 mg% with rhIGF-I, 80 ± 24 mg% with both peptides). It is concluded that: (i) IGF-I is able to stimulate growth in NX animals but suppresses endogenous GH production in the long run; (ii) the concomitant treatment with IGF-I and GH has additive effects on growth; and (iii) concomitant treatment with rhGH prevents hypoglycemia that is noted with rhIGF-I alone.

Uremic growth failure has been identified to be at least in part the consequence of secondary growth hormone insensitivity [1, 2]. The tissue resistance to the physiological action of growth hormone (GH) may be explained by reduced GH receptor expression [3], reduced somatotrope binding sites [4], low activity of GH binding protein [5] and low hepatic expression of insulin-like growth factor-I (IGF-I) mRNA [6, **Note added in proof**]. In addition, somatomedin bioactivity is diminished [2, 7] due to increased binding of IGF-I to its binding proteins [2, 8, 9] which accumulates in serum of the uremic organism. These changes result in impaired IGF-I-dependent growth processes.

In experimental chronic renal failure [1] as well as in children with chronic renal failure [7, 10] it is possible to stimulate growth by daily injections of rhGH in supraphysiological amounts. Supraphysiological doses of rhGH stimulated circulating IGF-I levels to a higher extent than IGFBP-3 levels [7]. The improvement of the relationship between IGF-I and IGFBP-3 resulted in a normalization of the IGF bioactivity [7, 11].

If the reduced availability of IGF-I is a major reason for GH insensitivity, one would expect that treatment with rhIGF-I might improve uremic growth failure. This has recently been confirmed [12]. On the other hand, IGF-I treatment may lead to serious side effects like hypoglycemia and circulatory instability [13–16]. In addition, high circulating levels of IGF-I may reduce pituitary GH secretion [15, 17, 18].

The combined treatment with rhGH and rhIGF-I may prevent some of the negative effects of a monotherapy with rhIGF-I. Because GH seems to increase anabolism without affecting catabolism [19, 20] and because IGF-I may have a positive effect on nitrogen balance by reducing catabolism [19, 21], an intensified effect on growth may be expected from a combined treatment with both peptides. In a recent experimental study in healthy animals, we demonstrated that maximally effective doses of IGF-I and of GH had additive effects on weight gain but not on length gain [22]. Although the additive effects of rhGH and rhIGF-I were discussed in a recent study for uremic animals [12], these studies are not conclusive because the doses of each peptide used for combined treatment were not maximally effective doses.

It was the aim of the present study to determine the maximally effective doses of rhIGF-I and of rhGH for body growth of uremic animals and to analyze whether the growth promoting effects of both hormones are additive. Furthermore, the effects of both peptides on glucose metabolism were evaluated.

Received for publication May 31, 1995
and in revised form November 15, 1995
Accepted for publication December 27, 1995

Methods

Animals

Female Sprague-Dawley (SD) rats (Charles River-Wiga, Sulzfeld/Allgäu, Germany), weighing 120 to 150 g, were used for the experiments. Female rats were chosen because of the better response to growth hormone in female rats, as previously documented [1]. One week prior to the studies, the animals were transferred to single cages at constant room temperature (24°C) and humidity (70%) on a 12 hours on/12 hours off light cycle. The diet contained 13,800 kJ/kg, 0.95% calcium, 0.8% phosphorus, 500 IU/kg vitamin D_3 and 18% protein (wt/wt). With the exception of pair-fed controls the animals had free access to food (Altromin C 1000, Altromin Company, Lage/Lippe, Germany) and deionized water.

The animals were subjected to a two-stage subtotal nephrectomy (NX) or sham-operation as described previously [23]. Subtotal nephrectomy of the left kidney was performed one week prior to surgical removal of the right kidney. At that moment, the animals had a mean body wt of about 150 g. Control aninmals (CO) were sham-operated (renal decapsulation). The CO group was pair-fed as previously described [23].

Recombinant peptide hormones (rhGH and rhIGF-I) were administered subcutaneously twice daily, in doses indicated under protocols. RhGH (Genotropin) and rhIGF-I (lot No. 77135/51) were provided by Pharmacia AB (Stockholm, Sweden).

Protocols

Dose response experiments. For dose response experiments, NX animals of 140 to 150 g were used. All animals were allowed free access to food and water. For determining the maximum dose of rhGH, groups of six animals each were injected with 0, 2.5, 5.0, 10.0 and 20.0 IU rhGH/kg daily in two divided doses s.c. during a period of ten days. For determining the maximum growth simulating effect of rhIGF-I, six animals per group were injected s.c. with 1, 2, 4, and 8 mg/kg/day in two divided doses during a period of ten days.

Experimental protocol for concomitant treatment with rhGH and rhIGF-I. The animals were randomized into four groups. Group A received solvent twice daily s.c. during 12 days, Group B received 2 × 5 IU rhGH/kg daily s.c., Group C received 2 × 2 mg rhIGF-I/kg daily s.c., Group D was injected with rhGH and rhIGF-I in doses indicated above. All groups (A, B, C, D) were additionally divided into two subgroups of nine animals each: uremic animals (NX) and sham-operated pair-fed control animals (CO).

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 was calculated from cumulative food intake and cumulative weight gain [1]. Nose-to-tail-tip distances were measured in anaesthesized animals and under complete muscle relaxation as described previously [23].

Organ weight

The animals were sacrificed by aortic puncture under general anaesthesia (100 mg/kg Ketanest, Park Davis Company, Berlin, Germany, and 10 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).

Biochemical measurements

Blood was obtained by aortic puncture from non-fasted animals at the end of the experiment, which was 14 to 15 hours after the last injection of peptide hormones. A 24-hour urine sampling was performed in a metabolic cage during the last 24 hours of the experiment. Serum and urine biochemistry were analyzed using a multichannel autoanalyzer (Beckmann Synchron CX7, Germany). Creatinine was determined by a kinetic method according to Jaffé without deproteinization; the within assay coefficient of variation was < 3%. Serum glucose concentration was determined photometrically using a Refloton analyzer (Boehringer, Mannheim, Germany). The within measurement coefficient of variation was < 3%. For these measurements blood was taken from the tail vein on day 1 immediately before the first injection of rhIGF-I, and one and two hours, respectively, thereafter. The measurements were repeated on day 11 before as well as one and two hours after the 21st injection.

Hormonal measurements

Measurements of insulin was performed by RIA as described earlier [24]. Measurements of rhGH and of rhIGF-I were done by RIA techniques as described earlier [7, 25]. The antibody for IGF-I determination was provided by Professor Peter Gluckman (New Zealand), and the standard preparation was recombinant hIGF-I (rhIGF-I; Pharmacia AB, Stockholm, Sweden). ^{125}I-iodinated rhIGF-I was used as a tracer. The cross reactivity with rat IGF-I was more than 90%. Rat GH was determined using a RIA technique [25]. Antibodies against rGH and a standard preparation of rGH (RP-2) were supplied by the National Hormone and Pituitary Program (Bethesda, MD, USA). Rabbit anti-monkey serum was prepared at Pharmacia AB, and rhGH for labeling was donated by Professor Paul Roos (Uppsala, Sweden). Incubation was performed at +4°C, initially with samples (in duplicate; intrassay Cv_w 2 to 8% for GH levels between 0,5 to 8 ng/ml) or standards together with primary antibodies overnight, followed by incubation with labeled rGH for a further 16 hours. The complexes were precipitated by second antibodies and centrifuged. The pellets were counted in a LKB-Wallac Gamma-counter. Concentrations were calculated. The standard curve ranged from 0.25 to 16 ng/ml.

Serum IGFBP-3 and IGFBP-4 were determined by Western ligand blotting performed according to the method of Hossenlopp et al [26] with slight modifications [27]. Two microliters of serum were processed by electrophoresis for five hours at 170 mV on SDS/15% polyacrylamide slab gels (15 × 15 × 0.15 cm) under non-reducing conditions (except the ^{14}C-labeled molecular weight markers; Rainbow marker, Amersham, UK). After electroblotting on nitrocellulose, membranes were processed as described in Zapf et al [27].

IGFBP-2 was identified by immunoblotting with IGFBP-2 antiserum: 20 μl of pooled serum of uremic and pair-fed normal rats were processed by electrophoresis on SDS/15% polyacrylamide gels (15 × 15 × 0.15 cm) under non-reducing conditions and transferred to Hybond C-Super Membranes (Amersham, UK) by electroblotting for two hours at 0.5 A. Membranes were processed according to the protocol for ECL Western blotting

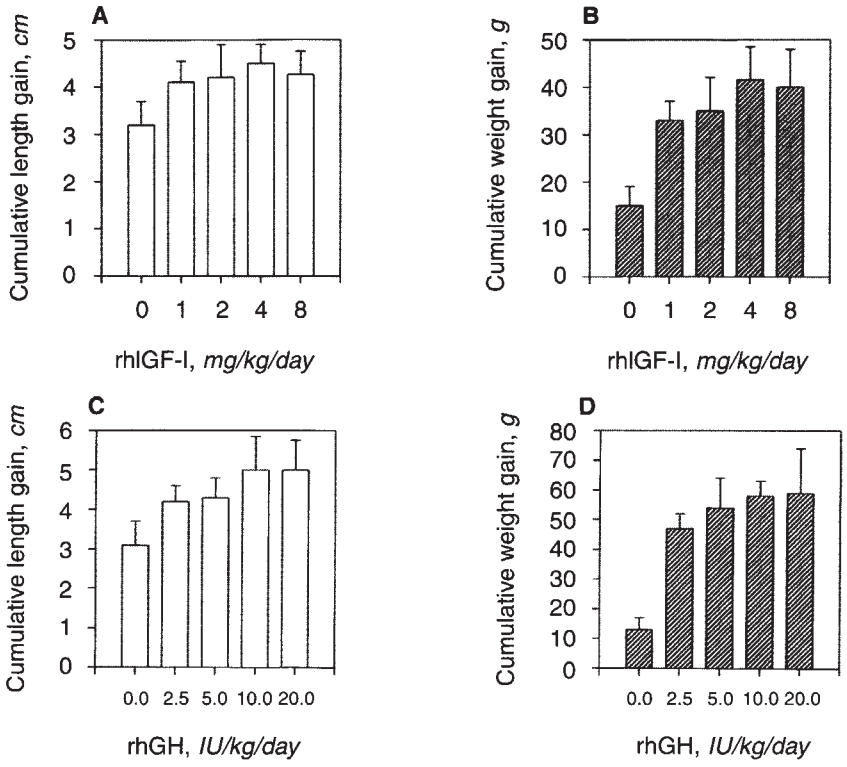

Fig. 1. *Growth response for weight gain and length gain in uremic rats following treatment with rhGH and with rhIGF-I.* Dose response curves. Increasing doses of rhIGF-I administered for 10 days had increasing effects on length gain (**A**) and weight gain (**B**); maximal effects were obtained with 4 mg/kg/day administered in two doses s.c. per day. RhGH administered for 10 days had also increasing effects on cumulative length gain (**C**) and weight gain (**D**); maximal effects were obtained with 10 IU/kg/day given in two doses s.c.

provided by Amersham. Rabbit anti-rat IGFBP-2 antiserum (provided by Dr. D. Clemmons, Chapel Hill, NC, USA) was used at 1:10,000 dilution and goat anti-rabbit IgG antiserum diluted 1:2000 was used as second antibody.

Histomorphometric analysis of proximal tibia

The proximal tibia growth zone was used for morphometric analysis. Five days prior to sacrifice, the fluorescent marker calcein was administered to animals in a single injection (15 mg/kg s.c.) [28]. The tibia was fixed in 4% formaldehyde with 0,1 M sodiumcacodylate-buffer at a pH of 7.4 for several days. It was then washed with H_2O and dehydrated in 70% ethanol (vol/vol) at 4°C for 24 hours; dehydration was completed in a graded series of ethanol at +20°C. Thereafter, tissue slices were embedded in methylmethacrylate and polymerization executed at +30°C. Five micrometer-thick sections were cut in frontal planes on a Jung microtome No. 1140. Sections were subsequently mounted on gelatin-coated glass slides and stained initially by the von Kossa reaction and subsequently with McNeill Tetrachrome [29] for morphometric estimation of growth plate height. Ten micrometer sections from each tibia, also mounted on glass slides, were used unstained for measurements of growth rates using incident light fluorescence microscopy [30].

Statistical analysis

Data are given as means ± SD if not indicated otherwise. Data were examined for normal and non-Gaussian distribution by the Shapiro-Wilk test [31]. For comparison between two normally distributed groups, unpaired Student's *t*-test (two-tailed) was used. For comparison of more than two normally distributed groups, one way ANOVA followed by pairwise multiple comparisons (Student-Newman-Keuls method) was used. For non normally distributed data, the non-parametric Kruskal-Wallis test, followed by all pairwise multiple comparison (Dunn's method), was used. $P < 0.05$ was accepted as statistically significant.

Results

Dose response experiments in uremic animals

Increasing doses of rhGH and rhIGF-I increased length gain and weight gain stepwise (Fig. 1). The maximally growth stimulating effect for length gain and weight gain in NX animals was obtained by injecting 4 mg rhIGF-I/kg in two divided doses s.c. daily and by injecting 10 IU rhGH/kg/day in two divided doses s.c.

Concomitant treatment with rhIGF-I and rhGH

Based on the dose response experiments, maximally growth promoting doses of rhIGF-I (2 × 2 mg/kg/bid) and rhGH (2 × 5 IU/kg/bid) were injected separately and concomitantly to NX animals and sham-operated CO animals ($N = 9$ animals per group). Cumulative weight gain in solvent treated NX animals was significantly less than in CO animals (Table 1). During the observation period of 12 days, peptide hormone treatment continuously improved both length gain and weight gain of NX animals and of CO animals (Fig. 2). The effect of rhGH on length gain was similar to the effect of rhIGF-I whereas the rhGH effect on weight gain was significantly greater. If both peptides were given concomitantly their growth promoting effects were nearly additive. Neither rhIGF-I nor rhGH increased the cumulative food intake of NX animals to a major extent. In contrast, the food efficiency ratio was significantly increased by rhGH and by concomitant treatment.

Table 1. Effect of 4 mg/kg/day rhIGF-I and 10 IU/kg/day rhGH given separately or concomitantly for 12 days on growth in uremic and pair-fed rats

	Cumulative food intake *g*	Food conversion ratio	Muscle (triceps surae) wet weight *g*	Cumulative weight gain *g*	Cumulative length gain *cm*
Uremia (N = 9)					
Vehicle	166 ± 15[a]	0.16 ± 0.05[c]	1.34 ± 0.07[a]	27.3 ± 10.2[c]	3.2 ± 0.5
rhIGF-I	172 ± 23[a]	0.23 ± 0.02[c] (+ 44%)	1.36 ± 0.06[a]	39.0 ± 10.1[c] (+ 43%)	4.2 ± 0.6[a] (+ 38%)
rhGH	166 ± 23[a]	0.33 ± 0.04[c] (+ 106%)	1.42 ± 0.11[a]	57.6 ± 13.4[c] (+ 111%)	4.1 ± 0.5[a] (+ 28%)
rhIGF-I + rhGH	181 ± 10[a]	0.38 ± 0.02[c] (+ 137%)	1.53 ± 0.07	68.9 ± 5.0[c] (+ 152%)	4.9 ± 0.5 (+ 53%)
Pair-fed (N = 9)					
Vehicle	166 ± 15[a]	0.28 ± 0.03[a]	1.32 ± 0.11[a]	47.6 ± 8.7[a]	3.4 ± 0.5
rhIGF-I	172 ± 23[a]	0.31 ± 0.04[a] (+ 11%)	1.43 ± 0.05[a]	54.9 ± 8.5[a] (+ 15%)	4.1 ± 0.4[a] (+ 20%)
rhGH	166 ± 23[a]	0.43 ± 0.03 (+ 54%)	1.50 ± 0.10[b]	71.9 ± 9.1 (+ 51%)	4.1 ± 0.6[a] (+ 20%)
rhIGF-I + rhGH	181 ± 10[a]	0.47 ± 0.03 (+ 68%)	1.56 ± 0.09[b]	84.7 ± 8.2 (+ 78%)	4.7 ± 0.5 (+ 38%)

Food conversion ratio is the cumulative weight gain (g)/cumulative food intake (g).

P values are from one way ANOVA followed by all pairwise multiple comparison (Student-Newman-Keuls method).

[ab] Significant differences between treatments within one experimental group (uremia or pair-fed); values sharing common superscripts are not significantly different, while values without common superscripts are significantly different

[c] Significant differences between uremia and the respective treatment group in pair-fed animals

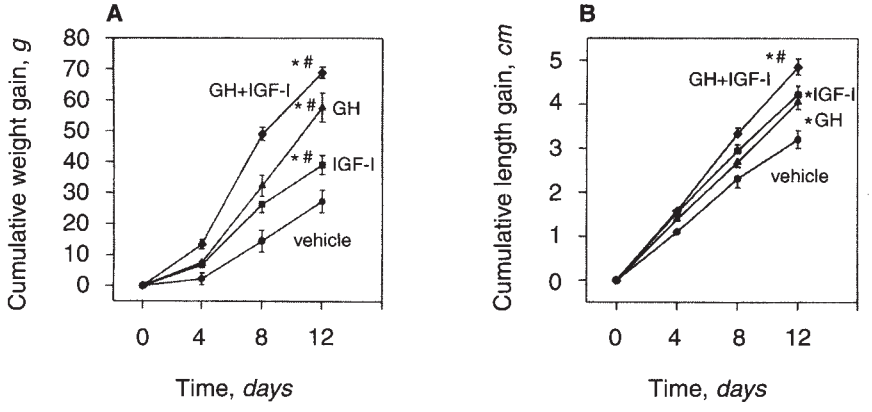

Fig. 2. *Effect of combined treatment for 12 days with maximally effective doses of rhGH and of rhIGF-I on weight gain and length gain in uremic animals.* Both 10 IU rhGH/kg/day and 4 mg rhIGF-I/kg/day increased mean cumulative weight gain (**A**) and length gain (**B**) compared to solvent controls. Co-administration of both hormones increased weight gain and length gain more than each single hormone. The growth stimulating effect was nearly additive if one analyzes the growth stimulating effects above baseline. Symbols are: (♦) GH + IGF-1; (▲) GH; (■) IGF-1; (●) control. Data are given as mean ± SEM. *Significant versus vehicle; #significant versus the other treatment modalities.

The striking effect of the combined treatment with rhIGF-I and rhGH on skeletal growth was confirmed by the micromorphometric analysis of the upper tibial metaphysis (Fig. 3A). In NX animals, the height of the epiphyseal plate was significantly increased by rhGH whereas the effect of rhIGF-I alone was only modest. Combined treatment with both peptides increased the height of epiphyseal plate significantly more than the treatment with rhGH or rhIGF-I alone. The growth rate per day was analyzed after s.c. injection of the fluorescence marker calcein five days prior to sacrifice. Measuring the distance from the calcification front to the calcein front within the primary spongiosa was taken as a marker of growth during the last five days of the experiment. In uremic animals, the growth rate with the combined treatment was significantly higher than with rhIGF I or rhGH alone. It is of note that rhIGF-I alone did not improve growth rate significantly.

Analysis of the histological changes of the growth plate in uremic animals documented an enlargement of the growth plate, which resulted from an inrease in cell number and cell size of all cell types of the proliferative and the hypertrophic cells including the cells of the calcified zone (Fig. 3B).

In parallel to the increase of body wt, the muscle weight of triceps surae increased under the influence of rhIGF-I and rhGH. The parallel increase of muscle wet wt and muscle dry weight gave evidence that the peptide hormones did not increase the water content out of proportion in these organs. In contrast, the ratio of dry weight over wet wt of the liver decreased slightly but significantly with the combined treatment, indicating that the water content was increased by about 2% in comparison to solvent treated animals.

Relevant biochemical and hormonal data are seen from Table 2. Serum creatinine was slightly increased in peptide hormone treated uremic animals but not in pair-fed controls. Peptide hormone treatment increased serum protein concentration in uremic but not in pairfed control animals. There was no change of urinary calcium excretion, whereas urea excretion decreased with each of the hormones. This effect was most expressed with the combined treatment. There was a tendency of increased protein excretion with the combination of rhIGF-I and rhGH treatment.

When endogenous GH was measured by a specific rat RIA, it became clear that the serum concentration of endogenous GH was significantly lower during treatment with both rhGH and rhIGF-I (Table 2). At the end of the experiment, IGF-I serum concentration was significantly increased with rhGH but not with rhIGF-I treatment. However, it has to be noted that blood samples were obtained at variable time points (14 to 19 hr) after

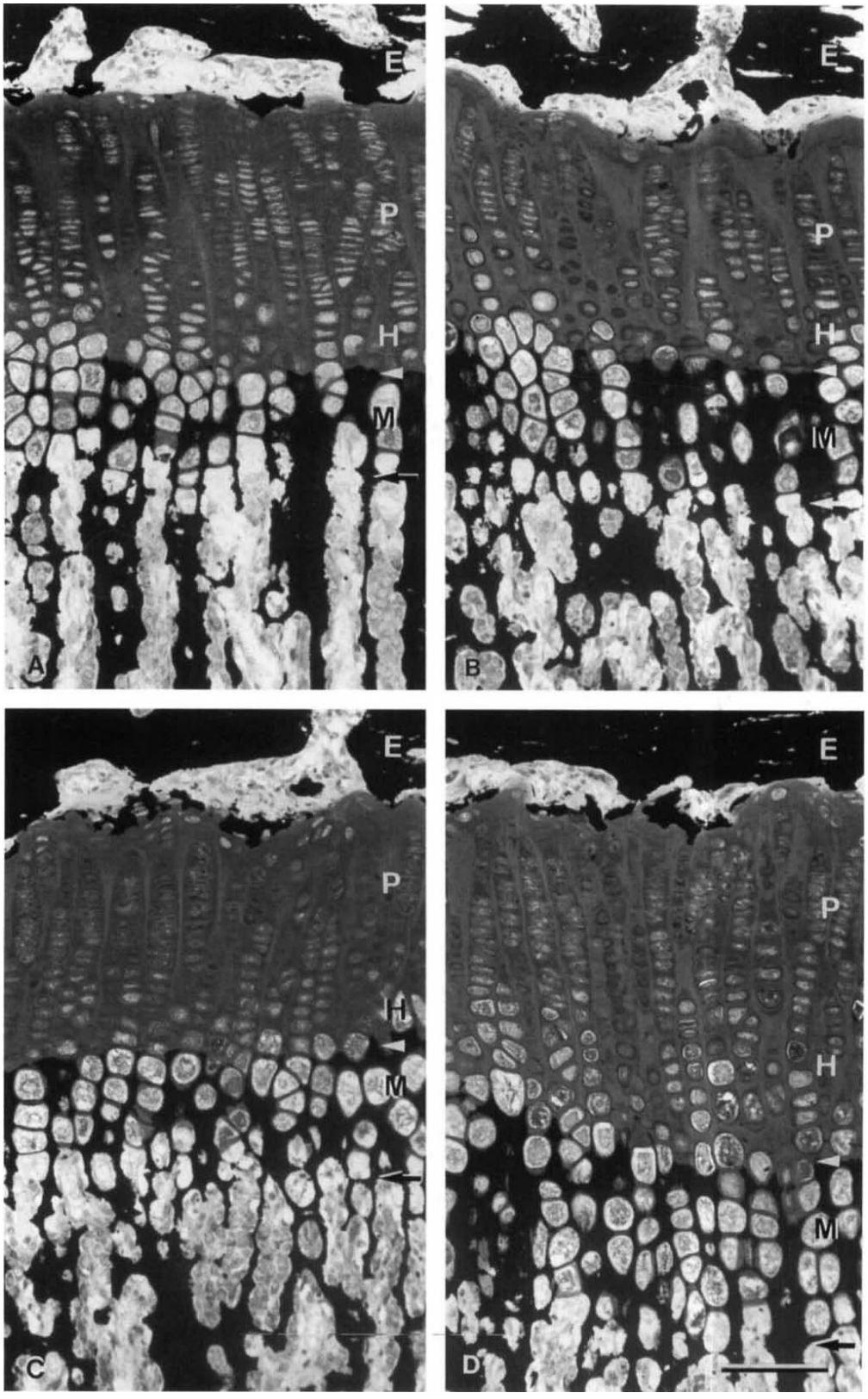

Fig. 3. A *Light micrographs of proximal tibial growth plate.* Thick sections (5 μm) of methylmetacrylate embedded proximal tibial growth plate. Staining: von Kossa and McNeil Tetrachrome. The growth plate heights of the two control groups (A and B) do not differ significantly, whereas the growth plate heights of uremic animals treated with rhIGF-I and of uremic animals treated with rhIGF I plus rhGH are significantly higher than those of the controls. Growth plate height increments are based both on largening of the proliferative zone height (to a major degree) and on an enlargement of the hypertrophic zone height (maturation, hypertrophy and mineralization zone). Abbreviations are: E, epiphyseal bone tissue; P, proliferating zone; H, hypertrophic zone; M, mineralization zone; arrowhead, mineralization front; horizontal arrow, vascular invasion front. Bar = 100 μm.

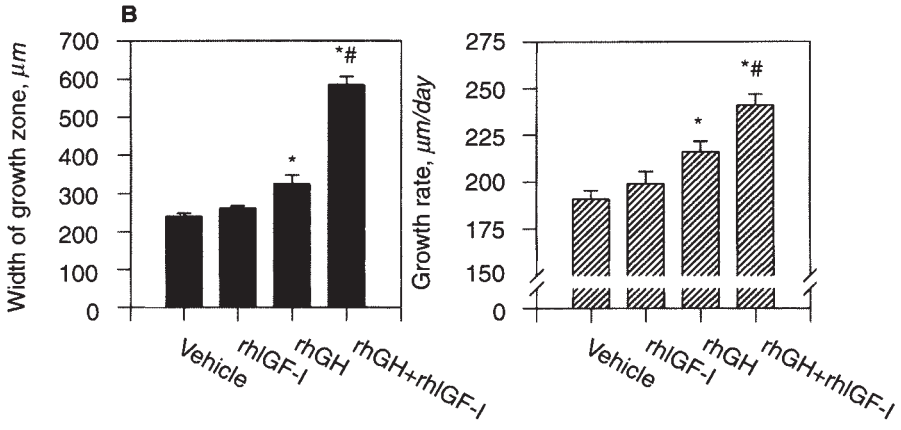

Fig. 3. B. Micromorphometric analysis of growth zone and of growth rate in uremic animals treated with peptide hormones. Five μm thick sections embedded in methylmetacrylate were used for measuring growth plate height and 10 μm sections for measurements of growth rates using incident light fluorescence microscopy. Width of growth zone and growth rate were significantly more increased with combined treatment than with rhIGF-I or rhGH alone. Data are given as mean ± SEM. *Significant versus vehicle; #significant versus the other treatment modalities.

Table 2. Serum and urine (U) biochemistry in uremic and pair-fed control rats treated with rhGH, rhIGF-I or a combination of both for 12 days

	Creatinine *mg/dl*	Urea *mg/dl*	Protein *g/liter*	Phosphate *mmol/liter*	GH *ng/ml* (rat assay)	IGF-I *ng/ml*	Insulin *μU/ml*	U-Urea *mg/100 g/d*	U-Calcium *mmol/100 g/d*	U-Protein *mg/100 g/d*
Uremia ($N = 9$)										
Vehicle	0.64 ± 0.10^{ad}	86.7 ± 13.0^{abd}	49.4 ± 2.5^{ad}	2.38 ± 0.20^{a}	14.6 ± 6.9	937 ± 78^{ad}	22.6 ± 2.7^{d}	288 ± 33^{ad}	0.03 ± 0.01^{a}	12.7 ± 8.0^{a}
rhIGF-I	0.71 ± 0.10^{abd}	93.0 ± 19.7^{ad}	55.2 ± 2.3^{ab}	2.96 ± 0.21^{b}	6.3 ± 4.1^{a}	1220 ± 75^{bd}	16.3 ± 2.3^{ad}	275 ± 37^{a}	0.02 ± 0.01^{a}	17.0 ± 8.7^{ad}
rhGH	0.71 ± 0.13^{abd}	80.8 ± 18.9^{ad}	53.1 ± 1.7^{ab}	3.40 ± 0.39^{c}	2.8 ± 4.1^{a}	1267 ± 72^{bd}	—	202 ± 34^{bd}	0.02 ± 0.01^{a}	17.6 ± 12.2^{ad}
rhIGF-I + rhGH	0.79 ± 0.10^{bd}	101 ± 18.8^{bd}	56.3 ± 2.4^{b}	3.45 ± 0.37^{c}	—	$1121 \pm 64^{a*}$	15.9 ± 2.3^{ad}	168 ± 48^{bd}	0.02 ± 0.01^{a}	27.7 ± 13.3^{d}
Pair-fed ($N = 9$)										
Vehicle	0.32 ± 0.07^{a}	35.1 ± 9.8^{a}	55.0 ± 4.1^{a}	2.69 ± 0.24^{a}	11.3 ± 4.2	840 ± 28^{a}	33.1 ± 3.8^{a}	356 ± 51	0.03 ± 0.02^{a}	5.89 ± 1.5^{a}
rhIGF-I	0.31 ± 0.03^{a}	30.0 ± 3.4^{a}	55.6 ± 1.8^{a}	2.90 ± 0.22^{a}	5.8 ± 3.9^{a}	861 ± 40^{a}	31.3 ± 3.5^{a}	254 ± 52^{a}	0.02 ± 0.02^{a}	5.89 ± 3.0^{a}
rhGH	0.29 ± 0.04^{a}	28.3 ± 5.9^{a}	55.0 ± 3.0^{a}	3.03 ± 0.22^{a}	1.4 ± 1.2^{a}	1001 ± 84^{b}	—	255 ± 34^{a}	0.02 ± 0.01^{a}	5.30 ± 1.0^{a}
rhIGF-I + rhGH	0.31 ± 0.03^{a}	29.6 ± 8.1^{a}	55.4 ± 5.0^{a}	3.35 ± 0.27^{b}	—	1035 ± 68^{b}	27.2 ± 3.4	235 ± 42^{a}	0.04 ± 0.02^{a}	6.70 ± 2.2^{a}

P values are from one way ANOVA followed by all pairwise multiple comparison (Student-Newman-Keuls method).
[abc] Significant differences between treatments within one experimental group (uremia or pair-fed); values sharing common superscripts are not significantly different, while values without common superscripts are significantly different
[d] Significant differences between uremia and the respective treatment group in pair-fed animals

the last hormonal injection, because the animals could not get sacrificed all at the same time.

Measurement of IGFBPs by Western ligand blot did not show a difference for IGFBP-3 between uremic and Co animals (Fig. 4A), whereas an increased activity for glycosylated (30 kDa band) and non-glycolysated (24 kDa band) IGFBP-4 was noted in uremic serum. By immunoblot, an increased staining for IGFBP-2 was noted in uremic animals (Fig. 4B). Treatment with rhGH and rhIGF-I did not influence these patterns.

Recombinant rhIGF-I had a major lowering effect on serum glucose concentration when measured one and two hours after the first injection (Table 3). This effect was significantly diminished if rhGH was injected concomitantly. When the effect of rhIGF-I was measured after the 21st injection on day 11, the glucose lowering effect of rhIGF-I was still present in uremic but not in control animals; however, it was much less expressed than on day 1. Again, concomitant injection of rhGH counterbalanced the effect of rhIGF-I. Serum insulin concentration was only measured at the end of the experiment at non-defined time points after the last hormonal injection in non-fasted animals according to the sacrifice protocol. This may be the reason why no systematic change for insulin was noted in uremic and control animals (Table 2).

Discussion

The present study demonstrates that both rhGH and rhIGF-I are able to improve growth in uremic rats in a dose-dependent way. Whereas maximally effective doses of rhIGF-I (4 mg/kg/day) and of rhGH (10 IU/kg/day) were equally effective on length gain, the effect on weight gain was higher with rhGH. This is the first report demonstrating that the effects of rhGH and rhIGF-I on length gain and weight gain are additive in uremic rats. This implies that IGF-I and GH improve growth, at least in part, via different mechanisms.

It is already known that the combination of rhGH and rhIGF-I treatment in rats [12] and humans [32] is substantially more anabolic than therapeutical doses of rhIGF-I or rhGH alone. In a recent paper, Hazel et al [12] could demonstrate that the enhancement of somatic growth in uremic rats was of great therapeutic significance if they used a combination of 1.7 mg rhIGF-I/kg/day and 5.6 IU rhGH/kg/day. Because they did not use maximally effective doses of both rhGH and of rhIGF-I, they could not decide whether the effects of rhGH and of rhIGF-I were indeed additive, because they could not exclude that increasing doses of either rhIGF-I or rhGH alone would have achieved the

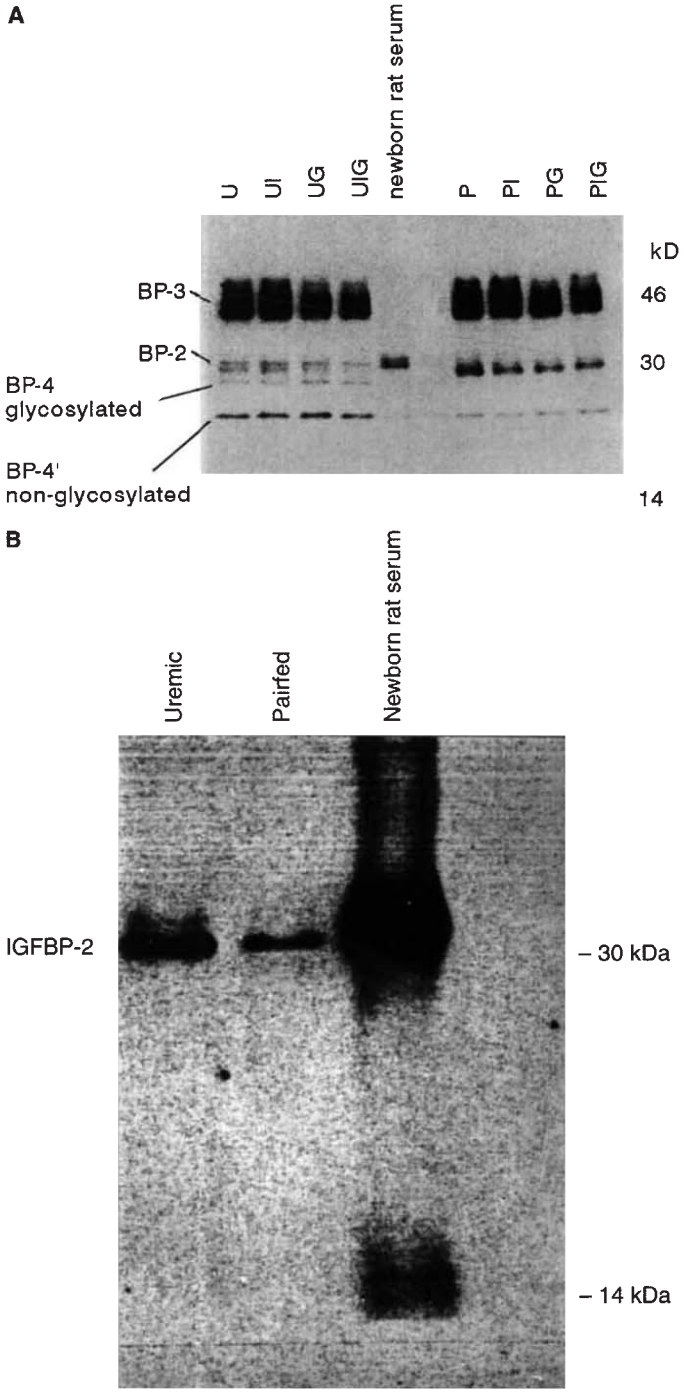

Fig. 4. A. *^{125}I-labeled IGF-II ligand blot of pooled sera from uremic (left panel) and control rats (right panel).* Abbreviations are: I, treatment with rhIGF-I; G, treatment with rhGH; IG, combined treatment. The 42/45/49 kDa triplets represent IGFBP-3. There is no difference between uremic and control animals and treatment with peptide hormones did not result in a significant change. For IGFBP-4 (24 kDa nonglycosylated and 30 kDa glycosylated) an increase of radioactivity was noted in uremic serum. **B.** Immunoblotting of pooled sera from uremic (U) and pairfed control rats (P) with IGFBP-2 antiserum. There was a two- to threefold increase of staining intensity for IGFBP-2 in uremic animals.

same improvement in growth as the combined treatment they had used. The same argument holds for the investigations of Kupfer et al in human [32].

Table 3. Effect of 2 mg/kg rhIGF-I and 5 IU/kg rhGH on serum glucose concentration measured one and two hours after s.c. injections

Experimental groups	Before injection	1 Hour after injection	2 Hours after injection
Day 1 (1st injection)			
Uremia			
rhIGF	120 ± 11.3[a]	51.2 ± 13.7[a]	49.2 ± 21.6[a]
rhIGF + rhGH	122 ± 15.5[a]	56.5 ± 9.1[a]	81.3 ± 24.7[b]
Pair-fed			
rhIGF	121 ± 12.2[a]	44.1 ± 8.5[a]	35.9 ± 7.6[a]
rhIGF + rhGH	124 ± 20.2[a]	43.6 ± 7.0[a]	89.2 ± 16.7[b]
Day 11 (21st injection)			
Uremia			
rhIGF	123 ± 9.5[a]	73.7 ± 15.3[ac]	123 ± 29.9[ac]
rhIGF + rhGH	119 ± 7.3[a]	111 ± 7.8[bc]	115 ± 15.0[ac]
Pair-fed			
rhIGF	135 ± 13.5[a]	114 ± 19.7[bc]	117 ± 7.5[ac]
rhIGF + rhGH	123 ± 16.4[a]	119 ± 19.2[bc]	121 ± 5.1[ac]

P values are from one way ANOVA followed by all pairwise multiple comparison (Student-Newman-Keuls method).

[ab] Significant differences between treatments within one experimental group (uremia or pair-fed); values sharing common superscripts are not significantly different, while values without common superscripts are significantly different

[c] Significant differences between day 1 and the respective treatment group on day 11

In an earlier study in healthy female SD rats [22], we have demonstrated that maximally effective doses of rhGH and rhIGF-I were additive on weight gain; however, the combined treatment did not result in additive effects on length gain. It is possible that in the healthy *ad libitum* fed animals growth was already maximally stimulated by a high dose of one of the two hormones and could not be further stimulated by the addition of the second hormone. In a situation of reduced growth velocity induced by uremia and/or reduced food intake (pair-fed controls), rhIGF-I and rhGH obviously have additive effects.

In the past, it has been assumed that all effects of IGF-I can be mimicked by GH treatment, which enhances the endogenous production of IGF-I. Growth plate chondrocytes express the growth hormone receptor [33, 34], and it has been speculated that GH acts selectively upon resting stem cells as a differentiation factor [35], the ensuing effect on proliferation being triggered by local production of IGF-I, that is, by an autocrine/paracrine mechanism (dual effect theory [35, 36]). In contrast, *in vivo* studies of Hunziker et al gave strong evidence that IGF-I is also able to stimulate stem cells [37]. In their studies both IGF-I and GH were found to stimulate all phases of chondrocyte differentiation, but IGF-I was less effective than GH. Our present study, demonstrating that GH increases length gain in addition to the maximal effect of rhIGF-I, suggests that in the uremic condition GH itself, in addition to circulating and locally produced IGF-I, can contribute to chondrocyte maturation and thus to skeletal growth.

The observed additive increase in the food efficiency ratio by both peptides without a major effect on the cumulative food intake is consistent with an increase in nitrogen balance, which was not directly measured. This is further supported by the observation that urea excretion rate decreased under the influence of both peptide hormones without a change in serum urea concentration. It is of interest that the maximal increase in the food efficiency ratio above baseline was two times higher with

rhGH than with rhIGF-I. Horber and Haymond have shown that rhGH treatment in healthy humans results in a positive protein balance by increasing protein synthesis whereas it did not influence the rate of protein catabolism [20, 38, 39]. In contrast, rhIGF-I appears to improve nitrogen balance by reducing protein breakdown without major effects on protein synthesis [19, 20]. If this is correct, both mechanisms may explain, at least in part, the additive effects of rhIGF-I and rhGH in nephrectomized and pair-fed CO animals in the present study. Exact measurements of the lean body mass were not performed; however, it is not very likely that an increase in total body water contributed to weight gain to a major extent. Whereas the relative amount of tissue water was unchanged by either hormone treatment for skeletal and heart muscle, the water content of liver increased slightly by 0.9% with rhGH and by 2% with combined rhGH and rhIGF-I treatment. The increase in total body water is much less than the total increase in body wt.

Recombinant hGH treatment increased slightly but significantly the serum IGF-I concentration. In animals injected with rhIGF-I, serum IGF-I concentration had returned to baseline 14 to 19 hours after the last injection. Ligand blotting and immuno blotting showed an increase of IGFBP-4 and IGFBP-2 in uremic serum (measured for the first time in uremic rats), whereas IGFBP-3 was unchanged. The increase of IGFBPs is analogous to the reported increased serum concentration of IGFBP-1, -2 and -3 in uremic children [40–42]. Treatment with peptide hormones did not change the pattern of binding proteins within the observation period of 12 days. Thus, the increase in IGFBP in uremic animals and the improvement of growth by exogenous IGF-I are compatible with the hypothesis that increased IGFBPs act as growth inhibitors in uremia [7, 8, 11, 42].

Analysis of growth rate by histomorphometry of the upper tibia metaphysis showed a minor stimulation of growth rate by rhIGF-I than recorded by the total body measurements. The histomorphometric analysis reflected the growth rate only for the last five days of the experiment (after staining for immunofluorescence). The discrepancies might therefore be explained by the different time intervals and/or differential effects of IGF-I on nose to tailtip length and on metaphyseal growth at the upper tibia, respectively. Another explanation might be the reduced endogenous production of GH following the treatment with rhIGF-I (Table 2) also observed in humans, such as in Laron dwarfism [17]. As a consequence, the effect of rhIGF-I on growth may decrease with time. In this respect, monotherapy with rhIGF-I may not be an ideal treatment for uremic growth failure. In humans, a variety of adverse events such as hypoglycemia and circulatory instability further limit IGF-I monotherapy, at least in high doses [13–16]. In our earlier rat experiments, most uremic but not control animals died within the first day of high-dose rhIGF-I treatment [43]. Our present investigations suggest that these animals may have died because of hypoglycemia. Measurements before, one and two hours after the first injection of rhIGF-I documented a drastic fall of blood glucose concentration.

As in the report of Hazel et al [12], rhGH did not increase serum glucose concentration but attenuated the hypoglycemia induced by IGF-I when given concomitantly. This was also reported for healthy volunteers [31]. Our results give the additional information that the potency of IGF-I to lower blood glucose levels decreases with time. After 11 days of treatment, only a minor reduction of serum glucose concentration was observed one hour after rhIGF-I injection (Table 3). Even after this time, the effect in uremic animals was more pronounced than in CO animals. These observations may explain in part the clinical observation that the adverse events of rhIGF-I in Laron dwarfism can be prevented to a certain extent if the starting dose of rhIGF-I is low and will be gradually increased to the final therapeutical doses [17]. The reason for the adaptation of the organism to exogenous IGF-I is not well understood and remains to be clarified. From experimental [44] and clinical studies it is expected that IGF-I treatment decreases insulin production. In the present experiments, we were not able to analyze how insulin production was influenced by rhIGF-I, since we measured serum insulin concentrations only at the end of the experiments, that is, 14 to 19 hours after the last injection of rhIGF-I.

In conclusion, it is possible to treat uremic growth failure and uremic growth hormone insensitivity in rats with rhIGF-I. However, the use of this peptide hormone is limited by the suppression of endogenous growth hormone secretion and by adverse events, such as hypoglycemia. When rhGH is given concomitantly with rhIGF-I to uremic animals, the growth promoting effects of both peptide hormones are additive and the hypoglycemia induced by rhIGF-I is attenuated. Since the combined treatment with rhIGF-I and rhGH seems to be safe and more effective than the treatment with rhGH alone, the combined treatment with both peptide hormones may become a new treatment modality for uremic growth failure also in humans.

Reprint requests to Otto Mehls, M.D., Department of Pediatrics, University of Heidelberg, Im Neuenheimer Feld 150, D-69120 Heidelberg, Germany.

Acknowledgments

This work was undertaken during the stay of Dr. G.T. Kovács in Heidelberg with a grant from the Deutscher Akademischer Austauschdienst (DAAD) and the stay of Dr. József Kovács with a grant of the Trans-European Mobility Scheme for University Studies (TEMPUS) and with a postgraduate scholarship from the Deutsche Forschungsgemeinschaft (DFG). We thank Pharmacia Company, Stockholm/Sweden for donation of recombinant growth hormone and IGF-I as well as Dr. A. Skottner-Lindun for performing rGH determinations. The secretarial help of Mrs. R. Greiffenhagen is acknowledged.

Note added in proof

TÖNSHOFF B, POWELL DR, ZHAO D, DOMENE HM, BLUM WF, MOORE LC, KASKEL FJ: Decreased hepatic insulin-like growth factor (IGF)-I and increased IGF binding protein (IGFBP)-1 and -2 gene expression in experimental uremia. (abstract) *J Am Soc Nephrol* 6:1032, 1995

References

1. MEHLS O, RITZ E, HUNZIKER EB, EGGLI P, HEINRICH U, ZAPF J: Improvement of growth and food utilization by human recombinant growth hormone in uremia. *Kidney Int* 33:45–52, 1988
2. BLUM WF, RANKE MB, KIETZMAN K, TÖNSHOFF B, MEHLS O: Growth hormone resistance and inhibition of somatomedin activity by excess of insulin-like growth factor binding proteins in uremia. *Pediatr Nephrol* 5:539–544, 1991
3. TÖNSHOFF B, EDEN S, WEISER E, CARLSSON B, ROBINSON ICAF, BLUM WF, MEHLS O: Reduced hepatic growth hormone receptor gene expression and increased plasma GH-binding protein in experimental uremia. *Kidney Int* 45:1085–1092, 1994
4. FINIDORI J, POSTEL-VINAY MC, KLEINKNECHT C: Lactogenic and somatotrope binding sites in liver membranes of rats with renal insufficiency. *Endocrinology* 106:1960–1965, 1980

5. Postel-Vinay MC, Tar A, Crosnier H, Broyer M, Rappaport R, Tönshoff B, Mehls O: Plasma growth hormone-binding activity is low in uremic serum. *Pediatr Nephrol* 5:545–547, 1991
6. Chan W, Valerie KC, Chan JCM: Expression of insulin-like growth factor in uremic rats: Growth hormone resistance and nutritional intake. *Kidney Int* 43:790–795, 1993
7. Tönshoff B, Mehls O, Heinrich U, Blum WF, Ranke MB, Schauer A: Growth-stimulating effects of recombinant human growth hormone in children with end-stage renal disease. *J Pediatr* 116:561–566, 1990
8. Powell DR, Rosenfeld RG, Sperry JB, Baker BK, Hintz RL: Serum concentrations of insulin-like growth factor 1, IGF-2 and unsaturated somatomedin carrier proteins in children with chronic renal failure. *Am J Kidney Dis* 10:287–292, 1987
9. Lee PDK, Hintz RL, Sperry JB, Baxter RC, Powell DR: IGF binding proteins in growth-retarded children with chronic renal failure. *Pediatr Res* 26:308–315, 1989
10. Lippe B, Fine RN, Koch VH, Sherman BM: Accelerated growth following treatment of children with chronic renal failure with recombinant human growth hormone. *Acta Ped Scand* 343(Suppl):127–131, 1988
11. Mehls O, Tönshoff B, Blum WF, Heinrich V, Seidel C: Growth hormone and insulin-like growth factor I in chronic renal failure–Pathophysiology and rationale for growth hormone treatment. *Acta Pediatr Scand* 370(Suppl):28–34, 1990
12. Hazel SJ, Gillespie CM, Moore RJ, Clark RG, Jureidini KF, Martin AA: Enhanced body growth in uremic rats treated with IGF-1 and growth hormone in combination. *Kidney Int* 46:58–68, 1994
13. Clemmons DR, Smith-Banks A, Underwood LE: Reversal of diet-induced catabolism by infusion of recombinant insulin-like growth factor-1 in humans. *J Clin Endocrinol Metab* 75:234–238, 1992
14. Lieberman SA, Bukar J, Chen SA, Celniker AC, Compton PG, Cook J, Albu J, Perlman AJ, Hoffman AR: Effects of recombinant human insulin-like growth factor-1 on total and free IGF-1 concentrations, IGF-binding proteins, and glycemic response in humans. *J Clin Endocrinol Metab* 75:30–36, 1992
15. Froesch ER, Zenobi PD, Hussain M: Metabolic and therapeutic effects of IGF-1 and growth hormone. *Horm Res* 42:66–71, 1994
16. Guler HP, Schmid C, Zapf J, Froesch ER: Effect of rhIGF-I on insulin secretion and renal function in normal human subjects. *Proc Natl Acad Sci USA* 86:2868–2872, 1989
17. Walker JL, van Wyk JJ, Underwood LE: Stimulation of statural growth by recombinant insulin-like growth factor 1 in a child with growth hormone insensitivity syndrome (Laron type). *J Pediatr* 121: 641–646, 1992
18. Cotterill AM, Camacho-Hubner C, Holly JM, Savage MO: The effect of recombinant human insulin-like growth factor-I treatment on growth hormone secretion in two subjects with growth hormone insensitivity (Laron syndrome). *Clin Endocrinol* 39:119–122, 1993
19. Clemmons DR, Underwood LE: Role of insulin-like growth factors and growth hormone in reversing catabolic stages. *Hormon Res* 38(Suppl 2):37–40, 1992
20. Haymond MW, Horber FF, Mauras N: Human growth hormone but not insulin-like growth factor 1 positively affects whole-body estimates of protein metabolism. *Horm Res* 38(Suppl 1):73–75, 1992
21. Hussain MA, Schmitz O, Mengel A, Keller A, Christiansen JS, Zapf J, Froesch ER: Insulin-like growth factor stimulates lipid oxidation, reduces protein oxidation and enhances insulin sensitivity in humans. *J Clin Invest* 92:2249–2256, 1993
22. Mehls O, Irzynjec T, Ritz E, Eden S, Kovacs G, Klaus G, Floege J, Mall G: Effects of rhGH and rhIGF-1 on renal growth and morphology. *Kidney Int* 44:1251–1258, 1993
23. Mehls O, Ritz E, Gilli G, Schmidt-Gayk H, Krempien B, Kourist B, Wesch H, Prager P: Skeletal changes and growth in experimental uremia. *Nephron* 18:288–300, 1977
24. Livessy JH, Hodgkinson SC, Roud HR, Donald RA: Effect of time, temperature and freezing on the stability of immunoreactive LH, FSH, TSH, growth hormone, prolactin and insulin in plasma. *Clin Biochem* 13:151–155, 1980
25. Kovacs G, Fine RN, Worgall S, Schaefer F, Hunziker EB, Skottner-Lindun A, Mehls O: Growth hormone prevents steroid-induced growth depression in health and uremia. *Kidney Int* 40:1032–1040, 1991
26. Hossenlopp P, Seurin D, Segovia-Quinson B, Hardouin S, Binoux M: *Anal Biochem* 154:138–143, 1986
27. Zapf J, Hauri C, Waldvogel M, Futo E, Haesler H, Binz K, Guler HP, Schmid C, Froesch ER: Recombinant human IGF-I induces its own specific carrier protein in hypophysectomized and diabetic rats. *Proc Natl Acad Sci USA* 86:3813–3817, 1989
28. Schenk R, Eggli P, Fleisch H, Rosini S: Quantitative morphometric evaluation of the inhibitory activity of new aminobiphosphonates on bone resorption in the rat. *Calcif Tis Int* 38:342–349, 1986
29. Cruz-Orive LM, Hunziker EB: Stereology for anisotropic cells: Application to growth cartilage. *J Microsc* 143:47–80, 1986
30. Hunziker EB, Schenk RK, Cruz-Orive LM: Quantitation of chondrocyte performance in epiphyseal plates during longitudinal bone growth. *J Bone Jt Surgery (Am Volume)* 69A:162–173, 1987
31. Zar JH: *Biostatistical Analysis.* Englewood Cliffs, Prentice-Hall, Inc., 1984
32. Kupfer SR, Underwood LE, Baxter RC, Clemmons DR: Enhancement of the anabolic effets of growth hormone and insulin-like growth factor I by use of both agents simultaneously. *J Clin Invest* 91:391–396, 1993
33. Isaksson OGP, Lindahl A, Nilsson A, Isgaard J: Mechanisms for the stimulatory effect of growth hormone on longitudinal bone growth. *Endocrin Rev* 8:426–438, 1987
34. Werther GA, Haynes KM, Barnard R, Waters MJ: Visual demonstration of growth hormone receptors on human growth plate chondrocytes. *J Clin End Metab* 70:1725–1731, 1990
35. Ohlsson C, Nilsson A, Isaksson O, Lindahl A: Growth hormone induces multiplication of the slowly cycling cells of the rat tibial growth plate (epiphyseal plate). *Proc Natl Acad Sci USA* 89:9826–9830, 1992
36. Green H, Morikawa M, Nixon T: A dual effector theory of growth hormone action. *Differentiation* 29:195–198, 1985
37. Hunziker EB, Wagner J, Zapf J: Differential effects of IGF-I and growth hormone on developmental stages of rat growth plate chondrocytes in vivo. *J Clin Invest* 93:1078–1086, 1994
38. Horber FF, Haymond MW: Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *J Clin Invest* 86:265–272, 1990
39. Bennett WM, Haymond MW: Growth hormone and lean tissue catabolism during long-term glucocorticoid treatment. *Clin Endocrinol* 36:161–164, 1992
40. Tönshoff B, Blum WF, Wingen AM, Mehls O, the European Study Group for Nutritional Treatment of CRF in Childhood: Plasma insulin-like growth factors (IGF) and IGF binding proteins 1, 2 and 3 in children with chronic renal failure: Relationship to height and glomerular filtration rate. *J Clin Endocrinol Metab* 80:2684–2691, 1995
41. Blum WF: Insulin-like growth factors and IGF binding proteins in chronic renal failure: Evidence for reduced secretion of IGFs. *Acta Pediatr Scand* 379(Suppl):24–31, 1991
42. Powell DR, Liu F, Baker B, Lee PDK, Belsha CW, Brewer ED, Hintz RL: Characterization of insulin-like growth factor binding protein-3 in chronic renal failure serum. *Pediatr Res* 33:136–143, 1993
43. Mehls O, Ritz E, Kovacs G, Fine RN, Worgall S, Mak RHK: Effects of human recombinant growth hormone and IGF-1 on growth and GFR in uremic rats. (abstract) *Kidney Int* 37:513, 1990
44. Jacob R, Barret E, Plewe G, Fagin KD, Sherwin RJ: Acute effects of IGF I on glucose and amino acid metabolism in the awake fasted rat. *J Clin Invest* 83:1717–1723, 1989