Effects of growth hormone and thyroxine on kidney insulin-like growth factor-I and renal growth in hypophysectomized rats

S. M. Marshall, A. Flyvbjerg, K. D. Jørgensen*, J. Weeke and H. Ørskov

Institute of Experimental Clinical Research, University of Aarhus, Nørrebrogade, DK 8000, Aarhus C, Denmark
*Department of Pharmacology, Biopharmaceutical Division, Novo-Nordisk A/S, Niels Steensensvej, DK 2930, Gentofte, Denmark
†Medical Department M (Diabetes and Endocrinology), Kommunehospital, Nørrebrogade, DK 8000, Aarhus C, Denmark

(Requests for offprints should be addressed to S. M. Marshall who is permanently at Department of Medicine,
The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, U.K.)

REVISED MANUSCRIPT RECEIVED 17 September 1992

ABSTRACT

The effects of treatment for 11 days with human growth hormone (hGH; 140 μg/day), thyroxine (T4; 3 μg/day) and hGH + T4 on renal growth and content of insulin-like growth factor-I (IGF-I) in hypophysectomized rats have been compared with saline-treated hypophysectomized animals and intact control animals. Right kidney weight and kidney weight/body weight ratio remained low in the saline-treated group (313 ± 9 mg vs 694 ± 28 mg in controls on day 11, P < 0.001 and 3.4 ± 0.12 × 10⁻³ vs 4.2 ± 0.10 × 10⁻³, P < 0.005 respectively). In T₄- and hGH-treated animals, kidney weight gain was similar (to 420 ± 14 and 450 ± 22 mg on day 11 respectively, P > 0.05), whilst the increase was greater in the group given hGH + T₄ (to 572 ± 34 mg, P < 0.001 compared with hGH- and T₄-treated groups). The kidney weight/body weight ratio became normal in the T₄- and hGH + T₄-treated animals but remained low in the hGH-treated group. The renal content of IGF-I was low in the saline-treated animals throughout the study (92 ± 10 ng/g on day 11 vs 219 ± 8 ng/g in control animals, P < 0.001), but increased to a maximum of 88% above baseline on day 1 in the group given T₄. In the hGH- and hGH + T₄-treated groups, renal IGF-I concentration rose to a peak of 317% above baseline on days 3 and 4, then fell to the values seen in control animals on day 11 (hGH: 242 ± 18 ng/g; hGH + T₄: 320 ± 41 ng/g; controls: 219 ± 8 ng/g; P > 0.05 for all comparisons). Thus treatment with hGH or T₄ results in similar kidney weight gain, despite a greater rise in the renal concentration of IGF-I in the hGH-treated animals. Treatment with both hGH + T₄ leads to an increase in the renal concentration of IGF-I similar to that seen with hGH treatment alone, but a larger increase in kidney weight, suggesting that T₄ does not stimulate renal growth via the IGF-I pathway and that growth promotion by hGH and T₄ is additive. Journal of Endocrinology (1993) 136, 399-406

INTRODUCTION

Studies using the hypophysectomized animal model have indicated that longitudinal growth is greatest when growth hormone (GH) and thyroxine (T₄) are replaced in combination (Simpson et al. 1950; de Groot, 1963). Thorngren & Hansson (1973) showed that the increase in the width of the tibial growth plate was greater than the expected additive effect, suggesting a synergistic effect. The mechanisms whereby T₄ interacts with the GH/insulin-like growth factor-I (IGF-I) axis are not well understood.

Rapid growth of the kidney, preceded by a rise in the renal concentration of IGF-I, is a common feature of many renal growth models, such as following the induction of diabetes, unilateral nephrectomy, potassium depletion and GH treatment of hypophysectomized rats (Flyvbjerg et al. 1991b) and dwarf GH-deficient animals (Lajara et al. 1989; Skottner et al. 1989). The rise in kidney IGF-I and the subsequent...
renal growth can both be inhibited by treatment with the somatostatin analogue octreotide, which inhibits GH secretion from the pituitary (Flyvbjerg et al. 1989), or insulin (Flyvbjerg et al. 1988) in the diabetic model, and by somatostatin after uninephrectomy (Flyvbjerg et al. 1989). Also, renal IGF-I mRNA levels, IGF-I content and renal growth are increased when pituitary-intact mice (Mathews et al. 1988) and rats (Miller et al. 1990) are implanted with GH-producing pituitary cells.

After hypophysectomy, followed the next day by unilateral nephrectomy, there is a small initial rise in the weight of the remaining kidney by the second day after uninephrectomy, before a decline over the next 8 days (Fogelman & Goldman, 1966). In hypophysectomized animals given GH and T4, the weight of the remaining kidney increases much more dramatically, to approximately 50% of that seen in uninephrectomized, non-hypophysectomized control animals. It would thus appear that the hypophysectomized animal is a useful model in which to examine the relative effects and modes of action of GH and T4 on kidney growth. We have therefore studied the effects on body weight, kidney weight, renal content of IGF-I and serum IGF-I of treatment with hGH, T4, and hGH + T4 in the hypophysectomized rat.

MATERIALS AND METHODS

Animals

Male Wistar rats were purchased from Charles River Wiga GmbH (Sulzfelt, Germany). The animals were housed at 22 ± 2 °C, 55 ± 10% relative humidity, with an air change 8–10 times per h and a cycle of 12 h light:12 h darkness (06.30–18.30 h light). The animals had free access to standard food (no. 1324; Altromin, Lage, Germany) and tap water. After acclimatization for 1 week, hypophysectomy was performed when the animals were 30–40 days old. Following anaesthesia with intraperitoneal amyl hydrate (400–500 mg/kg body weight) and metohexital (40–50 mg/kg body weight), hypophysectomy was performed by the transauricular method of Illhardt (1971), as previously described (Jørgensen, 1987). Only animals with a weight gain of less than 10 g and a weight loss of less than 4 g at 14 days after hypophysectomy were included in the experiment. Non-hypophysectomized animals, age-matched with the hypophysectomized rats, were used as controls.

Design

Fourteen days after hypophysectomy, the animals (with a body weight range of 82–106 g) were divided randomly into four groups. One group received a subcutaneous injection of 0.5 ml 0.154 mol NaCl/l (hypox + NaCl) once daily. A second group received a subcutaneous injection of 0.5 ml human GH (hGH; Norditropin, 140 μg/ml) (hypox + hGH) twice daily. The third group received a subcutaneous injection of 0.5 ml t-thyroxine (T4; 3 μg/ml) (hypox + T4) twice daily. The fourth group (hypox + hGH + T4) received the same doses of hGH and T4 as the second and third group twice daily. These doses of hGH and T4 were calculated from previous experiments in our laboratories (Thorlacius-Ussing et al. 1988) and from the work by Thorngren & Hansson (1973), and were chosen to achieve optimum growth rate and near-normal serum concentrations of IGF-I, T4 and triiodothyronine (T3). Hormone administration to the hypophysectomized animals was continued for 11 days. Non-hypophysectomized control animals had a body weight range of 150–180 g at the beginning of the study and did not receive any treatment during the subsequent 11 days.

All animals were weighed daily or every second day during the course of the experiment. Rats were used in groups of six for measurements. Control rats were killed on days 0 and 11. Rats from the other four groups were killed on days 0 (hypox + NaCl, only), 1, 2, 3, 4, 7 and 11. Animals were anaesthetized as for hypophysectomy and blood was collected from the abdominal aorta. After centrifugation, the serum was stored at −20 °C until further analysis. The right kidneys were removed, trimmed of fat, hilum, capsule and blood, weighed, snap-frozen in liquid nitrogen, and stored at −20 °C.

Hormones

Biosynthetic hGH (Norditropin, Nordisk Gentofte A/S, Gentofte, Denmark) was dissolved in distilled water to obtain an isotonic stock solution. Each day a fresh dilution was made in 0.154 mol NaCl/l to a concentration of 140 μg/ml t-Thyroxine (Sigma Chemicals, St Louis, MO, U.S.A.) was dissolved in 20 μl absolute ethanol. Prior to each injection, a fresh solution was made in 0.154 mol NaCl/l to a concentration of 3 μg/ml.

IGF-I extraction from kidney

Extraction of kidney IGF-I in 1 mol acetic acid/l was performed by the method of D’Ercole et al. (1984) as previously described (Flyvbjerg et al. 1988). Since IGF-I in both the renal tissue and the serum trapped in the tissue is measured, the tissue concentration of IGF-I has been corrected for the serum concentration as previously described (Flyvbjerg et al. 1991a). The amount of serum trapped in kidney tissue was 25–39 μl/g wet kidney weight.
IGF-I radioimmunoassay

IGF-I antibody UB 286 (raised by L. E. Underwood and J. J. van Wyk, Department of Pediatric Endocrinology, University of Carolina, Chapel Hill, NC, U.S.A.) was donated by the US National Hormone and Pituitary Program. For standards (0.5-10 pg/ml) and iodination, a full amino acid sequence IGF-I analogue (Amgen Biologicals, Thousand Oaks, CA, U.S.A.) was used, purchased from Amersham International plc, Amersham, Bucks, U.K. IGF-I was measured in rat serum after extraction in methanol/ acetic acid (Flyvbjerg et al. 1989). The intra-assay coefficient of variance (C.V.) on duplicates was 5% and interassay C.V. was 12% at a concentration of 1220 pg/l. The IGF-I antibody has 0.5% cross-reactivity with IGF-II and cross-reacts minimally with insulin at 1 μmol/l.

Assay of serum total T4 (TT4) and total T3 (TT3)

TT4 and TT3 were measured by radioimmunoassay (Weeke & Ørskov, 1973).

Statistical analysis

Results are given as means ± S.E.M. Differences between groups and differences with time within groups were analysed by one-way analysis of variance with the modified least-significant difference correction for multiple comparisons. Significant differences between experimental groups are shown in the figures, while the significant changes within groups with time are given in the text.

RESULTS

Body weight (Fig. 1)

The initial body weight of the hypophysectomized animals was significantly less than that of the control animals (93 ± 2 vs 166 ± 3 g, P < 0.001). The hypox + NaCl animals gained 4.3% (4 g; P > 0.05) of their initial body weight during the 11 days, whilst the hypox + T4 group gained 12% (10 g; P > 0.05 compared with day 0). The body weight of the hypox + T4 animals was similar to that of the saline-treated animals on day 11. In contrast, the hypox + hGH and hypox + hGH + T4 animals gained a similar amount of weight: 44 g (P < 0.05 compared with day 0) in the hGH-treated group and 53 g (P < 0.05 compared with day 0) in the hGH + T4 group.

Right kidney weight (Fig. 2a)

On day 0, the right kidney weight of the hypophysectomized animals was significantly lighter than that of the control animals (313 ± 9 vs 694 ± 28 mg, P < 0.001), the kidney weight/body weight ratio being 3.4 ± 0.12 × 10−3 in the hypox + NaCl animals and 4.2 ± 0.10 × 10−3 in the control group (P < 0.01). In the control group, the right kidney weight increased from 694 ± 28 to 1002 ± 78 mg (P < 0.001), an increase of 44% during the study period. The kidney weight in the hypox + NaCl animals remained constant for the duration of the study, as did the kidney weight/body weight ratio. The kidney weight increased in an identical manner in the hypox + T4 and hypox + hGH groups, reaching a mean of 420 ± 14 mg in the hypox + T4 group (P < 0.001 compared with day 0) and 450 ± 22 mg in the hypox + hGH group (P < 0.001 compared with day 0), increases of 32% and 41% respectively (P > 0.05). The ratio of kidney weight/body weight was 3.1 ± 0.11 × 10−3 in the hGH-treated group and 4.1 ± 0.08 × 10−3 in the T4 group (P < 0.001). Kidney growth was significantly greater in the hypox + T4 + hGH group, the weight reaching 572 ± 34 mg at 11 days, an increase of 83% (P < 0.001 compared with day 0). The ratio of kidney weight/body weight was 3.9 ± 0.19 × 10−3, similar to those in the controls and T4-treated animals and significantly greater than those in the saline-treated (P < 0.01) and hGH-treated animals (P = 0.01).

Kidney IGF-I content (Fig. 2b)

The initial renal concentration of IGF-I was significantly lower in the hypox + NaCl animals compared with the control animals (Fig. 2b) and fell to 92 ± 10 ng/g by day 11 (P = 0.002 compared with day 0). In the hypox + T4 animals there was a small but significant increase in the renal concentration of IGF-I on day 1 (P < 0.01 compared with day 0), followed by a steady decline, although values remained significantly higher than those in the hypox + NaCl animals at all time-points (Fig. 2b). In the hypox + hGH and hypox + hGH + T4 animals, there was a rapid and profound rise in the renal content of IGF-I, reaching a peak around 800 ng/g on days 2-4, an increase of 317% from baseline. Thereafter, concentrations declined to values equivalent to those in the control group by day 11. The renal concentrations of IGF-I were similar in the hypox + hGH and hypox + hGH + T4 groups at all time-points.

Serum IGF-I (Fig. 3)

At the beginning of the study, the serum concentration of IGF-I in the saline-treated hypophysectomized animals was significantly lower than that in the control group (6 ± 4 vs 113 ± 30 μg/l, P < 0.001) and remained low for the duration of the study. In the hypox + T4 group, the serum concentration was similar to values in the saline-treated group during the
study, apart from on day 11, when the concentration rose to 192±75 μg/l (P<0.05 compared with day 0). In the hypox + hGH and hypox + hGH + T₄ animals there was a very rapid, pronounced rise in the serum concentration of IGF-I on days 1 and 2, followed by a slower but sustained increase to reach values comparable with those in the control group on day 11 (hGH:1170±75 μg/l; hGH + T₄:1001±120 μg/l; control:1252±84 μg/l; no significant difference between any two groups).

Serum TT₄ and TT₃ (Table 1)
On day 0, the serum TT₄ and TT₃ concentrations were significantly lower in the hypox + NaCl animals compared with the controls (TT₄:24±0.5 vs 113±4 nmol/l, P<0.001; TT₃:0.11±0.03 vs 1.68±0.11 nmol/l, P<0.001) and remained depressed for the duration of the study. Serum TT₄ and TT₃ concentrations in the group treated with hGH alone were comparable with those in the saline-treated animals throughout the study. In the groups given T₄ or hGH + T₄, serum TT₄ concentrations rose very rapidly, reaching concentrations higher than those in the control group on days 1–4, but thereafter stabilizing around the concentrations seen in the control animals. The total T₄ concentrations observed in the hypox + hGH + T₄ group were significantly higher than those in the hypox + T₄ group on days 3, 4 and 7 (P<0.01). The serum concentration of TT₃ rose rapidly in both the hypox + T₄ and hypox + hGH + T₄ groups, entering the normal range on day 1 and remaining similar to values in the control group thereafter. Concentrations of TT₃ were identical throughout the study in the T₄- and hGH + T₄-treated groups.

DISCUSSION
The aim of our study was to investigate the regulatory mechanisms influencing the renotropic actions of GH, T₄ and hGH + T₄ in combination, with particular regard to the role of IGF-I. The model used proved suitable, the saline-treated hypophysectomized animals demonstrating the classical features of hypophysectomy. The chosen replacement doses of hGH and T₄ resulted in near-normalization of the serum concentrations of IGF-I, TT₄ and TT₃. Although the TT₄ concentrations were slightly higher in the hGH + T₄-treated animals compared with the group given T₄ alone on some days only, the concentrations of the active hormone T₃ were identical in the two groups at all time-points.

Treatment with hGH alone resulted in a very rapid and large rise in the renal concentration of IGF-I, to values greater than those in control animals on days
FIGURE 2. (a) Right kidney weight and (b) kidney concentration of IGF-I in hypophysectomized animals during 11 days of treatment with saline (○), human GH (140 μg/day; ●), thyroxine (3 μg/day; ■) and combined human GH and thyroxine (▲). Intact control animals (□). Results are expressed as means ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 compared with saline-treated animals.
TABLE I. Serum concentrations of total thyroxine (TT₄) and tri-iodothyronine (TT₃) in hypophysectomized animals treated for 11 days with saline (hypox + NaCl), human growth hormone (140 µg/day) (hypox + hGH), thyroxine (3 µg/day) (hypox + T₄) and combined human growth hormone and thyroxine (hypox + hGH + T₄), and in intact control animals. Values are means ± s.e.m. after 11 days of treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum TT₃ (nmol/l)</th>
<th>Serum TT₄ (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.51 ± 0.07</td>
<td>131 ± 8</td>
</tr>
<tr>
<td>Hypox + NaCl</td>
<td>0.04 ± 0.01***</td>
<td>27 ± 2***</td>
</tr>
<tr>
<td>Hypox + hGH</td>
<td>0.06 ± 0.01***</td>
<td>35 ± 5***</td>
</tr>
<tr>
<td>Hypox + T₄</td>
<td>1.45 ± 0.11</td>
<td>115 ± 10</td>
</tr>
<tr>
<td>Hypox + hGH + T₄</td>
<td>1.34 ± 0.12</td>
<td>144 ± 10</td>
</tr>
</tbody>
</table>

***P < 0.001 compared with control and T₄- and hGH + T₄-treated animals.

1–7, coupled with an increase in kidney weight. Previous studies have also demonstrated an increased renal concentration of IGF-I in hypophysectomized rats after 12 h (D'Ercole et al. 1984) and 3 (D'Ercole & Underwood, 1987) and 4 (Orlowski & Chernausek, 1988) days treatment with GH. This pattern of rapid increase in kidney weight along with a rise in the renal concentration of IGF-I is seen in many instances of rapid kidney growth, including experimental diabetes, uninephrectomy and potassium depletion (Flyvbjerg et al. 1991b), suggesting that local accumulation of IGF-I has a renotropic effect in many conditions of rapid kidney growth.

As expected, body weight also increased following hGH treatment, but the kidney weight/body weight ratio remained low, suggesting that body weight and kidney weight increased in parallel.

Treatment with physiological doses of T₄ produced an identical renal weight gain to that in the hGH-treated animals, with normalization of the kidney weight/body weight ratio. However, the renal concentration of IGF-I in the T₄-treated animals was only modestly increased above the levels seen in control animals, and declined during the study, whilst there was a very large and sustained rise in the concentrations observed in the hGH-treated animals. This suggests that the growth-promoting effects of T₄ are not primarily mediated through IGF-I but by an alternative mechanism. It has previously been demonstrated that treatment of hypophysectomized animals with similar doses of T₄ normalizes the renal content of T₃ and the activity of the iodothyronine deiodase, whereas hGH treatment has little effect on these parameters (Bjørn-Hansen Gøtzsche et al. 1991). Thus the growth-promoting effects of T₄ may well be mediated by a direct effect of thyroid hormones on peripheral tissues, T₃ itself stimulating protein gene
transcription by direct intranuclear action (Oppenheimer, 1979).

The increase in kidney weight during $T_4$ treatment, comparable with that seen with hGH treatment, is in contrast with the comparatively small gain in body weight. Thyroxine is known not to stimulate body weight gain (Thorngren & Hansson, 1973) and will also alter body composition, with loss of adipose tissue. Serum IGF-I concentrations were unchanged following treatment with $T_4$.

Combined replacement with hGH + $T_4$ resulted in a greater increase in kidney weight than was seen with each individual hormone, yet the renal content of IGF-I was similar to that in the group given hGH alone, again supporting the concept that the two hormones promote renal growth by different mechanisms, hGH acting via IGF-I and $T_4$, following conversion by deiodase to $T_3$, via a direct intranuclear effect on protein gene transcription. The magnitude of the increase in kidney weight with combined treatment suggests an additive effect on renal growth when both the IGF-I pathway and the thyroid system are fully activated. The kidney weight/body weight ratio was normalized, as it was by $T_4$ alone but not by hGH, suggesting differing effects of the two hormones on organ and whole body growth. Despite the incremental increase in kidney weight seen with combined treatment, body weight gain was identical to that in the hGH-treated group, again confirming the lack of effect of $T_4$ on body weight. Serum IGF-I concentrations were similar in the hGH- and hGH + $T_4$-treated groups. In a previous study, although chronic administration of low doses of $T_4$ had no effect on serum IGF-I, an acute dose of GH plus $T_3$ resulted in greater rises in liver IGF-I mRNA and serum IGF-I than were seen with GH alone (Wolf et al. 1989). Thus the effects of thyroid and growth hormones on the liver are complex.

Previous work has demonstrated that thyroid hormones interact with the GH/IGF-I axis at several levels. Thyroid hormones stimulate transcription of the GH and IGF-I genes in pituitary-derived cells (Melmed & Yamashita, 1986; Fagin et al. 1989), enhance the release of IGF-I from fetal mouse hypothalamus (Binoux et al. 1985) and increase the number of IGF-I receptors on anterior pituitary but not renal cells in thyroidectomized rats (Matsu et al. 1990). Our present study in hypophysectomized animals suggests that, although thyroid hormones in isolation stimulate kidney growth by an alternative pathway to the GH/IGF-I axis, when given along with GH, they produce tissue-selective additive effects with the GH/IGF-I axis in peripheral tissues.

We have demonstrated similar increases in renal weight following treatment with hGH or $T_4$ in hypophysectomized animals, coupled with a large increase in the renal content of IGF-I in the group given hGH only. Combined treatment with hGH + $T_4$ resulted in a greater increase in kidney weight than was observed with single treatment, but a rise in the renal content of IGF-I similar to that in the group given hGH alone. These results suggest that the renal growth-promoting effects of hGH are mediated via IGF-I, whereas those of $T_4$ are by an alternative mechanism, perhaps a direct effect on protein synthesis. The increase in kidney weight following treatment with both hGH and $T_4$ suggests an additive effect of simultaneous activation of the two mechanisms.

ACKNOWLEDGEMENTS

We are grateful to K. Nyborg for technical assistance and to Drs L. E. Underwood and J. J. van Wyk of the National Hormone and Pituitary Program for the gift of IGF-I antibody. The study was supported by the Danish Diabetes Association, the Danish Medical Research Council, the Ruth König Petersen Foundation, the Nordic Insulin Foundation, the Novo Foundation and the Aage Louis-Hansen Foundation. S. M. M. held a British Medical Research Council Travelling Fellowship.

REFERENCES


