Comparison of in vivo effects of insulin-like growth factors I and II and of growth hormone in hypophysectomized rats

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Abstract. Pure human IGF I (43 and 103 μg/day) and IGF II (131 μg/day) were infused into hypophysectomized rats during 6 days by means of sc implanted minipumps. Their effects on several growth indices were compared with those of various doses of sc infused human growth hormone. Growth hormone infusion produced a dose-dependent rise of endogenous rat IGF from 39 (without growth hormone) to 86 μU equivalents/ml (with 400 mU hGH/day) as determined by a competitive protein binding assay with a human IGF standard. In rats receiving the two doses of IGF I, total serum IGF levels rose to 83 and 99 μU equivalents/ml, respectively, in those receiving the IGF II dose the total serum IGF level rose to 146 μU equivalents/ml. These increases corresponded to steady state levels of 168 and 286 ng/ml of immunoreactive insulin-like growth factor (IR-IGF) I and 320 ng/ml of IR-IGF II. IGF I, but not IGF II led to an increase in body weight similar to that induced by the low doses of hGH (12.5 and 25 mU, respectively). The rise of endogenous rat IGF as well as the infused human IGF I and II caused a widening of the tibial epiphysis and an increase of the [3H]thymidine incorporation into costal cartilage. With respect to these two indices IGF II was clearly less potent than IGF I. When expressed in μU equivalents of the protein binding assay, endogenous rat IGF induced by hGH appeared to be relatively more effective than infused human IGF I or II.

Growth hormone infusion produced a change in the radiochromatographic [125I]IGF binding pattern of hypox rat serum: the gammaglobulin-sized specific IGF binding peak that is characteristic of normal rat serum but lacking in serum of hypophysectomized rats reappeared. Neither infused IGF I nor IGF II had this effect.

It may be concluded that the infusion of human IGF I or II in hypophysectomized rats produced qualitatively the same effects on growth indices as a growth hormone-induced increase of endogenous rat IGF. This supports the idea that IGF I and to some extent also IGF II are able to mediate some of the actions of growth hormone. Other growth hormone effects appear to depend on growth hormone directly.

Insulin-like growth factors (IGF) I and II belong to the polypeptide hormone family of the somatomedins. The structures of IGF I and II show considerable homology with the insulin A- and B-chain (Rinderknecht & Humbel 1978a,b). Somatomedin C (SM-C) has recently been shown to be identical to IGF I (Klapper et al. 1983). Due to their insulin-like structure, these growth factors possess insulin-like activity which, in contrast to insulin, is not abolished in the presence of insulin antibodies. They exert acute insulin-like effects on adipose tissue and muscle (Zapf et al. 1981a), fibroblasts (Zapf et al. 1981a), chondrocytes (Froesch et al. 1976) and calvaria cells (Canalis 1980; Schmid et al. 1983a) and the differentiation of cells of mesenchymal origin (Schmid et al. 1983b,c; Kurtz et al. 1982).

The levels of IGF I/SM-C and, to a lesser extent also those of IGF II in human serum depend on the growth hormone (GH) status (Furlanetto et al. 1977; Zapf et al. 1981b). Recently, pure IGF I has been shown to stimulate growth in hypophysectomized (hypox) rats (Schoenle et al. 1982a), a finding which strongly supports the somatomedin concept (Daughaday et al. 1972).

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The present study was designed to compare the in vivo effects of IGFII on growth and growth indices of hypox rats with the effects of IGF I and of human growth hormone (hGH).

Materials and Methods

\[^{3}H\]thymidine was from the Radiochemical Centre Amersham, UK. Human serum albumin (HSA) was supplied by the Swiss Red Cross, Bern. It was extensively dialyzed against three changes of distilled water and filtered under sterile conditions before use. Human growth hormone (Nanormon®, 2 U/mg) was from the Nordisk Insulin Laboratory, Gentofte, Denmark. Whale insulin (same amino acid sequence as pork insulin) and pure IGF I and IGF II were kindly provided by Dr. R. E. Humbel, Zürich. All chemicals of the highest purity available were from Fluka AG, Buchs, Switzerland and from Merck, Darmstadt, FRG.

The characteristics of the IGF antisera and the iodination of IGF I and IGF II have been described in detail earlier (Zapf et al. 1981b).

Assay procedures

Glucose, alkaline phosphatase and inorganic phosphorus were measured by automatized routine methods in the serum of each rat.

The tibia test

The tibiae were removed and stained as described by Greenspan et al. (1949). The proximal part was photographed under 25-fold magnification with a Polaroid camera. The width of the proximal epiphysis was measured on the photographs and the mean value of ten measurements was calculated.

Thymidine incorporating activity of costal cartilage

Segments of cartilage of the two lowest ribs were prepared free of perichondrium and incubated in 2 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing HSA (2 mg/ml), non-essential amino acids (1% v/v, code 5832, Difco Laboratories, Detroit), amino acids HeLA (1% v/v, code 5790), glycine (0.2 mM), glutamine (2 mM) and \[^{3}H\]thymidine (1µM, 1 µCi). The incubation was carried out for 24 h at 37°C. The cartilage segments were washed 4 times with 0.9% NaCl on a Vortex mixer and subsequently rinsed under running tap water overnight, dried, weighed, digested for 30 min at 90°C in 1 ml of 90% formic acid and counted in 10 ml of Instagel (Packard) in a beta counter.

Radioimmunoassays for human IGF I and IGF II

They were performed as described earlier (Zapf et al. 1981b). To separate IGF from the IGF carrier protein 0.5 ml serum samples were passed over a Sephadex G-50 (medium) column (1.5 x 100 cm) in 1 M acetic acid. The fractions eluting between 50 and 80% bed volume, devoid of IGF carrier protein, were lyophilized, washed in 2 ml of 0.1 M NH₂HCO₃ and re-lyophilized. The lyophilized material was dissolved in 1 ml of PBS/0.2% HSA buffer, and 1R-human IGF I or II was determined at 3 different dilutions where cross-reactivity of endogenous rat IGF was negligible.

Total serum IGF levels

Since no radioimmunoassay for rat IGF was available and because it cannot be determined in our IGF I or II RIAs (no significant cross-reactivity in the IGFII-RIA, non-parallel displacement in the IGF I RIA: Zapf et al. 1984) it was determined in a competitive protein binding assay (Zapf et al. 1977) using \[^{125}I\]IGF I as a tracer and a partially purified preparation of human IGF as a standard. This standard had itself been standardized in the rat fat pad assay (Froesch et al. 1963) and had a specific biological activity of 4.5 mU insulin equivalents per mg protein. It contained a 1:1 (wt/wt) mixture of immunoreactive IGF I and II. An IGF standard instead of an insulin standard has to be used in the protein binding assay because insulin does not cause any displacement of IGF tracer from the binding protein. The results of the protein assay are expressed as µU equivalents of the human IGF standard. The potency of pure IGFII in displacing \[^{125}I\]IGF I tracer from the binding protein is around 3-times higher than that of IGF I. Therefore, the activities of pure IGF I and II relative to that of the standard consisting of a mixture of the two factors are not identical: 1 µg of pure IGFI or II is equivalent to around 200 and 350 µU of the reference standard. Since the potency of rat IGF relative to that of human IGF I or II or to the human standard used in the protein binding assay is not known and since the serum of the IGF infused rats contains a mixture of endogenous rat IGF and infused human IGF total serum IGF levels are expressed as µU equivalents of the human standard.

\[^{125}I\]IGF binding patterns of rat sera

0.25 ml serum was incubated with 0.25 ml 0.1 M phosphate buffer, pH 7.4, and \[^{125}I\]IGF I (2 x 10⁵ CPM) for 17 h at 4°C and then chromatographed at 4°C on a Sephadex G-200 column (2 x 60 cm) in 0.1 M phosphate buffer, pH 7.4. Two ml fractions were counted in a gamma counter.

Animals

Male Sprague Dawley rats (100–120 g) were used for all experiments. They were kindly provided by Mr. Maier and Dr. Meier, Ciba-Geigy AG, Basel. The experiments were carried out 3–4 weeks after hypophysectomy. Rats whose body weight did not increase more than 0.1 g per day were considered totally hypophysectomized. They were kept on a 12 h light/dark cycle and had free access to drinking water and chow (Altromin International, Darmstadt, FRG).
Lage FRG) consisting of 23.5% protein, 6% fat and 5% carbohydrate. Hormones were administered by sc implanted minipumps (Alzet® minipumps, model 2001, Alza, Palo Alto). The mean pumping rate was 1.02 ± 0.15 µl/h, the mean filling volume 219.2 ± 8.7 µl. hGH (2 U/mg) was dissolved in saline, IGF I and IGF II in 0.1 M acetic acid. Hypox rats implanted with pumps containing saline or 0.1 M acetic acid did not differ in any of the measured parameters from the untreated (not implanted) hypox rats. Four rats were used for each experiment. Hormones were administered during 6 days. Body weight gain was checked every day at 8.00 a.m. After 6 days the rats were sacrificed. They were anaesthetized with 0.25 ml of pentobarbital and bled by aortic puncture. Testes, thymus, retroperitoneal fat, liver and soleus muscle were removed and weighed.

Statistics
Student's t-test has been used for statistical analysis of the data.

Results

Validity of the method of hormone administration
As shown previously, growth parameters of hypox rats were stimulated similarly whether a given dose of GH was administered ip by two daily sc injections or by continuous sc infusion (Schoenle et al. 1982b). In the experiments described here, IGF I and IGF II were determined by RIA in the solution before filling the minipumps and again in the remaining solution after removal of the minipumps from the site of implantation. The concentration of the higher dose of IGF I was 4.21 before and 4.28 µg/µl after 6 days under the skin, that of the lower dose 1.88 and 1.90 µg/µl, respectively. Only one dose of IGF II was administered. Its concentration was 5.22 before and 5.36 µg/µl after 6 days of infusion. Thus, IGF I and II were not destroyed in the minipumps during the implantation period.

The mean IR-IGF I levels determined in the serum pools of each group of rats after 6 days of infusion of 43 and 103 µg/day were 168 and 286 ng/ml. At an infusion rate of 131 µg/day of IGF II the IR-IGF II serum level was 320 ng/ml. The half-lives for IGF I and II calculated in first approximation from these data are 19 and 14 min for the lower and the higher IGF I dose, respectively, and 12 min for the IGF II dose, assuming a plasma volume of 5 ml.

Under the assumption that the disappearance of the infused IGF follows first order kinetics (which certainly represents a rough approximation towards a more complex elimination kinetics) the half-life $\frac{T}{2} = \frac{0.69}{k_{-1}}$, where $k_{-1}$ is the rate constant of disappearance. At steady state the disappearance rate $r_{-1}$ = infusion rate $r$. Furthermore $r_{-1} = r = k_{+1} \cdot Q$, where $Q$ is the total circulating amount of the infused IGF at steady state. Therefore, $k_{1} = \frac{r}{Q}$ and $\frac{T}{2} = \frac{0.69 \cdot Q}{r}$ can be calculated from the steady state serum level of the infused IGF and the circulating plasma volume (5% of the body weight, 5–6 ml for 100–200 g rats).

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Table 1.
Growth effects of IGF I and IGF II after administration during 6 days to hypox rats.

<table>
<thead>
<tr>
<th>Administered hormone µg/day</th>
<th>Serum IR-IGF I or II level ng/ml</th>
<th>Tibial epiphyseal width (µm) mean ± SEM (n = 4)</th>
<th>[3H]thymidine incorporating activity of costal cartilage (pmol [3H]thymidine incorporated/µg) mean ± SEM (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>–</td>
<td>153 ± 12</td>
<td>0.72 ± 0.07</td>
</tr>
<tr>
<td>IGF I 43</td>
<td>168</td>
<td>213 ± 8*</td>
<td>1.23 ± 0.09*</td>
</tr>
<tr>
<td>IGF I 103</td>
<td>286</td>
<td>257 ± 9*</td>
<td>1.72 ± 0.24*</td>
</tr>
<tr>
<td>IGF II 131</td>
<td>320</td>
<td>184 ± 4*</td>
<td>0.96 ± 0.09**</td>
</tr>
</tbody>
</table>

* $P < 0.001$ (compared with control). ** $P < 0.05$ (compared with control).

1 Determined in serum pools of 4 rats each.
Effects of IGF I and II on growth indices

Table 1 shows the effects of the infusion of IGF I and II on tibial epiphyseal width and on [3H]thymidine incorporating activity of costal cartilage. At IGF I concentrations of 168 and 286 ng/ml (infusion of 43 and 103 µg/day) tibial epiphyseal width was stimulated to 139 and 168%, and [3H]thymidine incorporating activity of costal cartilage was increased to 171 and 239%.

Less pronounced effects on growth indices were obtained with IGF II: although the serum IGF II level reached at an infusion rate of 131 µg/day was 320 ng/ml, no significant increase in body weight was observed. The tibial epiphyseal width was only stimulated to 120 ± 3% and [3H]thymidine incorporating activity of costal cartilage to 133 ± 13%. Nevertheless, these latter two effects were statistically significant ($P < 0.01$ and $< 0.05$, respectively).

The effects of a 6 day infusion of a) saline, b) hGH (25 mU/day), c) IGF II (131 µg/day) and d) IGF I (103 µg/day) on the tibial epiphyses of hypox rats are illustrated on the photographs of Fig. 1. The white band represents the epiphyseal cartilage. The black lines indicate where the epiphyseal width was measured. As compared to a), the epiphyses are visibly widened in b), c) and d).

Fig. 2 shows the body weight of the rats during hormone administration. Hypox rats treated with IGF I started to gain weight on the first day after implantation of the pumps. Hypox rats treated with 43 µg/day of IGF I put on weight similarly to those obtaining 12.5 mU/day of GH. The effects of 103 µg of IGF I/day and 25 mU of hGH/day were also comparable. IGF II did not significantly increase the body weight.

In Fig. 3 the growth effects elicited by infusion of

Fig. 1.
Tibial epiphyseal width of hypophysectomized rats after a 6 day sc infusion of (a) saline, (b) hGH 25 mU/day, (c) IGF II 131 µg/day, and (d) IGF I, 103 µg/day.
Body weight increase in hypox rats (▽ = untreated) after administration of: IGF I, 43 µg/day (▲); IGF I, 103 µg/day (●); IGF II, 131 µg/day (▼); hGH, 12.5 mU/day (Δ); hGH 25 mU/day (○). Each point gives the mean of the four rats of one group (± SEM).

Fig. 3.
Comparison of growth effects in hypox rats of insused IGF I (●) or IGF II (■) with those of endogenous rat IGF induced by hGH administration. Hormones were infused during 6 days at the following rates: IGF I: 43 µg/day, 103 µg/day; IGF II 131 µg/day; hGH 0, 12.5, 25, 200, 400 mU/day. Each point represents the mean ± SD of values obtained from four rats. Total IGF values were obtained by the protein binding assay from a serum pool of each group and are expressed as µU equivalents of the human IGF standard (see Materials and Methods).
human IGF I and IGF II are compared with those of endogenous rat IGF induced by administration of hGH. IGF I and IGF II are less effective than corresponding μU equivalents of GH-induced endogenous rat IGF on all three growth indices.

IGF I or IGF II infusions did not cause hypoglycaemia (results not shown). This is in contrast to the hypoglycaemic effects of IGF I or IGF II administered as a bolus (Zapf et al. 1983) and to the hypoglycaemic effect of an sc infusion of insulin (2.5 U/day) given to a control group of hypox rats.

None of the growth parameters was affected by insulin (not shown).

Inorganic serum phosphorus and alkaline phosphatase levels did not change significantly after IGF I and IGF II infusion. The organ weights expressed per 100 g body weight did not differ significantly between the various treatment groups and the hypox control rats (results not shown).

Fig. 4 shows the [125I]IGF I binding patterns of sera of various treatment groups after incubation with [125I]IGF I and chromatography on Sephadex G-200. Serum of normal rats yields five radioactive peaks: peak I represents non-specific, peaks II and III specific binding. The apparent molecular weights of peaks III and II are 40,000–50,000 and 150,000–200,000, respectively. Peak IV (85% bed volume) represents non-bound IGF and peak V (100% bed volume) free 125iodine or iodinated degradation products. When pre-incubation is carried out in the presence of an excess of unlabelled hormone, peaks II and III disappear, whereas peak IV increases markedly ('displacement' of bound [125I]IGFI from the binding proteins). Peaks I and V remain unchanged (not shown for the sake of clarity). A similar pattern is obtained with [125I]IGF II (not shown). After hypophysectomy peak II disappears and peak III increases. The pattern is 'normalized' by the infusion of hGH (25 mU/day during 6 days) to hypox rats. In contrast, IGF I and II infusion does not 'normalize' the binding pattern. The specific binding peak II is only present in normal or hGH treated hypox rats. Thus, it appears to depend on the presence of endogenous rat or exogenous human growth hormone in vivo.

Discussion

The main finding of this study is that in the absence of growth hormone IGF II, like IGF I, is able to stimulate two growth indices, tibial epiphyseal width and thymidine incorporation into costal cartilage. However, in contrast to the two IGF I doses given in these in vivo experiments, the IGF II dose did not cause a significant increase in body weight. The growth effects elicited by human IGFs in hypox rats resemble those brought about by hGH. The growth responses to IGF I are proportional to the administered dose of this growth factor. These results support the somatomedin hypothesis (Daughaday et al. 1972).
IGF I clearly is a more potent growth factor than IGF II: although the steady state serum concentrations of IR-IGF I and II after infusion of 103 and 131 µg/day of IGF I or II were similar (286 and 320 ng/ml, respectively) IGF I was considerably more effective than IGF II in stimulating tibial epiphyseal width (+ 68% by IGF I, + 20% by IGF II) and thymidine incorporating activity of costal cartilage (+ 139% by IGF I, + 33% by IGF II), and it caused a highly significant increase in body weight, whereas IGF II did not. The total serum IGF activities (endogenous rat and exogenous human IGF) as expressed in µU equivalents of the protein binding assay rose from a ‘background level’ of 39 µU/ml to 99 and 146 µU/ml respectively, after infusion of the above two doses of IGF I or II (Fig. 3). Thus, the resulting IGF I and II levels calculated from these data are 60 and 107 µU/ml. Multiplied with the corresponding conversion factor for the protein binding assay (1 µU of IGF I corresponding to 5 ng, 1 µU of IGF II corresponding to 2.86 ng) one obtains serum IGF I and II concentrations of 300 and 327 ng/ml which are similar to the concentrations determined by RIA (see above).

The effect of the steady state IGF II concentration measured after the 6 day infusion of 131 µg/day on tibial epiphyseal width and thymidine incorporating activity in costal cartilage can be roughly estimated from Fig. 3 to correspond to the effect of a steady state IGF I concentration of 20–25 µU/ml (total µU/ml minus the ‘background’ activity of 39 µU/ml on the IGF I dose-responsive curves extrapolated through the ‘saline points’, left and middle panel of Fig. 3). This latter concentration of IGF I would, however, not be expected to cause already a significant increase in body weight (right panel of Fig. 3).

When the growth stimulating µU equivalents (as determined in the protein binding assay) of infused human IGF I and of endogenous rat IGF produced under the influence of GH are compared (Fig. 3), rat IGF appears to be nearly twice as bioactive as human IGF I. However, since the potency of rat IGF relative to that of human IGF I in the protein binding assay is not known, no absolute comparison can be made. In vitro, rat IGF is 2–3 times more active than IGF I on DNA and RNA synthesis in rat calvaria cells (Schmidt et al. 1983c). This would be in keeping with our in vivo results. Additional direct effects of GH and/or GH fragments or indirect effects of GH mediated by other factors can, of course, not be excluded. Since endogenous rat or exogenous hGH changes the IGF binding in rat serum (Fig. 4) the bioavailability of IGF may also be changed.

Blood glucose levels in the IGF infused animals were not decreased, whereas hypox rats receiving 2.5 U/day of insulin by sc infusion were hypoglycaemic. When IGF is slowly infused, it is immediately bound by carrier protein. The latter inhibits the cross-reaction of IGF with insulin receptors (Zapf et al. 1979). Thus hypoglycaemia is prevented.

Although growth appears to be brought about mainly by IGF, GH may be an important modulator of IGF action. One of the possibilities of acting as a modulator is via its control of IGF binding proteins (Fig. 4). The glucose transport system in adipose cells is also modulated by GH (Schoenle et al. 1979), but not by infused human IGF I or II (Schoenle et al. 1983). Therefore, this latter in vivo effect of GH does not appear to be mediated by IGF.

The results of the present study support the concept that IGF I/SM-C is the major human somatomedin. The main role of IGF II, a much less potent growth factor, remains to be elucidated. GH is one of the major regulatory factors for the synthesis of IGF (Schwander et al. 1983), and it may modulate the effects of IGF on its target tissues.

References


Received on May 14th, 1984.