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Growth responses to patterned GH delivery

R.G. Clark[1], D. Mortensen[1], L.M.S. Carlsson[2], D. Carmignac[3] & ICAF Robinson[3]

[1]Endocrine Research Department, Genentech, Inc, 390 Pt. San Bruno Blvd., South San Francisco, California 94080, USA; [2]Research Centre for Endocrinology and Metabolism, Sahlgren's Hospital, Goteborg, Sweden, and [3]National Institute for Medical Research, Mill Hill, London, UK

We have investigated the effects of different patterns of administration of recombinant human growth hormone (rhGH) on weight gain, organ growth, serum GH binding protein (GHBP) and insulin-like growth factor-I (IGF-1) levels in a series of studies using hypophysectomized (Hx) or GH-deficient dwarf (dw/dw) rats. Animals were given rhGH either by subcutaneous (s.c.) injections (1 or 2 per day) or s.c. infusions and rhIGF-1 (2 mg/kg/day) by s.c. infusion. In Hx rats, all rhGH regimes increased body weight, tibial epiphyseal plate width, and organ weights in a dose-related manner. Dwarf rats showed a smaller growth response to rhGH than Hx rats, whereas rhGH induced greater elevations in serum GHBP in drarf rats. Growth responses depended on the pattern of rhGH administration (twice daily injections > continuous infusions > daily injections). The shape of the body growth curves also differed; rhGH injections increased weight gain linearly, whereas infusions gave an initial rapid weight gain which slowed with time (a curvilinear response). For both regimens, tibial epiphyseal plate width increased linearly with rhGH dose but infusions were 5-fold more potent than daily injections. Spleen and thymus weights were markedly increased by rhGH and were also affected by the pattern of GH exposure. At 5 mg rhGH/kg/day, thymus weights were 390 ± 35 mg for injections *vs.* 613 ± 34 mg for infusions ($P < 0.001$) compared with 248 ± 16 mg in vehicle-treated Hx controls. Infusions of rhIGF-1 also stimulated specific organ growth but caused less weight gain. RhIGF-1 additively increased the weight gain caused by rhGH injections but not by rhGH infusions. Circulating IGF-1 and GHBP levels were increased in a dose-dependent manner by rhGH infusion, whereas daily injections were ineffective. Thus, differential organ growth could be related to the higher serum IGF-1 concentrations induced by continuous rhGH administration. These studies show that whole body growth is best maintained by intermittent rhGH exposure, whereas, paradoxically, differential organ growth is most pronounced with continuous rhGH administration.

Keywords: GH; IGF-1; infusion; injection; differential growth

Introduction

In rodents the growth promoting and anabolic activities of administered GH have been shown to depend on the pattern of administration, but this relationship is complex depending on dose, frequency and route of administration (Cotes *et al.*, 1980, Jansson *et al.*, 1982a,b; Clark *et al.*, 1985; Groesbeck and Parlow, 1987; Robinson and Clark, 1987). GH secretion is highly episodic in the rat, and earlier studies have shown that the growth response to exogenous GH depends on the frequency of sc. injections (Jansson *et al.*, 1982a,b) and is more pronounced in response to multiple i.v. pulses compared with continuous i.v. infusion (Clark *et al.*, 1985). On the other hand, continuous s.c. infusion promotes weight gain more effectively in the short-term than single daily s.c. injections of GH (Cotes *et al.*, 1980). In humans GH is now usually given by single daily s.c. injections. Increasing the frequency to twice daily s.c. injections did not significantly improve the growth response (Smith *et al.*, 1988). Conversely, short-term continuous infusions of GH given to GH-deficient children were also effective in stimulating growth (Tauber *et al.*, 1993). This raises the possibility that depot formulations of GH, effective in animals (Klindt *et al.*, 1992), could have applications in humans.

It has long been recognized that GH actions are mediated in part by generating IGF-1 at the liver and other peripheral tissues (Daughaday, 1989) and there is some evidence that this also depends on the pattern of GH administration (Isgaard *et al.*, 1988; Maiter *et al.*, 1988). GH also has direct actions (Isaksson *et al.*, 1987), and GH receptors are also regulated by GH in a pattern dependent manner (Maiter *et al.*, 1988; Robinson *et al.*, 1993). GH and IGF-1 have differential effects on organ growth in GH deficient animals, particularly on lymphoid growth (Guler *et al.*, 1988; Skottner *et al.*, 1989) and function (Clark *et al.*, 1993; Robbins *et al.*, 1994). Previous studies in rodents and pigs (Wise *et al.*, 1994) have shown an increase in lymphoid tissue mass with either injections or infusions of GH, but the effects of different patterns of GH with or without IGF-1 have not been compared in the same study, and only a narrow range of doses of GH has been investigated.

In this study the efficacies of daily injections or continuous infusions of rhGH were compared over a wide range of doses measuring organ and body growth as well as effects on plasma IGF-1 and GH binding protein (GHBP) concentrations.

Results

Hypophysectomized rats

Hx rats treated with vehicle alone increased in body weight only 3 ± 1 g in 7 days. Administration of rhGH increased body weight in a dose-dependent fashion for both patterns of administration (Figure 1). These log dose response curves were linear for both injected and infused groups, but they were not parallel, as at the lowest two doses of rhGH the weight gains were equal, whereas at 1 or 5 mg/kg/day rhGH was significantly more effective at inducing weight gain by continuous infusion.

The body-weight *vs.* time curves (Figure 2) for continuous administration of rhGH were initially of a steeper slope than those for bolus injection, showing a highly significant ($P < 0.001$) curvilinearity in all but the lowest dose group. Thus curvilinearity is not simply due to the greater magnitude of the growth response in the infused groups. In contrast, bolus injections resulted in linear growth curves. Thus, potency comparisons for body weight increases induced by the same doses of rhGH given by infusion or injection change with time. Tibial epiphyseal plate widths showed the expected dose-related increase (Figure 3) and were linear for both regimes; but in this case the log dose response curves were parallel for both treatment regimes. A potency comparison showed that continuous s.c. delivery was

Correspondence: R.G. Clark
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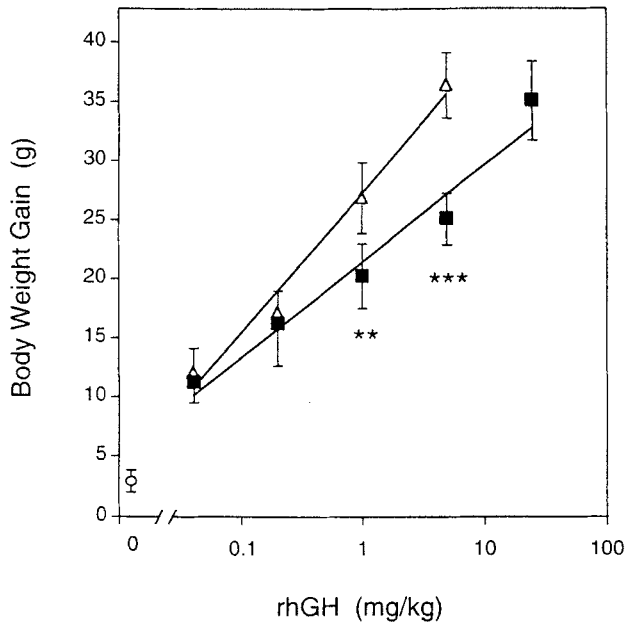

Figure 1 Body weight gains in hypophysectomized female rats treated with recombinant human GH (rhGH) at four doses by continuous s.c. infusion (△) or five doses by single daily s.c. injections (■) for 7 days. Mean±SD, $n=5$ per group, $^{**}P<0.01$, $^{***}P<0.001$ for injections *vs* infusions

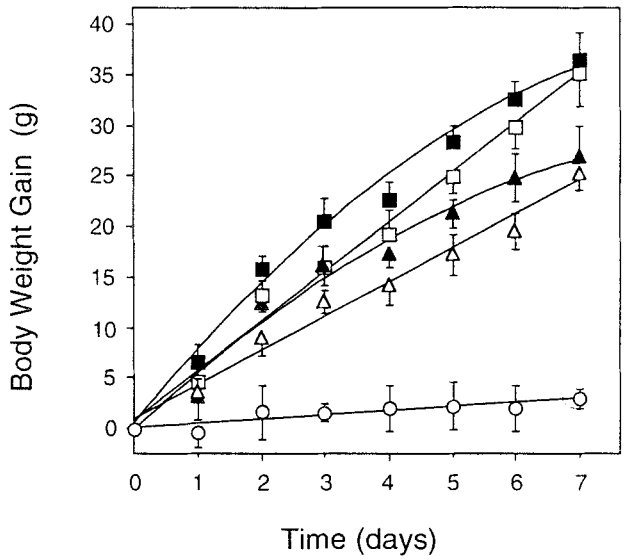

Figure 2 Cumulative body weight gains in hypophysectomized female rats given excipient (○) or rhGH by daily s.c. injections (□, △) at five (△) or 25 mg/kg/d (□) or rhGH by continuous s.c. infusion (■, ▲) at 1 (▲) or 5 mg/kg/d (■) for 7 days. Same experiment as in Figure 1. Mean±SD

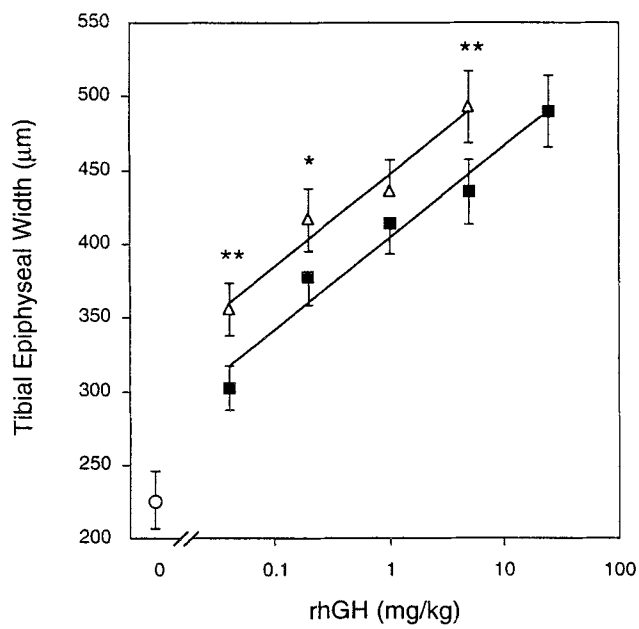

Figure 3 Tibial epiphyseal width (μm) in hypophysectomized female rats given excipient (○), or rhGH either by daily s.c. injections (■) or continuous s.c. infusions (△) for 7 days. Same experiment as in Figure 1. $^{*}P<0.05$, $^{**}P<0.01$ for injections *vs* infusions. Mean±SD

5-fold more efficacious than single daily s.c. injections of rhGH.

The body weights and organ weights measured at sacrifice (Table 1) all increased with increasing dose of rhGH. Again, continuous GH exposure was more effective than intermittent exposure. Since body weight was increased by rhGH, the data were expressed as percent body weight, also shown in Table 1. Kidney and heart showed proportional growth, whereas liver, spleen and thymus increased disproportionately to body weight, and this effect was more pronounced when GH was given by continuous infusion (Table 1). Serum GHBP also showed a differential response to rhGH administration pattern (Figure 4a), with a significant dose-dependent increase in serum GHBP with continuous rhGH infusion, but no change with injections of the same doses of hGH or even a much higher dose (2500 μg/rat). Growth hormone injections up to 500 μg/rat did not significantly increase IGF-1 levels 24 h later (Figure 4b). Daily injections of 2500 μg/rat were required to double serum IGF-1. In contrast, infusions of as little as 20 μg rhGH-1 per day significantly increased serum IGF-1, which was further increased in a dose-related manner (Figure 4b).

Effects of GH and/or IGF-1 in dwarf rats

In another experiment female dwarf rats were given rhGH by once or twice daily injections, or continuous infusions, with or without infusions of rhIGF-1. The different time course of response to continuous rhGH infusion compared with intermittent exposure is particularly apparent (Figure 5), confirming the data from Hx rats. The mean growth curve of the continuous rhGH treated group was best fitted by a third order polynomial ($r^2=0.99$), whereas the other groups showed a linear response with time. This shows that there is an initial rapid gain in body weight with continuous rhGH followed by a slower growth at a rate intermediate between once and twice daily injections (Figure 5).

The effects of the co-administration of rhIGF-1 with rhGH on final body weight gain and serum IGF-1 levels are shown in Figure 6 (a and b). rhIGF-1 alone caused a small increase in weight gain (Figure 6a) compared to the effect of rhGH. Twice daily injections of rhGH gave a greater weight gain than either continuous rhGH or once daily rhGH injections. Repeating the rhGH treatments combined with rhIGF-1 showed increased weight gain for the rhGH injection groups, but no significant improvement in weight gain was seen with continuous rhGH plus rhIGF-1. Serum IGF-1 levels (Figure 6b) were measured approximately 16 h after the last twice daily injection of rhGH and 24 h after the last daily injection. A comparison of serum IGF-1 and weight gain shows that serum IGF-1 does not predict the growth response. For example, although daily injections of rhGH led to a significant fall in serum IGF-1 levels, a greater growth res-

Table 1 Infusion *vs* Injections of rhGH: Absolute (a) and Relative (b) Organ Weight in Hx Rats

a) *rhGH (mg/kg/d)*	*Body Wt*	*Liver*	*Heart*	*Kidney*	*Spleen*	*Thymus*
			Absolute Weight (g)			
Excipient	96±4	4.0±0.1	0.36±0.03	0.70±0.04	0.20±0.04	0.25±0.02
Infusion						
0.04	105±5	4.1±0.3	0.36±0.02	0.72±0.05	0.20±0.02	0.36±0.05[a]
0.2	110±2	4.5±0.2	0.40±0.04	0.79±0.03	0.26±0.03	0.37±0.04
1.0	119±5[a]	5.2±0.3[b]	0.44±0.02[a]	0.84±0.06	0.32±0.03[a]	0.41±0.08
5.0	128±3[b]	5.7±0.6[c]	0.49±0.01[b]	0.89±0.07	0.38±0.04[a]	0.61±0.03[c]
Injection						
0.04	104±3	4.2±0.2	0.36±0.02	0.74±0.02	0.20±0.04	0.26±0.05
0.02	109±4	4.3±0.5	0.39±0.03	0.81±0.03	0.23±0.02	0.39±0.04
1.0	113±4	4.5±0.3	0.39±0.05	0.81±0.03	0.22±0.04	0.41±0.08
5.0	119±3	4.5±0.4	0.42±0.03	0.85±0.08	0.26±0.04	0.39±0.03
25	128±4	5.0±0.3	0.47±0.03	0.91±0.04	0.30±0.02	0.45±0.08
b)			*Relative Weight (% of Body Weight)*			
Excipient		4.2±0.1	0.37±0.03	0.70±0.04	0.21±0.04	0.25±0.02
Infusion						
0.04		3.9±0.2	0.34±0.01	0.72±0.05	0.19±0.02	0.36±0.05[b]
0.2		4.1±0.1	0.37±0.04	0.79±0.03	0.24±0.03	0.37±0.04
1.0		4.4±0.3[a]	0.37±0.02	0.84±0.06	0.27±0.02[a]	0.41±0.08
5.0		4.5±0.5[b]	0.38±0.02	0.89±0.07	0.30±0.03[a]	0.61±0.03[c]
Injection						
0.04		4.1±0.1	0.35±0.02	0.74±0.02	0.22±0.01	0.26±0.05
0.2		4.0±0.3	0.36±0.02	0.81±0.03	0.21±0.04	0.39±0.04
1.0		4.0±0.2	0.34±0.04	0.81±0.03	0.23±0.04	0.41±0.08
5.0		3.8±0.2	0.35±0.03	0.85±0.08	0.26±0.02	0.39±0.03
25		3.9±0.2	0.37±0.02	0.91±0.04	0.30±0.02	0.45±0.08

Organ weights at sacrifice in hypophysectomized (Hx) rats treated for 7 days with rhGH by daily s.c. injection or minipump infusion. Statistically significant differences from the same dose of rhGH given by injection are indicated by superscripted letters. [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$. Means and standard deviations are shown

ponse resulted than for rhIGF-1 infusions which greatly increased serum IGF-1 concentrations. Serum IGF-1 levels were significantly increased in all rhGH-treated groups if rhIGF-1 was co-delivered compared to treatment with rhGH alone.

The weights of organs recovered at sacrifice are shown in Table 2. Kidney and splenic growth were most sensitive to rhIGF-1 given alone or in combination with rhGH. In contrast, the liver showed no detectable growth with rhIGF-1 alone whereas rhGH increased relative liver size, particularly when given by continuous infusion (Figure 7a). Serum total protein (Figure 7b) and GHBP levels (Figure 7c) were also measured in these animals and showed dramatic increases in response to continuous rhGH infusions, whereas all other treatments were relatively ineffective. Both serum albumin and globulin were elevated by continuous GH and their ratio did not change significantly, nor did serum glucose change (data not shown). Serum IGF-1 levels confirmed the hepatic sensitivity to continuous rhGH exposure (Figure 6b).

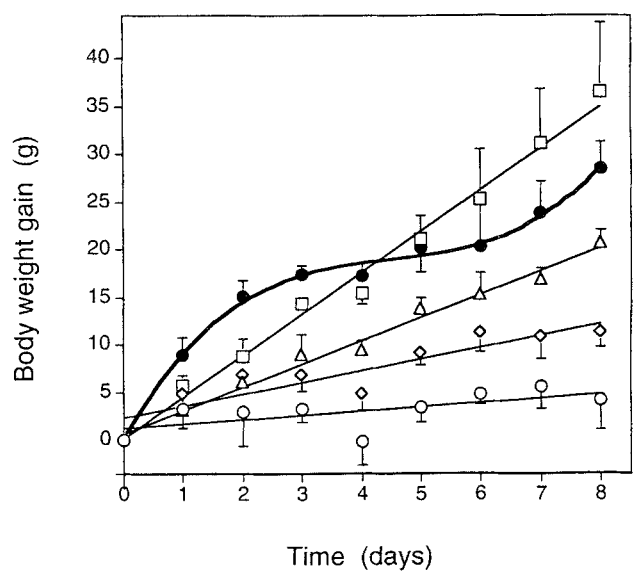

Figure 4 Cumulative body weight gains in female dw/dw rats given excipient (○), rhIGF-1 (s.c. infusion, 1 mg/kg/d, ◇) or rhGH at one dose (2 mg/kg/d) by daily s.c. injections (△), twice daily injection (□), or continuous s.c. infusion (●). Mean±SD

Discussion

It is well established that whole body growth in the rat is sensitive to the pattern of GH administration and that some tissues respond differentially to GH and IGF-1 (Jansson *et al.*, 1982a,b; Robinson and Clark, 1987; Guler *et al.*, 1988; Skottner *et al.*, 1989). We have now examined this in some detail, giving a wide range of doses of rhGH by injection or infusion in Hx rats, and have studied the effects of giving rhGH together with rhIGF-1 in GH-deficient dwarf rats. Although both continuous and pulsatile GH stimulated growth in a dose-dependent manner, the dose response curves for body weight gain were not parallel whereas tibial epiphyseal plate width, the classical index of skeletal growth in the rat, showed parallel dose-response curves. This implied a greater degree of soft tissue growth induced by continuous GH exposure, which was reflected in the weights of several organs at sacrifice. Furthermore, the body-weight growth curves were linear with time for rhGH injections but significantly curvilinear for continuous GH infusions, suggesting that tissue responsiveness to GH is maintained better with intermittent GH exposure. Studies in dwarf rats have

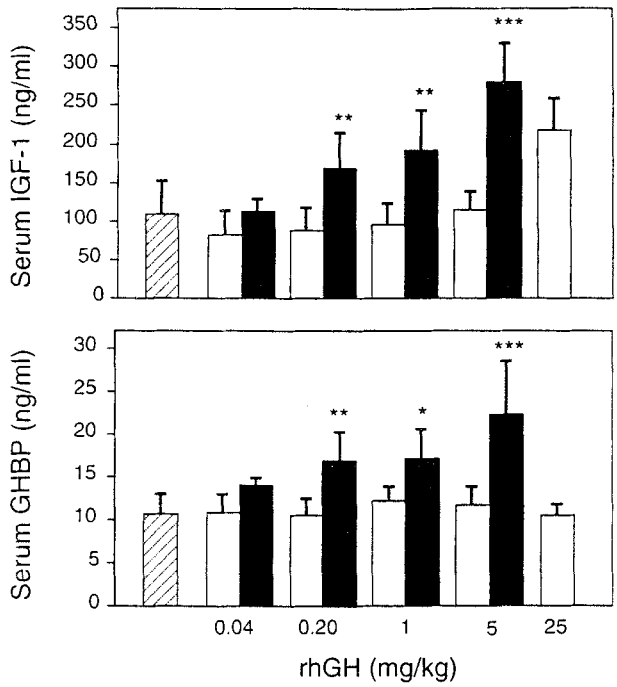

Figure 5 Serum IGF-1 (a) and GHBP (b) in hypophysectomized female rats given excipient (hatched bars), recombinant human GH (rhGH) at four different doses by continuous s.c. infusion (solid bars) or at five doses by single daily s.c. injections (open bars) for 7 days. Same experiment as in Figure 1. $*P<0.05$, $**P<0.01$, $***P<0.001$ for injections *vs* infusions. Mean±SD

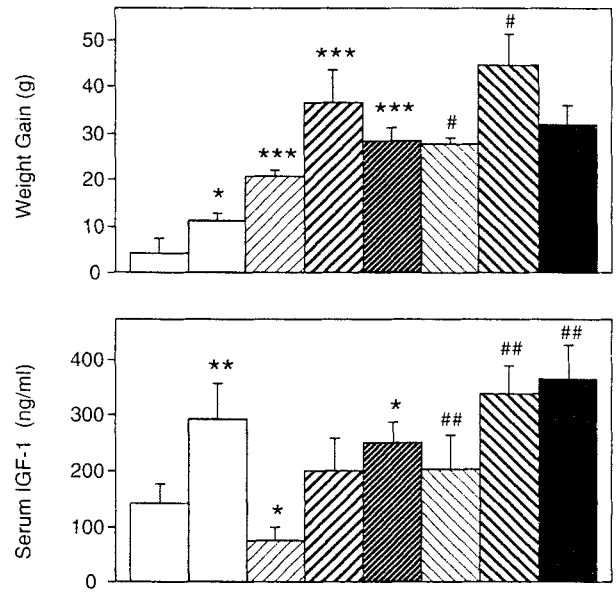

Figure 6 Body weight gain (a) and serum IGF-1 in dw/dw rats given either excipient (open bars), rhIGF-1 (1 mg/kg/d, s.c. infusion, light shading), rhGH (2 mg/kg/d, s.c.) by either daily injections (light ascending hatching), twice daily injections (dark ascending hatching) or continuous infusion (narrow ascending hatching). Another three groups of rats received s.c. infusions of rhIGF-1 plus rhGH given by either daily injections (light descending hatching), twice daily injections (dark descending hatching) or continuous infusion (solid bars). Same experiment as in Figure 5. $*P<0.05$, $**P<0.01$, $***P<0.001$ *vs* excipient; $\#P<0.05$, $\#\#P<0.01$, *vs* rhGH treatment in the same regime. Mean±SD

also shown that for body weight gain the dose response curves for pulsatile and continuous GH are non-parallel (Gevers *et al.*, 1995).

These results are in line with our earlier observations using i.v. administration of two doses of purified bovine GH in Hx rats, in which the response was greater in the first 5 days than the second 5 days of continuous, but not pulsatile, i.v. infusion (Clark *et al.*, 1985). There is one recent report apparently at variance with these conclusions, in which twice-daily GH injections or GH infusions were given to a different strain of dwarf rat (Gargosky *et al.*, 1994). Although the authors concluded that growth was superior with continuous GH, this study is difficult to interpret since only a single dose of GH was given for each group and a lower dose was given by injection than infusion. Furthermore, it is unclear whether the difference in weight gains between the two patterns of GH treatment was statistically significant.

Hypophysectomized rats lack all the pituitary hormones. We did not give thyroxin or corticosterone replacement therapy. It was therefore possible that some of the effects of rhGH that we observed were due to changes in peripheral endocrine balance independent of GH deficiency. This was not the case since similar results were obtained in our dwarf rats which show specific partial GH deficiency (Charlton *et al.*, 1988). In response to continuous s.c. rhGH infusions, drawf rats also showed an initial rapid weight gain that waned with time. Although differential organ growth was documented in this study (see below), it is possible that part of the initial weight gain seen with continuous GH infusion is due to increased water retention, since this is known to occur when GH is first given to GH-deficient subjects (Ho and Kelly, 1991). We did not see toxic effects on injecting high doses of rhGH (up to 2.5 mg/rat/d), unlike those reported by Groesbeck *et al.* (1987) who gave 5 mg/rat/d of purified rat GH by injection; these authors did not give equivalent doses by continuous infusion.

Although continuous GH infusions gave superior growth responses compared to single daily injections of GH, they were less efficient than twice daily injections of GH. RhIGF-1 also stimulated weight-gain and skeletal growth, but the responses were much smaller than to rhGH even though serum IGF-1 levels were at least as high as in the rhGH treated groups, confirming and extending previous observations (Schoenle *et al.*, 1982; Skottner *et al.*, 1989). Combining rhIGF-1 with rhGH injections produced an additive effect on growth, confirming previous data (Clark *et al.*, 1994), whereas continuous infusion of rhGH plus rhIGF-1 did not. This was not simply due to different effects on total serum IGF-1 levels since all groups showed the same increment with exogenous rhIGF-1, but it is possible that these combinations produce different effects on the circulating IGF binding proteins which are thought to be important in regulating IGF-1 bioavailability (Baxter, 1993). An additive effect of GH and IGF-1 on growth has also been reported in uremic rats (Hazel *et al.*, 1994).

Earlier studies have shown that IGF-1 causes differential organ growth, notably of the lymphoid organs and kidney (Guler *et al.*, 1988; Skottner *et al.*, 1989), and this was again observed in the present study. To our knowledge, however, this is the first report to show that the pattern of GH also differentially affects organ growth in the rat. As might be expected from earlier work with IGF-1, spleen and thymus were differentially affected by the pattern of GH exposure. However, we also noted dramatic effects on liver growth, which showed pronounced disproportionate stimulation by continuous rhGH infusion, and which was less marked for GH given by injections. In contrast, no effect on liver was seen with IGF-1 alone, nor did IGF-1 alter the selective effect of continuous rhGH infusion on liver growth.

What is the mechanism underlying this differential responsiveness to patterned GH exposure? It is unlikely to be due to different thresholds of sensitivity to plasma GH in various tissues, since the effects were maintained over a complete rhGH dose response. Continuous infusions of rhGH were more effective than injections in raising serum IGF-1 levels,

Table 2 Effects of rhGH and rhIGF-1: Absolute (a) and Relative (b) Organ Weight in Dwarf Rats

a)	*Absolute Weight (g)*					
Group	*Body Wt*	*Liver*	*Heart*	*Kidney*	*Spleen*	*Thymus*
Excipient	110±7	4.0±0.3	0.47±0.02	0.98±0.03	0.28±0.01	0.20±0.03
IGF-1	113±9	4.2±0.2	0.50±0.08	1.12±0.07[b]	0.39±0.03[a]	0.21±0.03
GH1/day	123±8[a]	4.9±0.3[b]	0.46±0.02	1.05±0.09	0.30±0.05	0.20±0.05
+ IGF-1	130±9[b]	5.4±0.3[b]	0.49±0.07	1.30±0.08[c]	0.47±0.06[c]	0.28±0.06[a]
GH2/day	143±13[c]	6.3±0.6[c]	0.50±0.06	1.17±0.09[b]	0.43±0.08[b]	0.26±0.06
+ IGF-1	148±11[c]	6.8±0.4[c]	0.54±0.05	1.42±0.10[c]	0.60±0.09[c]	0.28±0.08[a]
GH Pump	133±9[b]	6.7±0.4[c]	0.49±0.05	1.25±0.07[c]	0.37±0.05[a]	0.25±0.05
+ IGF-1	135±4[b]	7.0±0.3[c]	0.58±0.05[a]	1.38±0.10[c]	0.52±0.02[c]	0.32±0.07[a]
b)	*Relative weight (% or Body Weight)*					
Excipient		3.7±0.5	0.43±0.04	0.89±0.05	0.26±0.01	0.18±0.03
IGF-1		3.7±0.3	0.44±0.07	1.03±0.05[b]	0.35±0.01[b]	0.18±0.03
GH1/DAY		4.0±0.2	0.37±0.01[a]	0.85±0.04	0.25±0.04	0.16±0.03
+ IGF-1		4.1±0.2[a]	0.38±0.03	1.00±0.07[b]	0.36±0.04[c]	0.21±0.03
GH2/day		4.4±0.2[a]	0.35±0.02[a]	0.82±0.04	0.30±0.04[a]	0.18±0.03
+ IGF-1		4.6±0.4[b]	0.37±0.04[a]	0.96±0.07	0.41±0.04[c]	0.19±0.05
GH Pump		5.0±0.2[c]	0.38±0.02	0.94±0.03	0.28±0.04	0.19±0.03
+ IGF-1		5.2±0.3[c]	0.43±0.03	1.02±0.10[b]	0.38±0.01[c]	0.23±0.05

Organ weights at sacrifice in female dwarf rats treated for 8 days with excipient, rhIGF-1 (1 mg/kg/d), rhGH (2 mg/kd/d) or the combination of rhIGF-1 and rhGH (given daily and twice daily by s.c. injection and by minipump infusion). Statistically significant effects, compared to excipient treated rats, are indicated by superscripted letters [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$. Means and standard deviations are shown

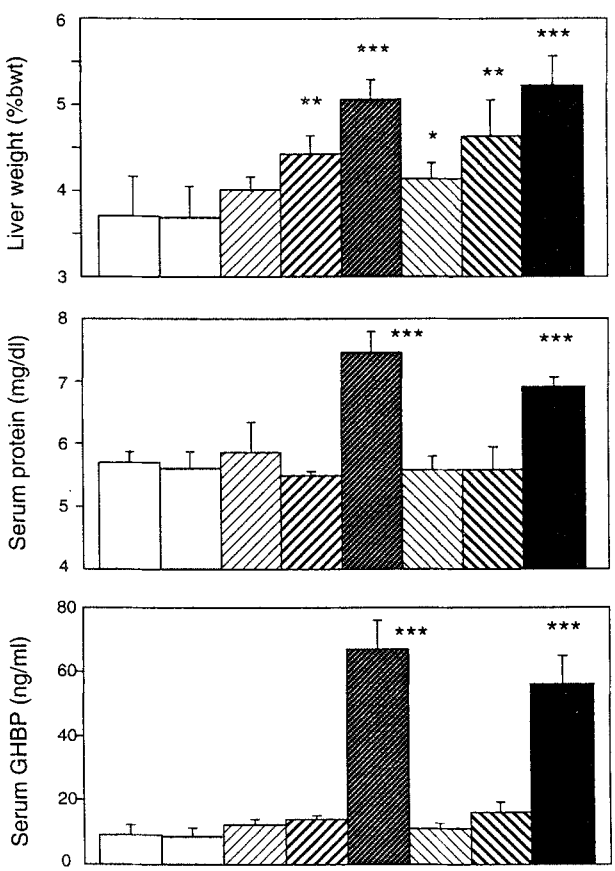

Figure 7 Relative liver weight (a), serum protein (b) and GHBP (c) in dw/dw rats given either excipient (open bars), rhIGF-1 (1 mg/kg/d, s.c. infusion, light shading), rhGH (2 mg/kg/d, s.c.) by either daily injections (light ascending hatching), twice daily injections (dark ascending hatching) or continuous infusion (narrow ascending hatching). Another three groups of rats received s.c. infusions of rhIGF-1 plus rhGH given by either daily injections (light descending hatching), twice daily injections (dark descending hatching) or continuous infusion (solid bars). Same experiment as in Figure 6. *$P<0.05$, **$P<0.01$, ***$P<0.001$ *vs* excipient. Mean±SD

confirming data in the literature (Maiter *et al.*, 1988), but it is unlikely that this is entirely responsible for mediating differential tissue growth responses since equivalent serum IGF-1 levels were produced by IGF-1 infusions. This does not exclude the possibility that local generation of IGF-1 in some tissues is more sensitive to the GH pattern than is the liver. For example, IGF-1 mRNA in skeletal muscle is affected differently by continuous *vs* pulsatile i.v. GH exposure, whereas both treatments are equally effective in increasing hepatic IGF-1 transcripts (Isgaard *et al.*, 1988).

Another possibility is that the tissue sensitivity to GH is altered by the different patterns of GH exposure changing the expression of the GH receptor itself (Robinson *et al.*, 1993). Hepatic GH receptors are up-regulated by continuous, but not by intermittent GH treatment, and this is reflected in an increase in the level of circulating plasma GHBP (Maiter *et al.*, 1988; Massa *et al.*, 1990), which is an alternatively spliced product of the GH receptor gene in the rat (Baumbach *et al.*, 1989). This increase is not directly related to growth since pulsatile GH injections, which effectively stimulated growth, did not raise serum GHBP (Maiter *et al.*, 1988). The present study has confirmed and extended this observation over a wide range of doses in Hx rats and in dwarf rats using direct RIA for GHBP. In addition to GH and prolactin receptors, many other liver proteins are sensitive to the pattern of GH administration (Jeffery *et al.*, 1986; Oscarsson *et al.*, 1991; Mode, 1993). Total serum protein was also increased dramatically by continuous GH administration but not by any other treatment. The combination of increased liver size, raised serum IGF-1, raised total protein, and serum GHBP all indicate that the liver is especially sensitive to continuous GH exposure, and that there is either a direct effect of GH on liver growth or it is mediated by growth factors other than IGF-1.

If these results in rodents also hold in man, it may be that some GH responses might be improved if GH exposure was made more continuous. There are conflicting reports on the effects of hGH injections in man on plasma GHBP (Fontoura *et al.*, 1992; Martha *et al.*, 1992; Ho *et al.*, 1993), but in a recent preliminary study of continuous *vs* intermittent hGH exposure in patients, plasma GHBP levels rose only in the continuously treated group (Tauber *et al.*, 1993). Whether this response will be sustained is less certain since GHBP levels are not grossly elevated in acromegalics (Baumann *et*

al., 1989). In view of the present experimental findings, it may be worthwhile to look for differential organ growth in man, particularly in liver and lymphoid tissue following continuous GH exposure, especially if these effects on organ size are associated with changes in physiological or pathological function.

Materials and methods

Animals

Young female hypophysectomized (Hx) rats (80–100 g, Taconic, NY) were weighed every 2–3 days for 10 days; any animal gaining more than 7 g during this period was excluded from the study. Treatment was started at 8 weeks of age or 15 days following surgery. Female dwarf (dw/dw) rats (Charlton *et al.*, 1988) were obtained from Simonsen (Gilroy, CA) and used at 8 weeks of age. Animals were fed a standard pelletted rodent diet and water ad libitum and kept in a room of constant humidity and temperature with controlled lighting (12 h light:12 h dark). The animals were randomized for both treatment group and cage to give groups of five with balanced equal mean initial body weights prior to treatment.

Hypophysectomized rats

All animals were anesthetized with ketamine/xylazine and, for continuous administration, osmotic minipumps (Model 2001, Alza, Palo Alto, CA) were inserted s.c. The pumps delivered vehicle phosphate buffer (5 mM), pH 7.8 containing 0.1% Tween 20) or rhGH at 4, 20, 100 or 500 μg/rat/d (i.e. 0.04–5 mg/kg/d for a 100 g rat). All animals were also given single daily s.c. injections (100 μl) of either vehicle or rhGH at 4, 20, 100, 500, 2500 μg/rat/d (i.e. 0.04–25 mg/kg/d for a 100 g rat). The control group therefore received infusions and injections of vehicle alone. Dosing continued for 7 days.

Dwarf rats

Animals were given rhGH (2 mg/kg/d, s.c.) in three regimens; continuously by osmotic minipump, or by injection once daily (at 8 AM) or twice daily (at 8 AM and 5 PM). Some groups also received continuous infusions of rhIGF-1 (1 mg/kg/d). All animals were subject to the same manipulations, i.e. every rat was given two minipumps and two injections per day delivering hormone or vehicle as appropriate. The control group therefore received two minipumps and two daily injections of vehicle per day. Dosing was for 8 days.

Measurements

Body weights were recorded daily and organs weighed at sacrifice. One tibia was fixed in formalin, longitudinally sectioned, and mounted for subsequent measurement of epiphyseal plate width using a light microscope fitted with an ocular micrometer. Two sections were mounted from each tibia and 4-6 measurements were read on each section and these values then averaged for each animal. A terminal blood sample was taken and the serum stored at −70°C for measurement of IGF-1 and GHBP as described previously (Carmignac *et al.*, 1992). Results are expressed as ng/ml in terms of recombinant hIGF-1 or recombinant hGHBP (Genentech, Inc.). Serum chemistries (glucose, total protein, albumen and globulin concentrations) were measured using an automated Monarch 2000 Chemical Systems Instrument (Allied Instrument Laboratories, Lexington, MA).

Data are presented as the mean ± SD. Statistical comparisons were made by an analysis of variance followed by Duncan's Multiple Range Test. A $P<0.05$ was considered significant. For each animal curvilinearity of the weight gain *vs* time plot was assessed by fitting orthogonal polynomials, and the resulting linear and quadratic components were subjected to one way analysis of variance and two-tailed *t* tests to assess differences among groups.

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