The episodic secretory pattern of growth hormone regulates liver carbonic anhydrase III

Studies in normal and mutant growth-hormone-deficient dwarf rats

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Carbonic anhydrase III (CAIII) occurs in male rat liver at concentrations twenty times those in the female, and is sensitive to the pattern of growth hormone (GH) release. Males release GH episodically and have high concentrations of CAIII; females produce GH in a more continuous fashion and have lower CAIII levels. In normal female rats, the endogenous GH secretory pattern was masculinized, either by regular injections of GH-releasing factor (GRF) or by intermittent infusions of somatostatin (90 min on/90 min off). Both treatments induced regular GH pulses and stimulated growth, but only intermittent somatostatin infusions raised CAIII levels (controls, 1.5 ± 0.5 ; somatostatin-treated, 9.0 ± 2.9 $\mu\text{g}/\text{mg}$; means $\pm$ s.d.). GRF pulses (4 μg every 4 h) did not however raise CAIII levels (controls 1.8 ± 0.5 ; GRF-treated 1.4 ± 0.4 $\mu\text{g}/\text{mg}$). Surprisingly, hepatic CAIII is also sexually dimorphic (males, 18.8 ± 3 ; females, 2.22 ± 0.4 $\mu\text{g}/\text{mg}$) in a GH-deficient dwarf rat strain which has low plasma GH levels without 3-hourly GH peaks. Intermittent somatostatin infusions in female dwarf rats partially masculinized hepatic CAIII, an effect reduced by co-infusion with GRF. This CAIII response was not secondary to growth induction, since neither somatostatin nor GRF stimulated growth in dwarf rats, and pulses of exogenous GH stimulated growth in female dwarfs without masculinizing CAIII levels. Furthermore, continuous GH infusion in male dwarf rats partially feminized hepatic CAIII levels (to 9.1 ± 2.4 $\mu\text{g}/\text{mg}$), whereas infusions of insulin-like growth factor-1, which induced the same body weight gain, did not affect hepatic CAIII (20.8 ± 6 $\mu\text{g}/\text{mg}$). These results show that hepatic CAIII expression is highly sensitive to the endogenous GH secretory pattern, independent of growth. They also implicate the low basal GH levels between pulses, rather than the peak GH levels, as the primary determinant of the sexually dimorphic hepatic CAIII expression in the rat.

INTRODUCTION

The pattern of release of growth hormone (GH) into the plasma differs in male and female rats. Male rats produce GH episodically, with GH levels that rise to peaks more than 100-fold greater than basal values (Tannenbaum & Martin, 1976), whereas females secrete GH in a more continuous manner (Eden, 1979; Clark *et al.*, 1987), with frequent irregular small peaks, although the absolute amounts of GH produced per day by males and females are similar (Eden 1979; Jansson *et al.*, 1985a).

The expression of several liver proteins [e.g. testosterone hydroxylases (Gustafsson *et al.*, 1983b), α_2 -microglobulin (Roy & Chatterjee, 1983) and carbonic anhydrase III (CAIII; Shiels *et al.*, 1983)] differs in male and female rats. This sexual dimorphism has been attributed, in part, to the sex difference in the GH secretory pattern (Gustafsson *et al.*, 1983a,b). In the male rat it is assumed that either the intermittent peak GH values or the troughs between the peaks produce a masculine pattern of liver protein synthesis, whereas the continuous presence of

GH in the female 'feminizes' hepatic protein expression. Hypophysectomy of female rats masculinizes some GH-dependent hepatic proteins, suggesting that it is the absence of GH which is important. However, hypophysectomy removes all the pituitary hormones, not just GH, and the resulting endocrine and nutritional changes are profound. Furthermore, in most studies, the method of administration of exogenous GH has not restored a physiological pattern of GH exposure.

We have studied the effects of various GH patterns on CAIII, a liver protein known to be both sexually dimorphic and GH-sensitive (Gorg, 1975; Carter *et al.*, 1981; Jeffery *et al.*, 1984, 1986), taking advantage of two recent developments in the study of GH secretory patterns. Firstly, we have developed methods for long-term intravenous administration of peptides which enable us to manipulate the endogenous GH secretory pattern in normal animals. A 'male-type' pulsatile GH pattern can be induced in normal female rats by giving either regular injections of GH-releasing factor (GRF) or intermittent infusions of somatostatin. During somatostatin infusion endogenous GH is suppressed, but as soon as the infusion

Abbreviations used: CAIII, carbonic anhydrase III; GH, growth hormone; GRF, growth-hormone-releasing factor; IGF, insulin-like growth factor.

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is interrupted a large rebound pulse of GH secretion occurs. Both these manoeuvres impose a male-type plasma GH profile in female rats and stimulate their growth (Clark & Robinson, 1985a,b, 1988). Secondly, we have used a recently discovered mutant dwarf rat which shows a specific partial pituitary GH deficiency (Charlton *et al.*, 1988). This animal model has enabled us to discriminate between the effects of GH patterns on hepatic protein synthesis and on growth promotion, and to assess the importance of basal levels of GH in regulating hepatic CAIII levels. The effects of the various peptide infusions on growth have been reported in detail elsewhere (Clark & Robinson, 1985a, 1988; Robinson & Clark, 1987; Skottner *et al.*, 1989), and will only be summarized here.

MATERIALS AND METHODS

Animals and surgery

Normal rats of the Sprague-Dawley strain (180–200 g) were obtained from the N.I.M.R. animal house. Dwarf rats were bred here as an AS strain derived from the original Lewis colony. Full details of this dwarf rat have recently been reported (Charlton *et al.*, 1988). Animals were equipped with an indwelling jugular venous cannula under halothane/N₂O/O₂ anaesthesia, and the cannula was brought to the exterior via a stainless steel spring and fluid-tight swivel. The cannulae were connected to a computerized system able to give intravenous infusions in continuous or pulsatile patterns over many days, while also performing automatic blood sampling. Full details of the cannulation, infusion and sampling system have been published elsewhere (Clark *et al.*, 1985, 1986, 1988). Animals were weighed daily and their food and water intake was monitored. Infusions or injections were given in a vehicle consisting of phosphate-buffered sterile isotonic saline, pH 7.4, containing 100 µg of rat serum albumin/ml.

Experimental treatments

Normal female rats were given intravenous injections of GRF [human GRF-(1–29)-NH₂; KabiVitrum AB, Stockholm, Sweden] at three different injection frequencies (2, 6 or 24/day) to give the same total daily dose (24 µg/day) for 7 days. Controls were given intravenous saline. Somatostatin-(1–14), (Ferring AB, Malmö, Sweden) was infused intermittently into another group of normal female rats, with infusion interrupted regularly by a 10, 30 or 90 min gap every 3 h; all rats received the same total daily dose of 480 µg of somatostatin for 8 days. A control group received saline throughout. Similar

experiments were carried out in male or female homozygous dwarf rats. Both male and female dwarf rats were given GRF injections (5 µg every 3 h) or somatostatin infusions (240 µg/day; 150 min on/30 min off). Other animals were given a combination of somatostatin (240 µg/day) and GRF (48 µg/day) in this intermittent infusion pattern, whereas controls received saline throughout the 8 day experiment. In dwarf female rats, bovine GH (kindly provided by Professor J. Kostyo, Ann Arbor, MI, U.S.A.) was given by intravenous infusion either continuously or in a pulsatile pattern (2 min bolus every 3 h) at the same total dose (36 munits/day) for 8 days. Other groups received pulsatile GRF {[Nle²⁷]GRF-(1–29)-NH₂; Ferring AB} or saline alone. Finally, male dwarf rats were given continuous intravenous infusions of recombinant human GH (200 munits/day) or recombinant human insulin-like growth factor-1 (IGF-1) (36 or 180 µg/day; Kabi-Vitrum AB) for 9 days; controls received saline alone.

Tissue collection

Livers were excised and frozen at –20 °C before use. Homogenates were made with distilled water (1:2, w/v) using an Ultra-Turrax homogenizer. Clear supernatants were obtained for radioimmunoassay and protein estimation (Bradford, 1976) after centrifugation at 30 000 g for 10 min at 4 °C.

Radioimmunoassay

Purified rat CAIII (5 µg/10 µl) was radioiodinated using 16 µg of chloramine-T in 10 µl of 0.25 M-phosphate buffer, pH 7.5, and 0.5 mCi of ¹²⁵I. Immediately after addition of chloramine-T to the reaction mixture, 56 µg of L-cysteine was added followed by 250 µl of 50 mM-phosphate buffer, pH 7.5, containing 1 g of BSA/litre. Iodination products were separated on a G-50 Sephadex column and eluted with the above phosphate/BSA buffer. Samples were assayed for CAIII (without extraction) in duplicate with standards in triplicate as described previously (Heath *et al.*, 1982). Results are expressed as µg/mg of soluble protein (means ± s.d.) and differences were assessed by analysis of variance (ANOVA) and/or Student's *t*-test as appropriate. Rat GH was assayed as previously described (Clark *et al.*, 1986, 1988) using reagents provided by NIADDK, Bethesda, MD, U.S.A.

RESULTS

A group of normal female rats were given a series of regular intravenous injections of GRF. Table 1 shows the results obtained after 7 days of such treatment.

Table 1. Effect of GRF on hepatic CAIII in normal female rats

Groups of female rats were given saline (controls) or GRF intravenously at different injection frequencies for 7 days. Hepatic CAIII was measured on killing the animals; growth data were recalculated from Robinson & Clark (1987). Results shown are means ± s.d. There were no significant differences in CAIII between any of the groups, whereas growth rates were stimulated significantly at the higher GRF injection frequencies (**P* < 0.05, ***P* < 0.02 versus controls).

Treatment (n)	CAIII (µg/mg)	Growth (g/day)
Control females (7)	1.82 ± 0.47	3.4 ± 0.7
2 × 12 µg of GRF per day (6)	1.95 ± 0.31	3.6 ± 0.3
6 × 4 µg of GRF per day (5)	1.41 ± 0.42	4.2 ± 0.4*
24 × 1 µg of GRF per day (6)	2.28 ± 0.94	4.5 ± 0.8**

Fig. 1. Effect of intermittent intravenous infusion of somatostatin in four conscious normal female rats

Blood samples (20 μ l) were withdrawn automatically and assayed for GH. Somatostatin (solid bars) was infused at 30 μ g/h in an intermittent pattern (150 min on/30 min off).

Normal rats gained more than 20 g over this period. Injections of GRF 6 or 24 times per day significantly stimulated weight gain, whereas two injections per day was without effect. Despite this stimulation of growth, there was no significant effect on hepatic CAIII in any group.

The effect of intermittent somatostatin infusions on endogenous GH secretion is illustrated in Fig. 1, which shows the plasma GH profile in four individual conscious female rats over a 12 h period. Spontaneous GH pulses are suppressed during somatostatin infusion, but a large

Fig. 2. Effect of GRF and somatostatin on hepatic CAIII in dwarf rats

Male and female dwarf rats were infused intravenously for 8 days in an intermittent pattern (150 min on/30 min off). A total of 24 animals were studied simultaneously, divided into groups of three of each sex receiving the following: saline (controls), GRF-(1-29)-NH₂ (GRF, 2 μ g/h), somatostatin (SS, 10 μ g/h), or a combination of GRF (2 μ g/h)+SS (10 μ g/h). Results are means $\pm$ s.d. Somatostatin infusions alone significantly increased CAIII levels in females (assessed by ANOVA), an effect reduced by co-infusion with GRF, which was without effect on its own.

rebound pulse of GH secretion occurs regularly after interruption of infusion. In a larger series of experiments, groups of normal female rats were given intravenous infusions of somatostatin (480 μ g/day), interrupted for 10, 30 or 90 min every 180 min for 8 days. Table 2 shows that these intermittent somatostatin infusions markedly increased hepatic CAIII concentrations. The masculinizing effect on CAIII was significantly greater with the longer breaks in somatostatin exposure, whereas the

Table 2. Effect of interrupted somatostatin infusion on female rat hepatic CAIII

Animals were infused with somatostatin (20 μ g/h), with the infusion interrupted for the time indicated on a 3-hourly cycle for 8 days. Growth data are taken from Clark & Robinson (1988) and represent growth rates calculated by linear regression for each animal. Livers were taken at the time the animals were killed and assayed for CAIII. CAIII levels in a separate group of male rats are shown for comparison. Results are means $\pm$ s.d. * P < 0.05, ** P < 0.01, *** P < 0.02 versus control females; † P < 0.02, †† P < 0.001 versus 10 min gap; n.d., not determined.

Treatment (<i>n</i>)	CAIII (μ g/mg)	Growth (g/day)
Control females (7)	1.3 $\pm$ 0.5	2.9 $\pm$ 0.6
10 min gap in somatostatin infusion (6)	3.2 $\pm$ 0.5**	4.3 $\pm$ 0.8**
30 min gap in somatostatin infusion (5)	8.7 $\pm$ 4.0***†	4.0 $\pm$ 1.0*
90 min gap in somatostatin infusion (6)	9.0 $\pm$ 2.9***††	3.8 $\pm$ 0.7*
Age-matched male controls (4)	22.0 $\pm$ 9.5***	n.d.

Table 3. Effects of GH or GRF on hepatic CAIII in dwarf female rats

Groups of female dwarf rats were given intravenous infusions of bovine GH either continuously or in 8×3 h pulses to give a total daily dose of 36 munits. Other animals received pulses of $[Nle^{27}]GRF-(1-29)-NH_2$ ($5 \mu g$ every 3 h) or saline alone. The experiment continued for 8 days. On killing the animals, the livers were assayed for CAIII activity; body weight changes were recorded during the experiment. Results are shown as means $\pm$ S.D.; * $P < 0.01$, ** $P < 0.05$, *** $P < 0.02$ versus controls.

Treatment (n)	CAIII ($\mu g/mg$)	Growth (g/day)
Control (5)	2.22 ± 0.36	0.7 ± 0.5
Continuous GH (6)	$1.63 \pm 0.49^{**}$	1.2 ± 0.3
Pulsatile GH (6)	$2.98 \pm 0.50^{***}$	$2.4 \pm 0.5^*$
Pulsatile GRF (6)	2.75 ± 0.77	1.0 ± 0.3

growth response was most pronounced with the shortest gap in somatostatin exposure, though the growth increments between the three somatostatin-treated groups were not significantly different from each other. Table 2 also shows CAIII levels in normal male rats, confirming the pronounced sex difference in expression of this enzyme.

This experiment suggested that a period of exposure to very low GH levels (induced in females by somatostatin infusion) might be important in determining hepatic CAIII levels, though it was not possible to rule out the effects of the regular high peak GH levels caused by the rebound GH release after somatostatin withdrawal. To address this point, CAIII levels were measured in comparable experiments with GRF and somatostatin carried out in dwarf rats, which are deficient in pituitary GH. Fig. 2 shows CAIII concentrations in livers from 24 male or female dwarf rats infused with saline, GRF, intermittent somatostatin or a combination of GRF and somatostatin for 8 days. The marked sex difference in CAIII levels is clearly also present in the dwarf control animals. Intermittent somatostatin infusions again dramatically increased hepatic CAIII levels in female rats towards that of the males, whereas pulses of GRF had no effect in either sex (Fig. 2). Addition of GRF to the somatostatin infusate had no effect in male dwarfs, but partially reversed the effect of somatostatin infusions in the females. None of these treatments stimulated growth in the dwarf rats. Both treatments elicit similar small peaks of GH secretion in dwarf rats, the main difference being that somatostatin infusions further suppress basal GH levels.

In the next experiment, female dwarf rats were given GH infusions at a low dose in either a continuous or a pulsatile pattern, whereas other groups received pulsatile GRF or saline (Table 3). There were small but significant effects of GH on hepatic CAIII levels; continuous GH lowered CAIII whereas pulsatile GH raised CAIII levels, but the effects were very small in comparison with those obtained with somatostatin. In contrast, pulsatile GH produced a marked stimulation of growth; continuous GH also stimulated growth but was less effective in this pattern. Pulsatile GRF had no effects either on growth or on hepatic CAIII levels in dwarf rats.

Finally, groups of five or six male dwarf rats were

given continuous infusions of human GH or of recombinant human IGF-1 for 9 days. Continuous human GH infusions (200 munits/day) stimulated growth (from 1.2 ± 0.7 to 2.1 ± 0.5 g/day, means $\pm$ S.D., $P < 0.05$) and significantly decreased hepatic CAIII levels (controls, $18.8 \pm 3 \mu g/mg$; continuous GH, $9.1 \pm 2.4 \mu g/mg$, $P < 0.01$). In contrast, infusions of IGF-1 (36 or 180 $\mu g/day$) had no effect on hepatic CAIII (22.2 ± 4.8 and $20.8 \pm 5.9 \mu g/mg$ respectively) even though the higher dose rate induced the same overall body weight gain (2.1 ± 1.2 g/day) as in the human-GH-infused group.

DISCUSSION

It is well recognized that GH controls the pattern of synthesis of some hepatic proteins in the rat. The degree of GH involvement varies between different proteins: steroid 5α -reductase activity can be completely 'feminized' in hypophysectomized rats by constant infusion of GH alone (Mode *et al.*, 1982); cytochrome $P-450_{15\beta}$ is partially feminized after similar treatment but fully feminized by GH infusion into intact male rats (MacGeoch *et al.*, 1985), whereas α_2 -microglobulin is partially feminized by GH infusion in intact males (Chatterjee *et al.*, 1986). The fact that, for many proteins, hypophysectomy produces a male pattern and GH infusion a female pattern, has led to the suggestion that GH is a feminizing factor (Mode *et al.*, 1981; MacGeoch *et al.*, 1985), whereas Noshiro & Negishi (1986) suggested that GH has a masculinizing influence on 15α - and 16α -reductases; thus it is still far from clear how the pattern of GH release affects synthesis of different hepatic proteins.

Part of these difficulties arises from exogenous GH replacement regimes which do not mimic the endogenous GH secretory pattern. We have taken an alternative approach, using chronically cannulated animals in which we have far greater control of the endogenous plasma GH profile. We have previously studied the pattern of GH secretion in both male and female rats over long periods in detail (Clark *et al.*, 1986, 1987), and have manipulated the endogenous GH secretory pattern by patterned administration of the hypothalamic peptides GRF or somatostatin, which stimulate or inhibit pituitary GH release (Clark & Robinson, 1985a, 1988; Robinson & Clark, 1987). CAIII was chosen for study, as a specific and convenient assay was available, and this enzyme was known to be fully masculinized in female rats after hypophysectomy, and partially feminized in intact males after GH infusion (Jeffery *et al.*, 1986). The questions we addressed were: (1) does the CAIII response to GH change in parallel with the growth response?, (2) can we masculinize or feminize CAIII in normal intact animals by altering endogenous GH release patterns?, (3) is it the peak or the trough GH level that is important?, and (4) are the effects of GH mediated by circulating IGF-1?

A male type of episodic GH secretion was induced in normal female rats, by giving either serial injections of GRF every 3 h or infusions of somatostatin, interrupted for a short period every 3 h. We have shown previously that female rats respond to regular pulses of GRF by releasing regular pulses of GH, and that such treatment increases their growth (Clark & Robinson, 1985a,b; Robinson & Clark, 1987). Similarly, infusions of somatostatin suppress endogenous GH release, but a large

rebound release of GH occurs on stopping infusion, again imposing a male pattern of GH secretion in female rats and stimulating growth (Clark & Robinson, 1988). Both GRF injections and repetitive somatostatin withdrawal produce regular male-type GH pulses, but only somatostatin treatment masculinized hepatic CAIII levels. This suggested that it was the suppression of basal GH levels rather than induction of GH peaks which masculinized CAIII in female rats. A 10 min gap in somatostatin infusion had the least effect on CAIII but the greatest effect on growth (Clark & Robinson, 1988), whereas the most pronounced effect on CAIII was observed with a 90 min gap in somatostatin infusion. Perhaps liver and skeletal GH receptors differ in their ability to resolve large changes in GH occurring over short time periods.

The conclusion that the basal rather than peak GH levels may determine the sexual dimorphism of CAIII is in line with previous studies showing that hypophysectomy in females masculinized CAIII, whereas hypophysectomy in males was without effect (Jeffery *et al.*, 1984). Hypophysectomy is a drastic procedure which disrupts many endocrine systems besides GH. Instead, we used our recently characterized dwarf rat that shows a specific pituitary GH deficiency (Charlton *et al.*, 1988). This animal model offers an number of advantages, both over surgically hypophysectomized animals and also over the phenotypically similar *lit/lit* mouse [used in earlier studies of hepatic GH-dependent proteins by Norstedt & Palmiter (1984) and Noshiro & Negishi (1986)], since its larger body size allows chronic intravenous cannulation and hence control of the plasma GH profile.

The pituitary GH contents in dwarf rats are only 5–10% of normal, and the plasma GH profiles of these animals show low but measurable GH concentrations (Charlton *et al.*, 1988), without the typical large 3-hourly bursts of GH secretion in the male. To our surprise, CAIII remained sexually dimorphic in dwarf males and females, with levels indistinguishable from those in normal rats. We can at once conclude that large peaks of GH are not necessary for masculinization of CAIII. It is more difficult to draw conclusions about basal GH levels in dwarfs, since the levels are so low in both sexes. If CAIII is responding to different basal GH levels in male and female dwarfs, the mechanism must respond over a very low range ($< 10^{-10}$ M) of plasma GH concentrations. However, it should be noted that several other factors [e.g. gonadal steroids; Jansson *et al.* (1985b)], could effect a sexually dimorphic CAIII expression in partially GH-deficient but otherwise normal animals, and that a sex difference exists in dwarf rat pituitary GH content, just as in normal rats (Charlton *et al.*, 1988).

Since dwarf rat pituitaries contain very small amounts of GH, it is unsurprising that GRF injections had no effect on growth or on hepatic CAIII levels. The dwarf rat pituitary can respond to GRF with a small release of GH in proportion to its GH content, but the amounts released were insufficient to cause growth or to masculinize CAIII. In contrast, intermittent somatostatin infusions were as effective in masculinizing hepatic CAIII in dwarf females as in normal females. Such infusions elicit small rebound GH secretory bursts in dwarf rats, but, more importantly, are able to suppress dwarf basal levels further (D. F. Carmignac, R. G. Clark & I. C. A. F. Robinson, unpublished work). We cannot

exclude the possibility that somatostatin acts directly at the liver, but feel that a more likely explanation is that somatostatin lowers basal GH levels, and it is the period of time without GH exposure that masculinizes hepatic CAIII: co-infusion with GRF partially reversed the masculinization caused by somatostatin, an interaction more likely to occur at the pituitary gland (to increase the amount of GH secreted) rather than at the liver itself. Similar conclusions were reached by Norstedt *et al.* (1983), who suggested that hypothalamic somatostatin could regulate sexually dimorphic liver enzymes via control of pituitary GH release.

The dwarf rat also provided a good model to test the effects of different patterns of GH administration. Pulsatile infusions of GH in dwarf females raised CAIII levels slightly, but did not masculinize hepatic CAIII despite effectively stimulating growth, again showing that large peaks of GH *per se* do not masculinize CAIII. On the other hand, continuous GH infusions in male dwarf rats markedly feminized CAIII levels, again showing that raising basal GH levels was sufficient to feminize CAIII, and that growth and CAIII stimulation can be dissociated. Furthermore, a slight but significant further feminization of CAIII levels was achieved by continuous GH infusions in female dwarf rats.

We also addressed the problem of whether GH acts directly or via the generation of IGF-1. Serum IGF-1 levels are GH-dependent, and the liver is a major site of IGF-1 production (Schwander *et al.*, 1983; van Wyk, 1984), so that hepatic effects of GH could well be mediated via IGF-1. However, infusions of IGF-1 in male dwarf rats had no effect on CAIII, even though they stimulated body growth (Skottner *et al.*, 1989) to the same extent as that induced by GH infusions, which did markedly feminize hepatic CAIII levels. Since in normal animals free blood IGF-1 levels change only slowly, it is unlikely that blood IGF-1 levels mediate the pattern-dependent effects of GH on CAIII. Although Norstedt & Palmiter (1984) suggested that the feminization of hepatic protein synthesis might be due to GH-induced IGF-1 production, they studied the mouse major urinary protein, which does not show the same response to GH as does CAIII. It is thus unwise to generalize about sexually dimorphic responses to GH of different liver proteins in different species.

If GH acts directly on the liver as it can in the skeleton (Isgaard *et al.*, 1986), then this hormone appears to exert pattern-dependent regulatory effects in several different tissues. IGF-1 mRNA generation in response to GH is more pattern-dependent in skeletal muscle than in the liver (Isgaard *et al.*, 1988), and it may be that different tissues obtain different signals from the same endogenous secretory pattern of GH. The simplest way to explain multiple effects would be the existence of at least two sets of receptors, high-affinity ones responding to low GH levels, and a lower affinity set which is primarily activated by high peaks of GH. The receptors identified by Leung *et al.* (1987) have an association constant of 10–30 nM, whereas other studies have suggested the existence of at least two receptor affinities (Baxter *et al.*, 1980), and several mouse GH receptors may arise from alternative mRNA transcripts (Smith *et al.*, 1988), which are regulated physiologically in the liver. It is important to establish the range of GH receptor subtypes in different rat tissues and to determine the physiological range of GH concentrations over which they operate. The

endogenous pulsatile GH rhythm in the rat has a much greater amplitude and sexual dimorphism than in other species, which probably explains why this species shows such marked sexual dimorphism in some of its GH-dependent hepatic proteins. The physiological relevance of such tight regulation of many hepatic proteins by the GH secretory pattern and the molecular basis of their GH control remain open for future study.

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