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# Growth Hormone Response of Bull Calves to Growth Hormone-Releasing Factor (42504)

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*Abstract.* Three experiments were conducted to determine serum growth hormone (GH) response of bull calves ($N$ = 4; 83 kg body wt) to iv injections and infusions of human pancreatic GH-releasing factor 1-40-OH (hpGRF). Peak GH responses to 0, 2.5, 10, and 40 μg hpGRF/100 kg body wt were 7 ± 3, 8 ± 3, 18 ± 7, and 107 ± 55 (mean peak height ± SEM) ng/ml serum, respectively. Only the response to the 40-μg dose was greater ($P < 0.05$) than the 0-μg dose. Concentrations of prolactin in serum were not affected by hpGRF treatment. In calves injected with hpGRF (20 μg/100 kg body wt) at 6-hr intervals for 48 hr, GH increased from a mean preinjection value of 3.1 ng/ml serum to a mean peak response value of 70 ng/ml serum. Differences in peak GH response between times of injection existed within individual calves (e.g., 10.5 ng/ml vs 184.5 ng/ml serum). Concentrations of GH in calves infused continuously with either 0 or 200 μg hpGRF/hr for 6 hr averaged 7.4 ± 3 and 36.5 ± 11 ng/ml serum, respectively ($P < 0.05$). Concentrations of GH oscillated markedly in hpGRF-infused calves, but oscillations were asynchronous among calves. We conclude that GH response of bull calves to hpGRF is dose dependent and that repeated injections or continuous infusions of hpGRF elicit GH release, although magnitude of response varies considerably. We hypothesize that differences in GH response to hpGRF within and among calves, and pulsatile secretion in the face of hpGRF infusion may be related to the degree of synchrony among exogenous hpGRF and endogenous GRF and somatostatin.


Human pancreatic growth hormone (GH)-releasing factor (hpGRF) (1) and some of its fragments specifically increase blood concentrations of GH in several species (2), including cattle (3–9). Considerable differences in sensitivity and magnitude of GH response are observed between animals given a single intravenous injection of a given dose of hpGRF (4, 6). It appears, therefore, that a single injection of hpGRF is not a reliable indicator of the GH response capacity of an animal. In addition, the single injection approach fails to determine capacity of the pituitary to respond to subsequent GRF stimulation. Indeed, work in humans (10) and rats (11) demonstrated a decline in GH responsiveness to repeated injections or continuous infusions of GRF. Results of clinical tests in humans and experimental studies with animals on GH responsiveness to a hpGRF challenge may be misleading if based solely on a single intravenous injection. Thus, alternative approaches are needed to evaluate GH responses to exogenous GRF. Two such approaches are (a) multiple injections and (b) continuous infusions of GRF. Previous GRF studies using cattle have primarily utilized steers (3, 6, 7) and dairy cows (5, 8, 9). However, GH response to hpGRF is affected by several factors such as gender (4, 12), age (4), and gonadal status (13). Thus, GH response to hpGRF in young prepubertal bulls is unknown and may differ from steers and lactating cows.

The overall objective of the present study was to determine the serum GH response and persistency of that response to administration of repeated injections and continuous infusions of hpGRF in bull calves.

**Materials and Methods.** *General.* Holstein bull calves weighing 66 ± 4, 81 ± 3, and 92 ± 4 kg were utilized in Experiments 1, 2, and 3, respectively. Calves were housed indoors in adjacent individual pens and exposed to approximately 15 hr of natural daylight daily and ambient temperatures of 12 to 33°C. Human pancreatic growth hormone-releasing factor 1-40-OH (hpGRF) was obtained from Bachem, Inc. (Torrance, CA). The placebo and vehicle for hpGRF was distilled sterile water.

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Blood samples were collected from each calf via an indwelling jugular cannula. Serum was decanted after centrifugation of blood samples and stored at −20°C until assayed for concentrations of GH (14) and prolactin (15). Calves were fed calf starter at 0600 hr and high-quality alfalfa hay at either 1500 hr (Experiments 1 and 2) or 1730 hr (Experiment 3). Feeding did not overlap with blood sampling, injections, or infusions. GH peak height (Experiments 1 and 2), mean GH (Experiment 3), and area under the GH response curve (Experiments 1, 2, and 3) were used as measures of hpGRF effects. Peak height was defined as the maximum concentration of GH achieved between 0 and 60 min (Experiment 1) or 0 and 30 min (Experiment 2) after injection of hpGRF. Area was defined as the integrated area under the GH response curve for 1 hr (Experiment 1) or 30 min (Experiment 2) after injection of hpGRF, and for the 6-hr infusion of hpGRF (Experiment 3). Preinjection and preinfusion means of GH were calculated from all samples collected before and including the 0-min sample.

Data were subjected to analyses of variance (16). Data expressed either as area or peak height provided the same statistical inferences; therefore, data expressed as area will be presented but not discussed.

*Experiment 1.* To determine a dose–response relationship between hpGRF and serum GH, a 4 (calf) × 4 (day) Latin square design balanced for residual effects was used. Treatments consisted of a single dose (volume of hpGRF solutions ranged from 5.9 to 7.6 ml) injected via an indwelling jugular cannula at 0915 hr on a given day. Doses of hpGRF were 0 (placebo), 2.5, 10.0, and 40.0 μg/100 kg body wt. Treatments were given on consecutive days. Blood samples were collected at −15, −10, −5, 0, 4, 6, 8, 10, 15, 20, 30, 60, 120, and 240 min relative to each injection. Comparisons between mean GH response to dose of hpGRF and placebo were made using Dunnett's *t* test (16).

*Experiment 2.* To determine the serum GH response to repeated injections of hpGRF, a complete-block design was used with four calves (blocks) given hpGRF intravenously at 6-hr intervals (i.e., treatments) for 48 hr starting at 2000 hr. The dose of hpGRF was 20 μg/100 kg body wt (injection volume ranged from 3.8 to 4.5 ml). Based on data from Experiment 1, 20 μg was a submaximal but effective dose of hpGRF. Blood samples were collected at −15, −10, −5, 0, 4, 6, 8, 10, 15, 20, and 30 min relative to each injection. Comparisons were made between treatments.

*Experiment 3.* To determine the serum GH response to continuous infusion of hpGRF, a single crossover design was used where four calves received continuous intravenous infusions of 0 and 200 μg hpGRF/hr for 6 hr. Treatments were given 2 days apart and started at 0930 hr each day. Two calves received the 0-μg dose and two received the 200-μg dose on each day. A Harvard infusion/withdrawal pump (model 954, Multi-Speed Transmission, Harvard Apparatus Co., Millis, MA) was used. Infusion volume rate was 4.2 ml/hr. Blood samples were collected at −75, −60, −45, −30, −15, 0, 5, 10, 15, 20, 25, 30, 45, and 60 min relative to the start of infusion, at 15-min intervals for the remaining 5 hr of infusion and at 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 min following completion of infusion.

**Results.** *Experiment 1.* Mean serum GH responses to hpGRF are presented in Table I. The 40 μg/100-kg dose of hpGRF increased peak height of GH 15-fold ($P < 0.05$) over that of the placebo. Although not significantly different, the 10-μg dose increased peak height of GH 2.5-fold above the placebo value. The 2.5-μg dose had no effect on serum GH. Preinjection mean GH did not differ between treatments and averaged 7.3 ng/ml serum. Peak height of GH in response to the 40 μg/100-kg dose of hpGRF occurred approximately 10 min postinjection. Mean concentrations of prolactin in serum were not affected by hpGRF administration (37.8 ± 2.9 vs 41.0 ± 3.3 ng/ml; mean prolactin before and after the 40-μg hpGRF dose, respectively).

*Experiment 2.* Preinjection mean GH did not differ ($P > 0.05$) between times of hpGRF injections and averaged 3.1 ng/ml serum across all calves and injections (Table II). Similarly, peak height of GH did not differ ($P > 0.05$) between times of hpGRF injections and averaged 70 ng/ml serum across all calves and injections (Table II). Based on the mean GH of all calves, no obvious diurnal pattern of response to sequential hpGRF injections was observed, and there was no evidence of reduced GH response following the eight con-

TABLE I. SERUM GROWTH HORMONE IN CALVES INJECTED WITH VARIOUS DOSES OF HUMAN PANCREATIC GROWTH HORMONE-RELEASING FACTOR (hpGRF 1-40-OH)

| GH trait[a] | Dose (μg) of hpGRF/100 kg body wt | | | | |
|---|---|---|---|---|---|
| | Placebo | 2.5 | 10 | 40 | SED[c] |
| Preinjection mean (ng/ml serum) | 5.8 | 7.2 | 4.8 | 11.3 | 3.2 |
| Peak height (ng/ml serum) | 7.2 | 8.3 | 18.3 | 107.3[d] | 33.3 |
| Area[b] ($ng \cdot min \cdot ml^{-1}$ serum) | 313 | 327 | 682 | 3534[d] | 1134 |

[a] Values are means of four calves.
[b] Area under 1-hr response curve.
[c] Standard error of differences of means.
[d] Different ($P < 0.05$) from placebo.

secutive hpGRF injections. However, considerable variation in GH response was observed between times of injection (up to 18-fold differences) within individual calves (Fig. 1).

*Experiment 3.* Mean GH concentration during the 6-hr infusion was five-fold greater ($P < 0.05$) for hpGRF-infused calves than for placebo-infused calves (Table III). Preinfusion mean GH did not differ between hpGRF and placebo treatments. There was no evidence of reduced GH response as the infusion continued for 6 hr and GH remained at elevated concentrations (3.3-fold above mean GH of controls) 90 min after infusion ceased. In three of the four hpGRF-infused calves GH pulsed at 1- to 3-hr intervals and pulses were asynchronous among calves. The fourth calf (7837; Fig. 2) showed little pulsatile activity. The serum GH response profiles for all calves depicting the markedly different patterns of response to hpGRF infusion are shown in Fig. 2.

**Discussion.** The serum GH response to single hpGRF injections in bull calves of these experiments fall within the range of mean responses reported for heifer calves (4), steers (3, 6), and lactating cows (8). In agreement with Al-Raheem *et al.* (3) and Mosley *et al.* (6), our lowest dose of hpGRF (2.5 μg/100 kg body wt) did not alter concentrations of GH. In contrast to our previous study using lactating cows (8), GH response of prepubertal bull calves in the present study to the 10-μg dose was markedly less than GH response to the 40-μg dose.

Similar to sheep (17) and cows (8), multiple injections of hpGRF consistently increases serum GH in bull calves. However, within in-

TABLE II. SEQUENTIAL RESPONSES OF SERUM GROWTH HORMONE IN CALVES TO REPEATED INJECTIONS OF 20 μg HUMAN PANCREATIC GROWTH HORMONE-RELEASING FACTOR (hpGRF 1-40-OH)/100 kg BODY WEIGHT

| GH trait[a] | Time of injection (hr) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2000 | 0200 | 0800 | 1400 | 2000 | 0200 | 0800 | 1400 | SED[c] |
| Preinjection mean (ng/ml serum) | 3.8 | 3.2 | 2.3 | 3.4 | 4.6 | 2.5 | 2.0 | 3.1 | 1.1 |
| Peak height (ng/ml serum) | 61.7 | 83.9 | 55.3 | 49.3 | 66.6 | 95.4 | 62.4 | 84.5 | 34.2 |
| Area[b] ($ng \cdot min \cdot ml^{-1}$ serum) | 1362 | 1670 | 914 | 1128 | 1347 | 1959 | 1233 | 1821 | 637 |

[a] Values are means of four calves.
[b] Area under 30-min response curve.
[c] Standard error of differences of means.

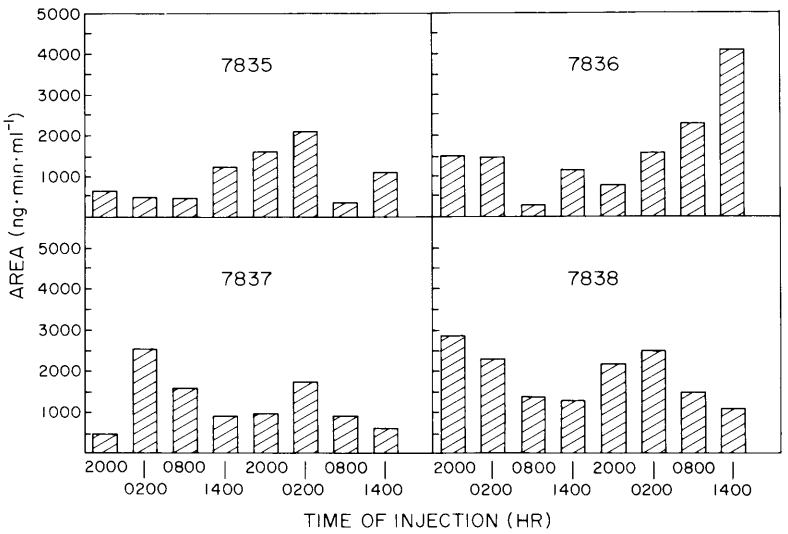


FIG. 1. Serum concentrations of growth hormone (expressed as area under the 30-min response curve) of individual calves given intravenous injections of 20 μg hpGRF 1-40-OH/100 kg body weight at 6-hr intervals (Experiment 2).

dividual bull calves the magnitude of GH response to multiple injections of hpGRF is variable. Indeed, individual lactating cows also show a variable GH response when hpGRF is administered at 4-hr intervals (8). The difference in GH response between hpGRF injections may be due to the timing of exogenous hpGRF administration relative to the animals endogenous GRF and somatostatin (SRIF) status. The single-injection protocol can not take into account the endogenous GRF/SRIF milieu and therefore interpretation of results using this type of protocol is difficult.

TABLE III. SERUM GROWTH HORMONE RESPONSE OF CALVES GIVEN A 6-hr CONTINUOUS INFUSION OF HUMAN PANCREATIC GROWTH HORMONE-RELEASING FACTOR (hpGRF 1-40-OH; 200 μg/hr) OR PLACEBO

| GH trait[a] | Placebo | hpGRF | SED[c] |
|---|---|---|---|
| Pre-infusion mean (ng/ml serum) | 5.1 | 4.3 | 1.6 |
| Infusion mean (ng/ml serum) | 7.4 | 36.5[d] | 8.4 |
| Area[b] (ng · min · ml$^{-1}$ serum) | 2622 | 13,451[d] | 2804 |

[a] Values are means of four calves.
[b] Area under 6-hr response curve.
[c] Standard error of differences of means.
[d] Different ($P < 0.05$) from placebo.

In agreement with data in lactating cows (9, 18), infusion of GRF to bull calves resulted in sustained GH response. In addition, GH profiles of GRF-infused animals were more pulsatile than controls (9, 18). Thus, it appears that hpGRF infusion induces events which increase episodic secretion of GH. Pulsatile activity of GH during constant infusion of hpGRF has also been reported in humans (19). In contrast, hpGRF infusion in steers does not increase pulse frequency, but augments the amplitude of naturally occurring GH pulses (7). Increased pulsatile activity during hpGRF infusion may be due to one or more of the following: episodic SRIF secretion (20); pituitary desensitization to GRF occurring in a cyclical manner (21); short-term pituitary GRF receptor down-regulation (22); GRF and SRIF inhibition of its own neurosecretion via a negative ultrashort loop feedback mechanism (23, 24); direct/indirect feedback of GH on its own secretion (25–27); and (or) feedback of GH-induced somatomedin on the pituitary (26, 28) or hypothalamus (28). Possibly, GRF and SRIF regulate their own release at the level of the hypothalamus (29) or interact at the level of the pituitary (30) to result in a distinct GH secretory profile.

The sustained GH response following multiple injections or continuous infusion of

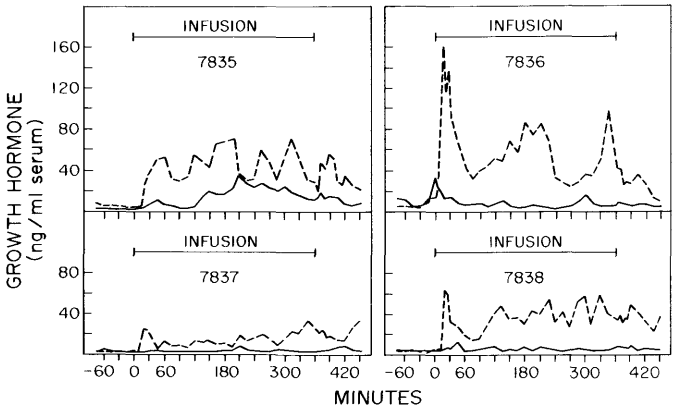


FIG. 2. Serum concentrations of growth hormone of individual calves given continuous intravenous infusions of 0 (—) and 200 (- - -) μg hpGRF 1-40-OH/hr for 6 hr (Experiment 3).

hpGRF agrees with data in lactating cows (8, 9), steers (7), and sheep (17). Indeed, work in cattle (7–9, 18, 31) suggests that GRF stimulates synthesis as well as release of GH, as has been demonstrated in rats (32). Evidence in cattle indicates that pituitary stores of GH do not become depleted and the pituitary does not become refractory to GRF during 19 days of infusion (18) or 57 days of consecutive daily injections (31). In contrast to results in ruminants, multiple injections (three at 2-hr intervals) of hpGRF to humans diminishes the GH response (10). However, GH response of humans to long-term treatment with repetitive injections of hpGRF is unknown.

We conclude that GH response of bull calves to hpGRF is dose dependent and that repeated injections or continuous infusions of hpGRF consistently elicit GH release, although magnitude of response varies considerably. We hypothesize that differences in GH response to hpGRF within and among calves, and pulsatile secretion in the face of hpGRF infusion, are related to the degree of synchrony among exogenous hpGRF and endogenous GRF and SRIF.

Based on the extensive variability of GH response to single injections of hpGRF seen within and among calves of the present study, multiple injections or continuous infusions of hpGRF provide a better indication of GH responsiveness in an animal.

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