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Growth Hormone-Releasing Factor on Growth Hormone Secretion in Prepubertal Calves[1] (42728)

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Abstract. The effects of iv administration of growth hormone-releasing factor (GRF) on growth hormone (GH) release and on nitrogen metabolism were measured in prepubertal calves. Crossbred beef heifers (111 kg) were used in a Latin square design to test the effects of 0, 0.01, 0.033, 0.067, and 0.1 μg human pancreatic (hp) GRF [hpGRF (1,40)OH]/kg body wt on plasma GH concentrations. When they were given doses of 0.067 and 0.1 μg hpGRF/kg body wt, plasma GH increased ($P < 0.05$) within 5–15 min, compared with injections of control buffer, and then returned to preinjection concentrations. The response to 0.067 μg hpGRF/kg body wt every 3 hr for 42 hr was studied in five heifers (137 kg body wt). The animals responded to 50% of the GRF injections with an increase in plasma GH during every 6-hr period measured. Nitrogen retention, hormone concentrations, and weight gain were measured in five bull calves (90 kg body wt) administered 0 or 0.067 μg Nle^{27} rat hypothalamic GRF (1,29)NH_2/kg body wt every 4 hr for 10 days. Metabolic parameters were interpreted to indicate an anabolic response to GRF even though increases of 16% in nitrogen retention, 23% in plasma somatomedin C concentrations, and 36% in weight gain with pulsatile GRF treatment were variable and statistically similar to those of controls. These results indicate that GRF induces peak GH secretion within 15 min in prepubertal calves and that calves can respond to multiple injections of GRF with an increase in plasma GH. © 1988 Society for Experimental Biology and Medicine.

Hypothalamic regulation of growth hormone (GH) secretion is mediated by a stimulatory factor, growth hormone-releasing factor (GRF), and an inhibitory factor, growth hormone-releasing-inhibiting hormone, somatostatin (SRIF). Intravenous administration of GRF results in a pulse release of GH into the plasma, similar to that occurring during endogenous GH secretion. It has been suggested that the pattern of GH secretion, frequency and amplitude of GH spikes, is important in the regulation of growth of certain species such as the rat (1, 2), but may not be important for others such as cattle (3). There are age-related changes in GH secretion in cattle, in which the secretion of plasma GH declines as animals age (4). To understand the GH physiology of young cattle, which secrete more GH, investigations were done to determine the acute response of young calves to exogenous GH-releasing peptides.

The objective of this study was to determine the role of GRF on the secretion of GH in prepubertal calves by studying the acute effects of GRF on GH secretion, the changes in plasma GH with multiple injections of GRF, and the effects of pulsatile GRF administration on nitrogen metabolism and plasma concentrations of other hormones in calves.

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Materials and Methods. *Animals.* All animals were maintained in an environmentally controlled room at 18°C with 12-hr photoperiods. Crossbred beef heifer calves individually penned with straw bedding were used in Experiments 1 and 2. The heifers were fed at 12-hr intervals with a diet consisting of 49% cracked corn, 39% dehydrated alfalfa, 5.6% solvent-extracted soybean meal, 5.6% cane molasses, and 1% salt, minerals, and vitamin A. In Experiment 3, five Holstein bull calves were adjusted for 3 weeks before experimentation to the diet and the metabolism crates. These calves were fed at 12-hr intervals with 45% cracked corn, 39% dehydrated alfalfa, 9.5% solvent-extracted soybean meal, 5.6% cane molasses, and 1% salt, minerals, and vitamin A. An indwelling catheter (Tygon microbore tubing, 1.27 mm i.d., Fisher Scientific, Pittsburgh, PA) was inserted in a jugular vein of the calves at least 1 day before experimentation. The catheters were filled with sterile saline containing 100 U heparin/ml between experiments and sampling periods and with sterile saline with 40 U heparin/ml when blood samples were being taken. Blood samples were treated with heparin (4 U/ml), and plasma was stored at −15°C until analyzed. All samples were analyzed for GH (5). The GH assay had intra- and interassay coefficients of variation of 1.7 and 9.0%, respectively.

Peptides. Animals received bolus iv injections of human pancreatic GRF [hpGRF(1,40)OH] in Experiments 1 and 2 (6) and Nle^{27} rat hypothalamic GRF [Nle^{27} rGRF(1,29)NH_2] in Experiment 3 (7). The GRFs were dissolved in 0.1% acetic acid (1 μg/μl) and then diluted with a sterile buffer solution. The buffer was physiological saline (0.818% NaCl) containing 0.01 *M* $Na_2PO_4{*}H_2O$, pH 7.0; 0.01% ascorbic acid; and 1% bovine serum albumin. Solutions for injection and infusion were prepared on the day of experimentation.

Dose titration (Experiment 1). Five 4-month-old heifers (111 kg body wt) were assigned randomly to a Latin square design to test the plasma GH response to 0, 0.01, 0.033, 0.067, and 0.1 μg hpGRF/kg body wt. The doses of GRF tested were based on unpublished results from preliminary experiments. The Latin square was completed over 3 days, with one block of the Latin square conducted each morning and another block in the early afternoon. Animals were fed the evening before the experiment and were allowed several hours to eat before the feed was removed. Blood samples were collected 20, 10, and 1 min before injection and 5, 10, 15, 20, 40, 60, 80, 100, and 120 min after injection of GRF. Samples taken before injection were averaged to represent the animals preinjection GH concentrations. Characteristics of the GH response to GRF included a preinjection mean, the mean of the acute GH release within 20 min after GRF injection, and the GH postinjection means. Samples after injection were averaged into periods of 5–20, 40–60, 60–80, 80–100, and 100–120 min. The means were analyzed as a Latin square design with dose, period, and animal as main effects (8, 9).

Multiple injections (Experiment 2). Five 5-month-old heifers (137 kg body wt) were assigned randomly to a crossover design testing 0 or 0.067 μg hpGRF/kg body wt administered at 3-hr intervals for 42 hr. The treatments were then reversed, and the experiment was repeated after 1 week. To monitor acute GH changes in response to GRF after multiple injections, blood was sampled during four periods of 5 or 6 hr during which two injections of GRF were administered. Each 5- or 6-hr sampling period was separated by an 8-hr interval during which three unmonitored GRF injections were administered. Within each sampling period, the animals were fed between the GRF injections. Blood samples were collected at 20-min intervals except immediately after a GRF injection, when they were taken at 5-min intervals for 20 min. The GH mean concentrations before GRF injection, 0–20 min after injection, and during sampling periods were analyzed as a crossover design with treatment (0 or 0.067 μg GRF/kg body wt), replicate, and animal as main effects (8, 9).

Nitrogen balance (Experiment 3). Five Holstein bull calves at 3.5 months of age (90 kg body wt) were assigned randomly to a crossover design to test the effects of administration of GRF (0.067 μg Nle^{27} rGRF (1,29)NH_2/kg body wt) or control buffer

every 4 hr for 10 days on nitrogen metabolism. Similar to one report (10), but contrary to another (11), we have observed no difference in the GH-releasing activity of Nle^{27} rGRF (1,29)NH_2 and hpGRF (1,40)OH in heifers (data not shown). After the 10-day period, the animals were rested for 7 days; then treatments were reversed. Blood was sampled at −20, −10, −1, 5, 10, 15, 20, 40, and 60 min after injection of GRF at 1400 hr daily. Preinjection GH concentrations from Days 0 to 10 were averaged to obtain an estimate of baseline GH concentrations and for statistical analysis. The 5- to 20-min GH concentrations and the 40- to 60-min GH concentrations were averaged daily. The daily GH means following injection of GRF were averaged for statistical comparisons among Days 0–5, 6–10, and 0–10.

In addition to GH, all plasma samples from Experiment 3 were analyzed for insulin (12), plasma urea nitrogen (Micro-urea, Technicon-N-10a, autoanalyzer), and plasma glucose (Worthington Flozyme Glucose, Worthington Diagnostic Systems, Inc., Freehold, NJ). Intra- and interassay coefficients of variation for the insulin assays were 1.1 and 1.3%, respectively. Coefficients of variation for plasma urea nitrogen were 5.8%, intraassay, and 7.3%, interassay. The plasma glucose intraassay coefficient of variation was 3.1%, whereas the interassay coefficient of variation was 7.1%. Insulin, plasma urea nitrogen, and plasma glucose concentrations of plasma samples taken surrounding an injection of rGRF were averaged daily for preliminary statistical analysis, but because no daily differences were observed, all samples were averaged in the final statistical analysis. The plasma samples taken before GRF injection were pooled daily for analysis of somatomedin C (Sm-C, Immuno Nuclear Corp., Stillwater, MN, as modified by Plouzek (13), triiodothyronine (Amerlex T-3 RIA kit, Amersham Corp., Arlington Heights, IL), thyroxine (Amerlex T-4 RIA kit, Amersham Corp.), and cortisol (Cortisol Double Antibody, Diagnostics Product Corp., Los Angeles, CA). The pooled samples were analyzed in one assay. The resulting intraassay coefficients of variation were 1.9% for Sm-C, 1.5% for thyroxine, 1.8% for triiodothyronine, and 1.4% for cortisol. Sm-C, thyroxine, triiodothyronine, and cortisol were analyzed statistically on a daily basis and among Days 0–5, 6–10, and 0–10, but due to the lack of significant differences among days or between Days 0–5 and 6–10, all samples were averaged in the final analysis.

Samples of feed, feces, urine, and orts were collected daily, composited every 5 days, and stored at −15°C until analysis. Feed, feces, and orts were chopped and then ground frozen in liquid nitrogen by using a heavy duty blender (Waring, Model 32BL75, Waring Commercial, New Hartford, CT). Urine samples were thawed and mixed, and an aliquot was removed for chemical analysis. Nitrogen was determined by the Kjeldahl procedure (14) using a Kjeltec System II (Tecator, Hoganas, Sweden). Dry-matter contents of feed, orts, and feces were determined by drying at 60°C (14). The chemical composition of the feed, feces, orts, and urine allowed for the calculation of dry-matter intake, fecal dry matter, apparent dry-matter digestibility, nitrogen intake, urinary nitrogen, fecal nitrogen, nitrogen retention, apparent biological value, apparent nitrogen digestibility, and intake nitrogen excreted in urine.

Data were analyzed as a crossover design with treatment (0 or 0.067 μg rGRF/kg body wt) and replicate as main effects and animal nested within treatment as the error term to test treatment effects (8, 9). As no differences between Days 0–5 and 6–10 were observed in the nitrogen data as well as the endocrine data, the data presented are the means of Days 0–10.

Results. *Dose titration (Experiment 1).* Plasma GH increased in a dose-dependent manner, with a peak of GH occurring between 5 and 15 min after injection of hpGRF, which declined rapidly to basal levels within 60 min (Fig. 1). One animal did not respond to any dose of hpGRF. Overall, the animals responded to 66% of the GRF injections. Before injection of hpGRF, GH averaged 5.5 ± 1.2 ng/ml (±SE). During the first 20 min after injection of hpGRF, GH averaged 7 ± 1.9, 19 ± 11, 20 ± 8, 43 ± 17, and 54 ± 17 ng/ml for 0, 0.01, 0.033, 0.067, and 0.1 μg hpGRF/kg body wt, respectively. Growth hormone levels during the 20-min period after injection of 0.067 ($P < 0.05$) and 0.1 μg hpGRF/kg body wt ($P < 0.025$) were

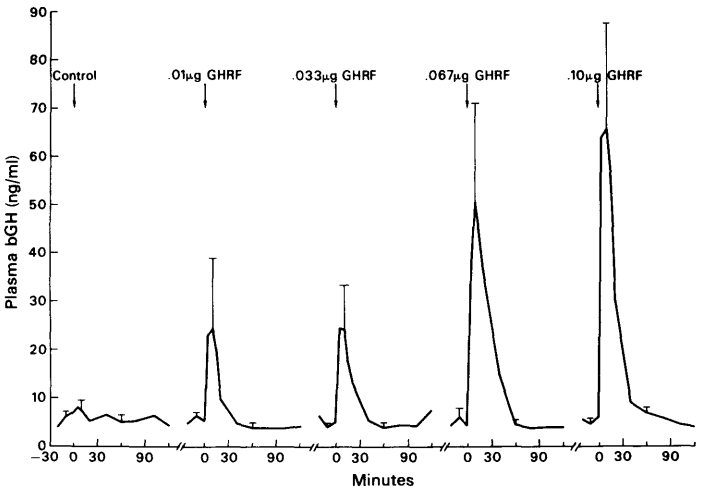

FIG. 1. Mean concentrations of plasma GH in crossbred beef heifers in response to iv injections of 0, 0.01, 0.033, 0.067, and 0.1 μg hpGRF (1,40)OH/kg body wt (n = 5). The dose of hpGRF is indicated above the arrow at the time of administration. To indicate representative SE at baseline and peak concentrations, the samples 10 min before and after injection of hpGRF have SE marked by the bars.

greater than controls, whereas 0.01 and 0.033 μg hpGRF/kg body wt were similar to controls. There were no differences between treatments 40–120 min after hpGRF injection. The 40- to 120-min GH mean was 6 ± 1.9 ng/ml.

Multiple injections (Experiment 2). The characteristic GH peak and 5- to 20-min mean of the GH response to iv injections of 0.067 μg hpGRF/kg body wt every 3 hr for 42 hr (Fig. 2) were similar to those observed in Experiment 1. Animals responded with an

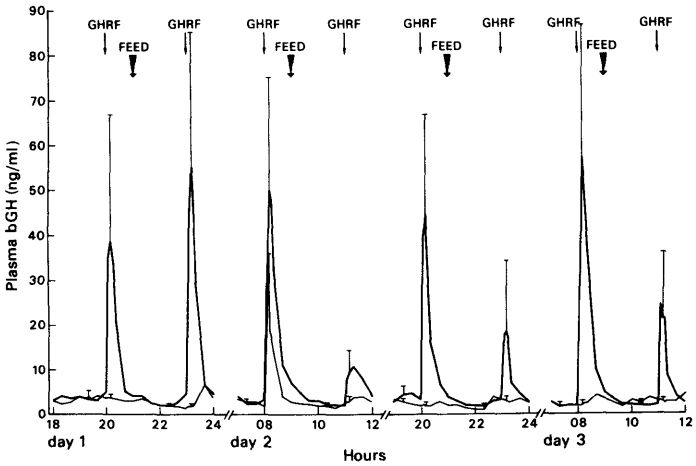

FIG. 2. The average plasma GH concentrations of crossbred beef heifers receiving 0 or 0.067 μg hpGRF (1,40)OH/kg body wt at 3-hr intervals for 42 hr. The thick line illustrates the response of animals receiving hpGRF (n = 5), and the thin line illustrates the plasma response of animals receiving buffer without hpGRF (n = 5). To represent SE before hpGRF injection and during the peak response of GH after hpGRF injection, SE are shown by the bars for samples 40 min before hpGRF injection and 10 min after injection. Times of feeding and hpGRF administration are indicated by the arrows.

increase in plasma GH by at least doubling their preinjection concentrations. During each 5-hr sampling period, heifers responded with at least a doubling of their plasma GH levels to only one of the two hpGRF injections. Of the 40 hpGRF injections monitored, only 20 actually produced a GH response, of which 12 occurred after the first hpGRF injection, and 8 responses were observed after the second. Three of the five animals responded to the first injection of hpGRF, but they did not respond to hpGRF after feeding. The injection that a given animal responded to with a rise in GH remained consistent throughout the experiment with two exceptions: (1) One animal responded to both injections of hpGRF in one period, and (2) one animal did not respond to hpGRF during one sampling period. The average 20-min GH response within the four sampling periods to the first hpGRF injection was 37 ± 20 ng/ml (±SE) and 22 ± 16 ng/ml to the second hpGRF injection. The response to hpGRF from one sampling period to another was the same. The overall GH mean was 3 ± 2.0 ng/ml for control animals.

Nitrogen balance (Experiment 3). Mean concentrations of plasma hormones in animals injected with buffer or rGRF for 10 days are given in Table I. The mean GH response in animals given rGRF was 25 ng/ml ($P < 0.05$), compared with 7 ng/ml in control calves. The calves responded to 46 (77%) of the 60 rGRF injections which were monitored each afternoon. The GH mean for the 5- to 20-min period after rGRF was similar on Days 0 and 10 as compared by Student's *t* test (9). GH averaged 6.5 and 8.7 ng/ml before injections of rGRF and buffer. Although concentrations of Sm-C were 23% greater in calves given rGRF, these differences were not statistically significant. Insulin, triiodothyronine, thyroxine, and cortisol concentrations were not changed with administration of rGRF.

Although nitrogen retention increased 16% in calves given rGRF, it was not different ($P > 0.05$) from that of controls. Urinary, fecal, and intake nitrogen did not significantly change with rGRF treatment (Table II). However, nitrogen intake increased 12% and urinary nitrogen increased 4%, which caused a decrease of 3 percentage units of intake nitrogen excreted in the urine with rGRF treatment. Apparent nitrogen digestibility and biological value were not affected by rGRF treatment. Plasma urea nitrogen, which declined 6% with rGRF treatment, and glucose were not significantly different between treatments. Although body weight gain increased 36% with rGRF administration, the increase was not significant. Dry-matter intake also increased 14%, but it was not statistically different from control values. Fecal dry matter and dry-matter digestibility were not altered by treatment.

TABLE I. EFFECT OF MULTIPLE INJECTIONS OF Nle^{27} rGRF (1,29)NH_2 ON PLASMA HORMONE MEANS OVER 10 DAYS IN BULL CALVES

	rGRF	Control	SE
GH (ng/ml)[a]	6.5	8.7	1.5
GH (ng/ml)[b]	25.1[c]	7.0	8.0
GH (ng/ml)[d]	9.8	7.8	1.8
Sm-C (nmole/liter)	8.1	6.6	2.4
Insulin (ng/ml)	0.54	0.51	0.12
Triiodothyronine (ng/dl)	1.31	1.34	0.22
Thyroxine (μg/dl)	7.7	7.4	1.1
Cortisol (μg/dl)	2.2	1.8	0.3

[a] Mean of 20, 10, and 1 min before rGRF injection.
[b] Mean of 5, 10, 15, and 20 min after rGRF injection.
[c] Mean significantly different ($P < 0.05$) from corresponding control mean.
[d] Mean of 40 and 60 min after rGRF injection.

Discussion. Prepubertal calves responded to acute injections of GRF with a dose-dependent increase in plasma GH concentrations. No diminution of the GH response was observed to GRF injected at 4-hr intervals for 10 days. These results are similar to studies with older cattle and sheep (13, 15–18). In another study with prepubertal (15 kg) lambs there was a response to GRF, but when the animals were given multiple iv injections, the magnitude of the response declined over 3 days (19). In contrast, Kensinger *et al.* (18) demonstrated that 48-kg lambs responded without a decline in GH amplitude to every separate GRF subcutaneous injection given during a 24-hr period when two, four, or eight injections were administered.

Five-month-old heifer calves receiving GRF every 3 hr for 42 hr responded to 50% of the GRF injections measured (Experi-

TABLE II. EFFECT OF MULTIPLE INJECTIONS OF Nle^{27} rGRF (1,29)NH_2 ON NITROGEN METABOLISM, WEIGHT GAIN, AND FEED INTAKE IN BULL CALVES

	rGRF	Control	SE
Nitrogen intake (g/10 days)	306	274	53
Urinary nitrogen (g/10 days)	98	94	10
Fecal nitrogen (g/10 days)	27	25	5
Apparent nitrogen digestibility (%)	91.2	90.0	0.4
Nitrogen retention (g/10 days)	181	156	41
Apparent biological value (%)	63.5	60.4	4.9
Intake nitrogen excreted in urine (%)	33.2	35.9	4.4
Plasma urea nitrogen (mg/dl)	6.5	6.9	0.8
Plasma glucose (mg/dl)	95.3	90.3	5.3
Weight gain (kg/10 days)	2.66	1.95	0.79
Dry-matter intake (kg/10 days)	12.5	11.0	2.2
Apparent dry-matter digestibility (%)	69.0	69.1	1.5

ment 2), whereas a greater degree of responses (77%) was observed in 3.5-month-old bull calves given GRF every 4 hr for 10 days (Experiment 3). The greatest difference between these two experiments was that all the samples from the bull calves were taken 8 hr after feeding, whereas in the heifer calves, half of the samples were taken before feeding and half were taken immediately after feeding. The change in the number of animals responding to GRF between experiments may have also been due to sex differences, the different interval between administration of GRF, or inhibition by SRIF. Although some sex differences have been reported in the GH response to GRF administration (20), others have not consistently observed a greater GH response to GRF in males than in females (13).

The amplitude of the GH response to GRF may also have been related to feeding. The GH response to GRF was greater before feeding than after feeding in Experiment 2. In an experiment with sheep in which GRF was administered at 3-hr intervals, the GH response was highest before feeding, decreased after feeding, then gradually increased in amplitude with increasing time after feeding (13). Since animals in both these studies were adjusted to feeding at 12-hr intervals, both psychologically and physiologically, the feeding regimen may have been responsible for the differences in amplitude of the GH response to GRF. It has been demonstrated that feeding acutely reduces plasma GH within minutes in sheep and goats (21–23). Furthermore, plasma GH concentrations started to decline in some animals before feeding, suggesting that the anticipation of being fed caused the reduction (22, 23). In a study with GRF, fed sheep, those that had feeding simulated by a water-filled balloon in the cranial sac of the rumen, and those which were anticipating being fed showed a reduced GH response to GRF compared with fasted controls (24).

Administration of GRF every 4 hr for 10 days to 90-kg calves increased nitrogen retention 16%, but this difference was not statistically significant. Although the endocrine and metabolic data were not significantly changed with GRF treatment, the changes in the values indicated that a minor increase in the postabsorptive nitrogen utilization occurred in the calves. In the study of Moseley *et al.* (25), nitrogen retention of calves was significantly increased by 18% and urinary nitrogen declined by 14% between Days 9 and 14 of the 20-day continuous infusion of GRF. In this study, the lack of significance of nitrogen retention may have resulted from the limited number of animals in the study, the increased variability of the results, the low concentration of GRF administered, or the GRF being administered as a pulse rather than as a continuous infusion. Since continuous infusion of GRF enhances the endogenous episodic secretion of GH and increases plasma GH concentrations in prepubertal ruminants (17, 25, 26), the method of ad-

ministration may have limited the GH response. However, in lactating cattle multiple injections of GRF have effectively elevated serum GH and milk yield (27, 28).

In conclusion, prepubertal bull and heifer calves were responsive to GRF administered as either discrete single or multiple iv injections. Considerable variation occurred in the number of calves responding to the GRF injections with a noticeable lack of responsiveness following feeding. Nitrogen retention in calves increased by 16% and weight gain increased by 36% in response to GRF pulsed at 4-hr intervals for 10 days. However, there was considerable variability and, thus, GRF treatments remained statistically similar to the controls. Pulsatile administration of GRF has not always been successful in eliciting an anabolic response in ruminants (19). Multiple injections of GRF in lactating dairy cows increased milk yields in some studies (27, 28); in contrast, others have not demonstrated an anabolic response (29). However, in a study in which adult sheep were given the same dose of GRF at similar intervals as the calves in the present study, a significant decrease in urinary nitrogen occurred (13). The dissimilar effect of GRF in both of these studies may result from differences due to age of the animals in as much as younger animals secrete more GH than do adults (13).

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