NOTE

Response of Growth Hormone Release to Human Growth Hormone-Releasing Factor and Its Analogs in the Bovine

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Abstract

Responses of growth hormone (GH) release to synthetic human growth hormone-releasing factor (hGRF)-44-NH₂ analogs were determined, and the GH-releasing potency based on dose per kg of body weight (bw) was compared with that of hGRF-44-NH₂ in female dairy calves. Four- and 12-month-old calves were injected intravenously with 0.25 µg of hGRF-44-NH₂ or its analogs per kg of bw. Blood samples were collected before, and during 180 min after each injection, and plasma GH concentrations were measured by radioimmunoassay. Areas under the GH response curves for 180 min after injection of hGRF-44-NH₂ and its analogs were used as an index of the GH-releasing potency of each peptide. The GH-releasing potency of hGRF(1-26)-NH₂ was significantly lower than that of hGRF-44-NH₂ ($P < 0.05$). On the other hand, hGRF(1-29)-NH₂ possessed similar potency to hGRF-44-NH₂. [D-Tyr¹]-hGRF-44-NH₂ showed prolonged GH-releasing activity, though its potency was similar to that of hGRF-44-NH₂. Also, [D-Ala²]-hGRF(1-29)-NH₂ exhibited prolonged GH-releasing activity, and its potency was 2.5 ($P < 0.05$) and twice ($P < 0.05$) as great as that of hGRF-44-NH₂ and hGRF(1-29)-NH₂, respectively. These results demonstrate that the N-terminal 29 amino acid residues of hGRF possess the activity site required for full GH release *in vivo*, and [D-Ala²]-hGRF(1-29)-NH₂ has longer and greater activity, on a dose basis, than hGRF-44-NH₂ in the calves.

Recently, growth hormone-releasing factors (GRFs) have been isolated, purified and characterized (Guillemin *et al.*, 1984). Synthetic human GRF (hGRF)-44-NH₂ specifically stimulates the release of growth hormone (GH) in humans (Guillemin *et al.*, 1984), rats (Guillemin *et al.*, 1984), rabbits (Chihara *et al.*, 1984), chickens (Leung and Taylor, 1983; Harvey and Scanes, 1984), goats (Hodate *et al.*, 1984, 1985a; Hart

et al., 1984), sheep (Hodate *et al.*, 1985a), cattle (Hodate *et al.*, 1984; Johke *et al.*, 1984; Moseley *et al.*, 1984) and pigs (Hodate *et al.*, 1985b). To elucidate the structure-activity relationships of GRFs, the relative GH-releasing potencies of several kinds of analogs or fragments of hGRF-44-NH₂ have been studied mainly in the rat (Guillemin *et al.*, 1984; Rivier *et al.*, 1982; Wehrenberg and Ling, 1983; Ling *et al.*, 1984; Ohashi *et al.*, 1984; Lance *et al.*, 1984; Heiman *et al.*, 1985) and also in domestic

animals (Hodate *et al.*, 1985c; Lance *et al.*, 1984; McCutcheon *et al.*, 1984). In the rat, Rivier *et al.* (1982) found that hGRF(1-29)-NH₂ has full intrinsic GH-releasing potency *in vitro*. Lance *et al.* (1984) reported that [D-Ala²]-hGRF(1-29)-NH₂ was approximately 50 times more potent than hGRF(1-29)-NH₂ in eliciting GH secretion *in vivo*. The GH responses to hGRF(1-29)-NH₂ in lactating cows (McCutcheon *et al.*, 1984) and pigs (Lance *et al.*, 1984; Kraft *et al.*, 1985) and to [D-Ala²]-hGRF(1-29)-NH₂ in pigs (Lance *et al.*, 1984) have been also determined. However, the relative GH-releasing potencies of these peptides to hGRF-44-NH₂ are not known in the farm animals.

In the present experiments, we studied the GH-releasing potencies of hGRF(1-29)-NH₂, hGRF(1-26)-NH₂, [D-Ala²]-hGRF(1-29)-NH₂, [D-Tyr¹]-hGRF-44-NH₂ and hGRF-44-NH₂ in female dairy calves.

Materials and Methods

Experimental animals

Four 4-month-old and eight 12-month-old Holstein female dairy calves, 128 kg and 330 kg mean body weight (bw) respectively, from the institute herd were used. The animals were fed at 0830 h, and synthetic hGRF-44-NH₂, its analogs, or saline were given at 1100 h.

Preparations of synthetic hGRF-44-NH₂ and its analogs for injection

The hGRF-44-NH₂ and its analogs: hGRF(1-26)-NH₂ and hGRF(1-29)-NH₂, fragments with 18 and 15 amino acids deleted from the C-terminal of hGRF-44-NH₂ respectively; [D-Tyr¹]-hGRF-44-NH₂, an analog with D-Tyr substitution in N-terminal Tyr of hGRF-44-NH₂; [D-Ala²]-hGRF(1-29)-NH₂, an analog with D-Ala substitution in position-2 Ala of hGRF(1-29)-NH₂, were synthesized by the solid-phase method and purified by HPLC (Ohashi *et al.*, 1984). These preparations were dissolved in 3 ml of sterilized saline before use and injected intravenously (iv) through an indwelling catheter inserted into one of the external jugular veins.

Blood sampling method

The jugular venous blood of the experimental animals was taken from an indwelling catheter previously inserted into one of the external jugular veins. The blood samples were collected before, and during over a period of 180 min immediately following the injection of hGRF-44-NH₂ and its analogs, or saline. Serial 3-4 ml blood samples were collected into centrifuge tubes containing heparin, and immediately chilled with ice. Individual plasma samples were obtained after centrifugation and stored at -60°C until assayed.

Measurement of plasma GH and statistical analysis

The concentrations of plasma GH were determined by radioimmunoassay (Johke, 1978). Areas under the GH response curves for 180 min after each injection were determined and regarded as the total amount of GH released in response to hGRF-44-NH₂, its analogs, or saline. These areas under the GH response curves were used as an index of the GH-releasing potency of each peptide. The paired *t*-test was used to assess the significance of the differences in the data (Snedecor and Cochran, 1980).

GH responses to a single injection of hGRF-44-NH₂, hGRF(1-26)-NH₂, and [D-Tyr¹]-hGRF-44-NH₂

Four 4-month-old calves were injected iv with 0.25 µg of hGRF-44-NH₂, hGRF(1-26)-NH₂, or [D-Tyr¹]-hGRF-44-NH₂ per kg of bw, and 3 ml saline (as a control). These experimental animals were repeatedly used for each treatment. The blood samples were drawn at -10, 0, 5, 10, 20, 30, 45, 60, 90, 120, 150 and 180 min after injection. The blood sample at 0 min was obtained just before each injection. The experiments were carried out between late March and early April, and treatments were carried out at 1-week intervals.

GH responses to a single injection of hGRF-44-NH₂, hGRF(1-29)-NH₂, and [D-Ala²]-hGRF(1-29)-NH₂

Eight 12-month-old calves were divided into two equal groups. The four calves (337 kg mean bw) in one group were given a single iv injection of 0.25 µg of hGRF-44-NH₂, hGRF(1-29)-NH₂, or [D-Ala²]-hGRF(1-29)-NH₂ per kg of bw. These animals were repeatedly used for

each treatment. The blood samples were drawn at -30, -15, 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, and 180 min after injection. In another group (322 kg mean bw), 3 ml saline (as a control) was injected iv into each of the four calves. The blood samples were drawn at 15-min intervals. The experiments were carried out between late November and early December, and treatments were carried out at 1-week intervals.

Results

GH responses to hGRF-44-NH₂, hGRF(1-26)-NH₂, and [D-Tyr¹]-hGRF-44-NH₂

The responses of plasma GH in the 4-month-old calves to a single injection of

hGRF-44-NH₂, hGRF(1-26)-NH₂ and [D-Tyr¹]-hGRF-44-NH₂, or saline are shown in Fig. 1. Table 1 shows the areas under the GH response curves for 180 min after injection of each peptide or saline.

The mean plasma GH concentration in each saline injected control calf varied within the range 2.8 to 6.8 ng/ml, and no elevation of the plasma GH level after injection was detected throughout the blood sampling period. In contrast, the concentration of plasma GH after the injection of hGRF-44-NH₂ increased from the value of 4.5 ± 0.5 ng/ml (Mean $\pm$ SE) at 0 min to a maximum value of 107.2 ± 37.8 ng/ml ($P < 0.05$) at 10 min.

Fig. 1. Plasma GH responses to a single injection of hGRF-44-NH₂ (○), hGRF(1-26)-NH₂ (△) and [D-Tyr¹]-hGRF-44-NH₂ (▲), or saline (●) in 4-month-old calves. An arrow indicates the time of peptide (0.25 μ g per kg of bw) or saline injection. Asterisks indicate the statistical differences; * $P < 0.05$, ** $P < 0.01$ as compared with the corresponding levels of hGRF-44-NH₂. Each point with a bar represents the mean $\pm$ SE ($n = 4$).

Table 1. Area under GH response curve for 180 min after injection of saline, hGRF-44-NH₂ and its analogs in calves (4- and 12-month-old). The value is expressed as mean $\pm$ SE (n=4). Asterisks indicate the statistical difference; *P<0.05 as compared with the value of hGRF-44-NH₂ in each group.

Treatment	Area (ng $\cdot$ min/ml)	
	4-month-old	12-month-old
Saline	809.9 $\pm$ 83.9*	683.4 $\pm$ 128.9*
hGRF-44-NH ₂	3,022.4 $\pm$ 606.7	2,165.4 $\pm$ 703.5
hGRF(1-26)-NH ₂	1,162.6 $\pm$ 208.6*	—
hGRF(1-29)-NH ₂	—	2,670.1 $\pm$ 305.7
[D-Tyr ¹]-hGRF-44-NH ₂	3,616.6 $\pm$ 427.8	—
[D-Ala ²]-hGRF(1-29)-NH ₂	—	5,407.3 $\pm$ 922.8*

Fig. 2. Plasma GH responses to a single injection of hGRF-44-NH₂ (○), hGRF(1-29)-NH₂ (△) and [D-Ala²]-hGRF(1-29)-NH₂ (▲), or saline (●) in 12-month-old calves. An arrow indicates the time of peptide (0.25 μ g per kg of bw) or saline injection. Asterisks indicate the statistical differences; *P<0.05, **P<0.01 as compared with the corresponding levels of hGRF-44-NH₂. Each point with a bar represents the mean $\pm$ SE (n=4).

Injection of hGRF(1-26)-NH₂ caused an elevation in the plasma GH level from the value of 4.7 ± 0.5 ng/ml at 0 min to a peak value of 20.1 ± 3.9 ng/ml ($P < 0.05$) at 5 min. The peak value was only 19% of the peak produced by hGRF-44-NH₂. The concentrations of GH at 10, 20 and 30 min in the calves given this fragment were significantly lower ($P < 0.05$) than the corresponding concentrations of GH in the calves given hGRF-44-NH₂. Also, the area under the GH response curve was significantly smaller ($P < 0.05$) than that obtained by hGRF-44-NH₂.

Injection of [D-Tyr¹]-hGRF-44-NH₂ also caused a significant increase in the plasma GH level from the value of 2.9 ± 0.4 ng/ml at 0 min to a maximum value of 39.6 ± 15.6 ng/ml ($P < 0.05$) at 45 min. The plasma level for GH at 60 min (29.7 ± 9.7 ng/ml) was significantly higher ($P < 0.05$) than the corresponding value for GH (4.8 ± 0.7 ng/ml) in the animals given hGRF-44-NH₂. Although the maximum plasma concentration of GH in the calves given this analog was 37% of that produced by hGRF-44-NH₂, the area under the GH response curve was larger than that obtained by hGRF-44-NH₂, though neither the difference between the maximum values nor the difference between the areas was statistically significant.

GH responses to hGRF-44-NH₂, hGRF(1-29)-NH₂, and [D-Ala²]-hGRF(1-29)-NH₂

Fig. 2 and Table 1 show the responses of GH release in the 12-month-old calves to a single injection of hGRF-44-NH₂, hGRF(1-29)-NH₂ and [D-Ala²]-hGRF(1-29)-NH₂, or saline and the areas under the GH response curves for 180 min after injection of each peptide or saline, respectively.

The mean plasma GH concentration in each saline injected control calf varied within the range 2.8 to 6.3 ng/ml, and no elevation of the plasma GH level after injection was detected throughout the blood sampling period.

The concentration of GH after injection

of hGRF-44-NH₂ rose to a maximum value of 47.0 ± 17.1 ng/ml ($P < 0.05$) at 15 min from 1.4 ± 0.2 ng/ml at 0 min. Also, injection of hGRF(1-29)-NH₂ caused a significant increase ($P < 0.05$) in the plasma GH up to the level produced by hGRF-44-NH₂. Both the response of plasma GH and the area under the GH response curve in the calves given hGRF(1-29)-NH₂ were very similar to those of GH in the calves injected with hGRF-44-NH₂.

The plasma GH concentration after [D-Ala²]-hGRF(1-29)-NH₂ injection increased from 2.0 ± 0.6 ng/ml at 0 min to a maximum of 53.2 ± 16.0 ng/ml ($P < 0.05$) at 20 min, and then continued above 30 ng/ml until 120 min. The concentrations of GH at 60-150 min were significantly higher ($P < 0.05$) than the corresponding values for GH in the calves given hGRF-44-NH₂. Although the maximum level of GH obtained after this analog injection was similar to that produced by hGRF-44-NH₂ and hGRF(1-29)-NH₂, the area under the GH response curve was 2.5 ($P < 0.05$) and twice ($P < 0.05$) as large as that obtained by hGRF-44-NH₂ and hGRF(1-29)-NH₂, respectively.

Discussion

Our results demonstrate that an analog with D-Ala substitution in position-2 Ala of hGRF(1-29)-NH₂, [D-Ala²]-hGRF(1-29)-NH₂, exhibits the prolongation of GH-releasing activity and a significant increase in potency based on dose per kg of bw compared with that of hGRF-44-NH₂ or hGRF(1-29)-NH₂ in the 12-month-old calves. Similar phenomena were also observed in the lactating cows (Johke *et al.*, unpublished data) and goats (Hodate *et al.*, unpublished data). In the rat, this analog was approximately 50 times more potent than hGRF(1-29)-NH₂ in eliciting GH secretion both *in vivo* (Lance *et al.*, 1984) and *in vitro* (Heiman *et al.*, 1985). The reason for the

increased *in vivo* GH-releasing potency of [D-Ala²]-hGRF(1-29)-NH₂ in the calves is not known, but the increased potency could be ascribed either to the increased biological half-life or the increased affinity of the receptor to this peptide.

Although [D-Tyr¹]-hGRF-44-NH₂ showed prolonged GH-releasing activity compared with hGRF-44-NH₂ in the 4-month-old calves, its potency was similar to that of hGRF-44-NH₂. This result agreed with the *in vivo* result reported previously in 10-month-old calves or wethers (Hodate *et al.*, 1985c) and the *in vitro* result in the rat (Ohashi *et al.*, 1984).

Our results also demonstrate that deletion of 18 amino acids from the C-terminal of hGRF-44-NH₂ causes a significant decrease in the *in vivo* GH-releasing potency in calves, but deletion of 15 amino acids does not reduce the potency. In the rat, deletion of as many as 13 amino acids failed to reduce the *in vivo* GH-releasing activity; however, deletion of 16 amino acids resulted in a significant decrease (Wehrenberg and Ling, 1983). Moreover, it has been reported that hGRF(1-29)-NH₂ is approximately one half as potent as hGRF(1-40)-OH in the *in vivo* assay in the rat (Lance *et al.*, 1984), but has similar potency to hGRF(1-40)-OH when tested *in vitro* with rat pituitary cell cultures (Rivier *et al.*, 1982). In a previous study, we suggested that the amino acid residues above 27 from the N-terminal of hGRF-44-NH₂ are essential for exerting sufficient GRF activity *in vivo* in cattle and sheep (Hodate *et al.*, 1985c). The present results support the above findings, and suggest that the N-terminal 29 amino acid residues of hGRF possess the activity site required for full bovine GH release *in vivo*.

In previous studies, the responses of GH release to hGRF-44-NH₂ in cattle (Johke *et al.*, 1984), goats (Hodate *et al.*, 1985a) and sheep (Hodate *et al.*, 1985a) have been shown to decrease with advancing age. Both the peak level of plasma GH and the area

under the GH response curve for 180 min after injection of hGRF-44-NH₂ in the 4-month-old calves tended to be larger than in the 12-month-old calves, though the differences were not statistically significant in the present experiments. In cattle, it has been reported that the basal GH level and peak level of plasma GH after injection of thyrotropin releasing hormone did not differ significantly between May and October (Johke, 1978). Therefore, the response of bovine GH release to hGRF-44-NH₂ may not be affected by the season.

We conclude that, on the basis of the dose per kg of bw, the *in vivo* GH-releasing potency of hGRF(1-29)-NH₂ is equal to that of hGRF-44-NH₂, and [D-Ala²]-hGRF(1-29)-NH₂ has longer and greater activity than hGRF-44-NH₂ or hGRF(1-29)-NH₂ in stimulating GH release in calves.

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