EFFECTS OF HUMAN GROWTH HORMONE-RELEASING FACTOR ANALOGS ON GROWTH HORMONE RELEASE AND LACTATION IN DAIRY COWS

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Introduction

It is well known that exogenous growth hormone (GH) increases milk production in dairy cows (Peel and Bauman, 1987), and that human growth hormone-releasing factor (hGRF) stimulates specifically GH release in cattle (Johke et al., 1984). Recently, it has been reported that exogenous hGRF stimulated milk production in dairy cows (Enright et al., 1986; Pelletier et al., 1987; Lapierre et al., 1988).

In the present experiments, we studied the effects of subcutaneous (sc) injections of two synthetic hGRF analogs, [D-Ala^2]-hGRF(1-29) NH_2 (DAGRF) and [D-Ala^2, Ala^{15}]-hGRF(1-29) NH_2 (A15-DAGRF), on GH release and lactation in Holstein dairy cows.

Materials and Methods

Experiment 1: Effect of a single sc injection of hGRF analogs on GH release in dry cows

Four dry cows fed at 0830 and 1630 h daily, 6 years of mean age, 735 kg in mean body weight (bw), were injected subcutaneously either 5 ml saline or 2 mg of DAGRF and/or A15-DAGRF in 5 ml saline per cow, at 1130 h. These cows were used repeatedly for each treatment carried cut at intervals of 1-week. Jugular venous blood samples were collected into centrifuge tubes containing heparin via an indwelling catheter at 10-30 min intervals from −0.5 h to 24 h after the injection, and immediately chilled with ice. Plasma samples were obtained after centrifugation and stored at −60°C until assayed for GH.

Experiment 2: Effect of daily sc injections of hGRF analogs on milk production in lactating cows

Sixteen lactating cows (5 years of mean age, 638 kg in mean bw, 184 days of mean stage of lactation) were used. The experiment was carried out over 36 days, which were divided into 3 periods: a preinjection (day 0 to 10), an injection (day 11 to 24), and a postinjection period (day 25 to 35). During the experimental period, the cows were individually fed corn silage, alfalfa hay cube, and concentrate based on milk production prior to the experiment in order to meet nutrient requirements of Japanese Feeding Standard (MAFF) at 0930 and 1930 h daily. The amount of concentrate fed for each cow remained constant, but corn silage fed in sufficient amounts to allow *ad libitum* intake. The cows were milked twice daily at 0830 and 1830 h. Milk samples were collected 9-11 times during the experimental period for composition analyses.

The 16 cows were divided into two groups; Group A and B. Moreover, the 8 cows of Group A or B were divided into two equal groups; control and analog group. In Group A, during 14 consecutive days of the injection period, the cows of the control and the analog group received a daily sc injection of 5 ml saline and 2 mg of DAGRF in 5 ml saline at 1130 h, respectively. In Group B, the cows of the control and the analog group received a daily sc injection of 5 ml saline and 2 mg of A15-DAGRF in 5 ml saline at 1130 h, respectively. Jugular venous blood samples were collected into evacuated tubes containing heparin by venipuncture at 1130 (just before injection) and 1330 h (2 h after injection), and immediately chilled with ice. Plasma samples were obtained as described for Experiment 1.

Measurement of plasma GH and milk components, and statistical analysis

Concentrations of plasma GH were determined

by radioimmunoassay. In Experiment 1, as an index of the amount of GH released in response to saline or hGRF analogs, areas under the GH response curves (AUC) for 24 h after injection were determined. Milk fat, protein, and lactose were measured using an infrared milk analyzer. The t-test was used to assess the significance of the differences in the data.

Results and Discussion

Experiment 1: Effect of a single sc injection of hGRF analogs on GH release in dry cows

The mean plasma GH concentration in saline injected cows varied within the range 1.3 to 4.7 ng/ml. In contrast, the mean concentrations of GH after injection of DAGRF and A15-DAGRF rose to maximum values of 22.0 and 28.3 ng/ml at about 5 h from 1.4 and 1.7 ng/ml at 0 h, respectively. Thereafter, the GH concentrations declined, and returned to the levels before injection after 10-12 h. Mean AUC for hGRF analogs (DAGRF, 7,797.8; A15-DAGRF, 10,549.0 ng·min·ml^{-1}) were 3-4 times ($p < 0.01$) as large as that for saline (2,540.9 ng·min·ml^{-1}).

The present data were resulted by the dose of peptides only one fifth of that employed by Lapierre et al. (1988). The difference between the results obtained by the present experiment and by Lapierre et al. (1988) seems to be the difference between half-life of DAGRF or A15-DAGRF and that of hGRF(1-29)NH_2.

Experiment 2: Effect of daily sc injections of hGRF analogs on milk production in lactating cows

In the cows of Group A and B, during the last 7 days of injection period (day 18 to 24), milk production averaged 22.6 and 19.9 kg/day for the control and increased to 25.1 (+11.1 %; $p < 0.001$) and 23.7 kg/day (+19.1 %; $p < 0.001$) for the analog group, respectively (table 1). Both Group A and B, during the experimental period, percent of fat, protein, and lactose in milk of the analog group did not show significant differences as compared with those for the control. The gross efficiency of milk production increased by DAGRF or A15-DAGRF treatment.

During the injection period, concentrations of plasma GH just before injection in both Group A and B were not significantly different between the control and the analog group. In contrast, in Group A and B, overall mean values of GH at 2 h after injection were 2.5 ($n=8$) and 1.7 ng/ml ($n=6$) for the control and increased to 31.0 ($p < 0.001$) and 11.3 ng/ml ($p < 0.001$) for the analog group, respectively. Moreover, the GH concentrations at 2 h after injection for the analog group in both Group A and B did not show significant differences among the injections. The result suggests that responsiveness of pituitary in the cows received daily sc injections of 2 mg of DAGRF or A15-DAGRF during 14 consecutive days does not decrease. Therefore, during the injection period, the lactating cows of the analog

TABLE 1. MEAN MILK YIELD (kg/d) OF DAIRY COWS DURING PREINJECTION, INJECTION, AND POSTINJECTION PERIODS OF SALINE OR hGRF ANALOGS

		Experimental period			
		Preinjection	Injection		Postinjection
Group	Treatment[1]	D 0 to 10	D 11 to 24	D 18 to 24	D 25 to 35
A	Saline	22.8	22.7	22.6	20.9
	DAGRF	22.7	24.7[a] (+8.8%)	25.1[a] (+11.1%)	21.7
B	Saline	20.4	20.4	19.9	19.6
	A15-DAGRF	20.3	22.8[a] (+11.8%)	23.7[a] (+19.1%)	20.1

[a]Indicate significant difference from Saline within each period. a, $p < 0.001$.
[1]*Saline = 5 ml; DAGRF = 2 mg [D-Ala^2]-hGRF(1-29)NH_2; A15-DAGRF = 2 mg [D-Ala^2, Ala^{15}]-hGRF* (1-29)NH_2 injected daily during injection period.

group in Group A and B probably show the similar GH profiles obtained by the dry cows received a single sc injection of 2 mg DAGRF or A15-DAGRF in Experiment 1.

In conclusion, daily sc injections of 2 mg of DAGRF or A15-DAGRF over 14 days increase milk production in mid- to late lactation cows. The increase of endogenous GH via hGRF analogs seems to mediate these milk production responses.

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(**Key Words**: hGRF Analog, GH, Lactation)

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