Age-Associated Changes in Bovine Growth Hormone Release via Human Growth Hormone-Releasing Factor

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Abstract Responses of growth hormone (GH) release to synthetic human growth hormone-releasing factor-44-NH₂ (hGRF-44) in Holstein dairy cattle of various ages were compared. Blood samples of the experimental animals were taken from an indwelling catheter inserted into one of the external jugular veins before, and during a 90 min period following the injection of hGRF-44 (0.25 µg per kg of body weight). Plasma GH concentrations were measured by radioimmunoassay. Areas under GH response curves for the 90 min period after the hGRF-44 injection were determined and used as an index of the amount of GH released in response to this peptide. The GH releases of the experimental animals were significantly ($P < 0.01$) stimulated by hGRF-44 injection in all cases. The magnitudes of GH release via hGRF-44 were not significantly different between cattle from 6-12 hours to 6 months of age. On the other hand, the response in 12 month-old calves decreased significantly ($P < 0.05$) as compared with that of the 6 month-old calves. Similarly, the response in heifers of 24 months of age decreased significantly ($P < 0.05$) as compared with that of the calves of 12 months of age. Also, the response in 48 month-old dry cows was significantly ($P < 0.05$) lower than that for 24 month-old heifers. These results indicate that somatotrophs of pituitary in just postnatal calves have an ability to respond to exogenous GRF, and pituitaries in cattle above 12 month-old exhibit a certain age-related decline in GH response to exogenous GRF.

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Key words : age-associated change, growth hormone, hGRF-44, cattle

Synthetic human growth hormone-releasing factor-44-NH₂ (hGRF-44) specifically stimulates the release of growth hormone (GH) in several farm animals, i.e. cattle¹⁻³⁾, sheep⁴⁻⁶⁾, goats^{1,5,7)}, pigs^{4,8)} and chickens^{9,10)}. In these animals, generally, there is a decline in the circulating basal level of GH with advancing age¹¹⁻¹⁸⁾. Also, it appears that there is an age-related change in the response to stimuli of GH release. For example, it has been reported that the responses of GH release to exogenous thyrotropin releasing hormone (TRH) in heifers¹⁹⁾ and young fowls^{18,20)} were greater than those in lactating cows and adult fowls, respectively. In previous studies, we also suggested that the GH responses to hGRF-44 in cattle, sheep and goats decrease

with age^{2,5)}. In rats and humans, however, there is still controversy as to whether the response of GH to GRF decreases with age or not²¹⁻²³⁾.

In the present experiments, we studied in order to get further information regarding the influences of age on the responses of GH release to synthetic hGRF-44 and on the basal levels of plasma GH in Holstein dairy cattle.

Materials and Methods

Experimental animals

Holstein dairy cattle from 6-12 hours to 58 months of age from the Institute herd were used. These animals were fed at 0830 h.

Synthetic hGRF-44 for injection

The hGRF-44, synthesized by the solid-phase method and purified by HPLC²⁴⁾, was dissolved in 2-5 ml sterilized saline before use, and administered intravenously to each experimental animal through an indwelling catheter inserted into one of the external jugular veins between 1030 and 1130 h (2-3 hr after feeding).

Blood sampling method

The collections of blood samples from the experimental animals were carried out throughout the year. The jugular venous blood was taken from an indwelling catheter previously inserted into one of the external jugular veins. Serial 3-5 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 radioimmunoassay¹⁹⁾. Areas under the GH response curves for 90 min after hGRF-44 injection were determined and used as an index of the amount of GH released in response to this peptide. The t-test²⁵⁾ was used to assess the significance of the differences in the data.

Basal levels of plasma GH in relation to age

The basal levels of plasma GH in three 1 month-old male calves, 58 kg mean body weight (bw), four 6 month-old female calves, 199 kg mean bw, and four 58 month-old dry cows, 717 kg mean bw, were determined. The blood samples were drawn at 15 to 30 min intervals between 1015 and 1700 h of the day.

GH responses to hGRF-44 in relation to age

The responses of GH release to hGRF-44 (0.25 µg per kg of bw) in six neonates of 6-12 hours of age (3 female and 3 male; 38 kg mean bw), five 7 day-old calves (3 female and 2 male; 40 kg mean bw), eight 1 month-old female calves (59 kg mean bw), seven female calves of 4 months of age (129 kg mean bw), eight 6 month-old female calves (159 kg mean bw), seven 12 month-old female calves (344 kg mean bw), eight heifers of 24 months of age (429 kg mean bw), and six 48 month-old dry cows (672 kg mean bw) were compared. The blood samples were drawn at -15, -10, 0, 5, 10, 15, 20, 30, 45, 60, and 90 min after hGRF-44 injection.

Results

Basal levels of plasma GH in relation to age

The mean plasma GH concentration in the 1 month-old calves varied within the range 6.9 to 30.7 ng/ml (Fig. 1), and the overall mean value was 15.7 ± 1.7 ng/ml (mean $\pm$ SE; $n = 16$). The mean plasma GH concentrations in the cattle of 6 and 58 months of age varied within the ranges 3.5 to 12.3 ng/ml and 2 to 3.9 ng/ml (Fig. 1), and the overall mean values were 5.9 ± 0.6 ng/ml ($n = 19$) and 2.6 ± 0.1 ng/ml ($n = 16$), respectively. The overall mean value of plasma GH in the 1 month-old calves was significantly ($P < 0.001$) higher than that from the calves of 6 months of age. On the other hand, the overall mean value of GH in the cows of 58 months of age was significantly ($P < 0.001$) low compared with that from the 6 month-old calves.

Thus, the basal plasma GH levels decreased with age.

GH responses to hGRF-44 in relation to age

The changes of plasma GH levels in cattle from 6–12 hours to 48 months of age in response to a single intravenous injection of hGRF-44 are shown in Fig. 2. Table 1 shows the areas under the GH response curves for 90 min after injection of the peptide.

The GH releases to hGRF-44 in the experimental animals were significantly ($P < 0.01$) stimulated in all cases. The peak plasma GH values after hGRF-44 injection and the areas under the GH response curves were not significantly different

Fig. 1. Mean plasma GH levels in cattle of 1 month (○), 6 months (●), and 58 months (△) of age. Sampling was at 15 to 30 min intervals between 1015 and 1700 h of the day. Each point with a bar represents the mean $\pm$ SE. The figure in parentheses represents number of determinants.

Fig. 2. Plasma GH levels in response to a single intravenous injection of hGRF-44 ($0.25 \mu\text{g}$ per kg of bw) in cattle of 6-12 hours ($\circ$), 7 days ($\bullet$), 1 month ($\bullet$), 4 months ($\triangle$), 6 months ($\blacktriangle$), 12 months ($\blacktriangle$), 24 months ($\square$), and 48 months ($\blacksquare$) of age. Each point with a bar represents the mean $\pm$ SE. The figure in parentheses represents number of determinants.

Table 1. Areas under GH response curves for 90 min after a single intravenous injection of hGRF-44 ($0.25 \mu\text{g}$ per kg of bw) in cattle from 6-12 hours to 48 months of age.

Age	n	Areas ($\text{ng} \cdot \text{min} \cdot \text{ml}^{-1}$)
6-12 hours old	6	$4,673.4 \pm 1,125.1$
7 days old	5	$3,695.7 \pm 992.1$
1 month old	8	$3,683.5 \pm 683.6$
4 months old	7	$3,231.5 \pm 484.3$
6 months old	8	$4,079.9 \pm 455.0$
12 months old	7	$2,798.7 \pm 247.9^*$
24 months old	8	$1,662.7 \pm 250.1^{***}$
48 months old	6	$745.3 \pm 64.3^{***}$

The value is expressed as the mean $\pm$ SE. Asterisks indicate the statistical difference; $^*P < 0.05$, $^{***}P < 0.001$ as compared with value of 6 months old calves.

between cattle from 6-12 hours to 6 months of age. In contrast, the peak plasma GH values and the areas in the cattle of 12, 24 and 48 months of age were significantly ($P < 0.05$) lower than those obtained by the 6 month-old calves. Similarly, the peak values and the areas in the 24 and 48 month-old cattle were significantly ($P < 0.05$) lower than those from the calves of 12 months of age. The area in the 48 month-old

cows was also significantly ($P < 0.05$) low compared with that obtained by the 24 month-old heifers.

Thus, the responses of GH release to hGRF-44 in cattle above 12 month-old decreased significantly with increasing age.

Discussion

These data demonstrate that the somatotrophs of pituitary in the calves of 6-12 hours of age have an ability to respond to hGRF-44, and the pituitaries in cattle above 12 month-old exhibit a certain age-related decline in the GH response to hGRF-44. The former finding, as far as we know, has not yet been reported in cattle. The latter finding confirms the preliminary results^{2,5)} and extended. The present results also indicate that the basal level of plasma GH in cattle decreases with age. This result agreed with that of previous reports^{11,14,16,17)}.

Since the pituitary in 6-12 hour-old calves exhibited to the responsiveness to hGRF-44 in the present study, the GH secretion in neonatal calves seems to be regulated by the hypothalamic GRF. COXAM *et al.*²⁶⁾ reported that in 3 day-old calves the increase in plasma GH concentration by hGRF-44 was dose-related. In rats, hypothalamic GRF is critical in regulating serum GH concentrations in neonatal animals²⁷⁾. OHMURA *et al.*²⁸⁾ reported that somatotrophs in fetal sheep in mid- and late gestation have receptors for GRF, because hGRF-40 markedly stimulated GH release in fetuses between 71 to 134 days of gestation, and suggested that ovine GH secretion may be modulated by GRF at this stage of gestation. GLUCKMAN²⁹⁾ also reported that the somatotroph in fetal sheep can respond to exogenous GRF from at least 77 days of gestation. It is not known whether somatotrophs in bovine fetuses have an ability to respond to exogenous GRF or not.

The reason for the decline in the bovine GH response to hGRF-44 and/or that in the basal plasma GH level with advancing age is not clear at the present time, but a decrease in endogenous GRF and an increase in somatostatin secretions may occur with advancing age. SHIZUME *et al.*³⁰⁾ demonstrated that there were a significant decrease in content of hypothalamic GRF and a significant increase in somatostatin content in old rats (24 to 28 month-old) as compared with those obtained in young rats (3 month-old), and that the secretion of hypothalamic GRF in the old rats decreases significantly as compared with that from the young rats, and suggested that an age-related decline in rat GH secretion may be due to decreased hypothalamic GRF secretion. In addition, as other possible explanations, it may be that the sensitivity of bovine pituitary to GRF or somatostatin, i.e. number and affinity of GRF or somatostatin receptor, changes with age; the sensitivity of pituitary to GRF decreases, and the sensitivity to somatostatin increases with age. In rats, CEDA *et al.*³¹⁾ observed that both *in vivo* and *in vitro*, the responses of GH release to hGRF-44 in young animals (2 to 5 month-old) are larger than in aged animals (14 to 18 month-old), and suggested that the age difference in GRF responsiveness may be due to both hypothalamic and pituitary influences. Furthermore, an age-related decline in

bovine GH synthesis in the pituitary may occur. In cattle, it has been reported that the pituitary GH content or its secretion per unit of bw decreased with increasing age and/or body size^{11,13)}.

In the present study, neither the responsiveness of bovine GH to hGRF-44 nor the basal plasma GH level difference of sex was observed in the calves of 6-12 hours, 7 days, and 1 month of age. It has been reported that sex of the calf did not influence the basal level of serum GH during the week after birth³²⁾. The collections of blood samples in the present experiments were carried out throughout the year. In previous studies, it has been reported that both the basal plasma GH level and the GH response to exogenous TRH in the bovine were not significantly affected by season or environmental temperature^{19,33,34)}. In goats, it has been also reported that the concentration of GH in the blood remained relatively constant throughout the year, and that no correlation was found between the average weekly concentration of GH and the changes in air temperature and daylength throughout the year³⁵⁾. Therefore, the data obtained in the present study seem to be not significantly influenced by sex of the experimental calves below 1 month-old and the season of blood sampling.

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人成長ホルモン放出因子による牛の成長ホルモン放出 におよぼす年齢の影響

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合成人成長ホルモン放出因子 (hGRF-44) による牛成長ホルモン (GH) 放出反応を比較した。hGRF-44 (体重 kg 当り 0.25 μ g) の静脈注射前後の血液を頸静脈カテーテルから経時的に採取し，血漿 GH 濃度をラジオイムノアッセイで測定した。また，hGRF-44 投与後 90 分間の GH 反応曲線下の面積を測定し，このペプチドに反応して放出された GH の総分泌量の指標とした。hGRF-44 注射後，いずれの場合においても，有意な牛 GH 放出反応が観察された。6-12 時間齢から 6 カ月齢の子牛における hGRF-44 に対する GH 放出反応は，各群間で統計的に有意な違いを示さなかった。これ

に対して，12 カ月齢子牛の反応は 6 カ月齢子牛の反応に比べ有意に減少した ($P < 0.05$)。同様に，24 カ月齢の未経産牛の反応は 12 カ月齢子牛の反応より有意に小さく ($P < 0.05$)，さらに，48 カ月齢の乾乳牛の反応は 24 カ月齢未経産牛の反応に比べ有意に減少した ($P < 0.05$)。これらの結果は，1) 牛下垂体の GH 分泌細胞は出生直後でも外因性の GRF によく反応する機能を持つこと，2) 12 カ月齢以降の牛下垂体の外因性 GRF に対する反応性は加齢に伴って減少することを示している。

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