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Growth hormone and insulin-like growth factor I concentrations in bulls of various growth hormone genotypes

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Abstract A leucine/valine substitution at amino acid position 127 was identified by the polymerase chain reaction and restriction fragment length polymorphism in the bovine growth hormone gene. Genotyping was performed in 84 AI bulls of three different breeds, in which plasma concentrations of growth hormone (GH) and insulin-like growth factor I (IGF-1) were also measured. Gene frequencies of variants L (leucine) und V (valine) were 0.80/0.20 (Black and White), 0.90/0.10 (Brown), 0.71/0.29 (Simmental). Hormone concentrations were measured during different physiological conditions (normal feeding, fasting, realimentation) in the majority of animals. Generally, genotype LL was associated with higher concentrations of GH than LV. This difference was significant in Black and White bulls ($P < 0.05$). In contrast, IGF-1 concentrations were higher in LV than in LL animals. This was most pronounced in mature, realimented Simmental bulls. We conclude that the various GH alleles influence the circulating concentrations of GH and IGF-1.

Key words Growth hormone · Insulin-like growth factor I · Restriction fragment length polymorphism · Polymerase chain reaction

Introduction

The use of detectable markers, such as genetic polymorphisms, in breeding programmes could render the selection of animals more accurate and efficient. Plasma growth hormone concentrations were elevated in cows selected for high milk yield in contrast to those selected for low milk yield or to control animals (Kazmer et al. 1986; Klemetsdal et al. 1991). Robinson et al. (1992) reported positive correlations between GH levels and breeding values in dairy bulls but negative relations were found by Kazmer et al. (1991). As for growth characteristics, both positive and negative relations have been reported (Wheaton et al. 1986; Ohlson et al. 1987; Arthur et al. 1990). The plasma level of insulin-like growth factor I was found to be positively correlated with weight and daily gain (Groenewegen et al. 1990; Davis and Bishop 1991). Ronge et al. (1988) described positive correlations between the energy and protein balances and IGF-1 in cows. High concentrations of GH were associated with low concentrations of IGF-1 and a high milk yield (Schams et al. 1991a). In an experiment with lactating Holstein cows, recombinant-derived bovine GH variants with valine at amino acid position 127 elicited a greater milk response than did leucine GH variants (Eppard et al. 1992). Up to now, the relations between GH genotypes and GH and IGF-1 concentrations have not been investigated. The scope of the present study was to analyze relations between the two alleles at amino acid position 127 and concentrations of GH and IGF-1 in bulls. The substitution of valine for leucine at amino acid position 127 (Seavey et al. 1971) was genotyped by the polymerase chain reaction and by restriction fragment length polymorphism.

Materials and methods

Animals and blood sampling

Blood samples were collected at five sampling periods from 84 unrelated bulls (23 German Black and White, 20 Bavarian and Tyrolean Brown, 41 Simmental) located in an Austrian and four German AI stations. Plasma GH concentrations were measured in 497 samples of 79 bulls and IGF-1 concentrations in 467 samples of 56 bulls. Blood samples of 54 animals were taken in normal feeding status, after 3-days food restriction, and at day 4 after refeeding. Samples were taken three times a day (11 am, 2 pm, 5pm) or at 15-min intervals between 7 am and 5 pm. Plasma GH was determined from each sample, and IGF-1 every 150 min when more samples were available. The concentrations of the 11 am, 2 pm, and 5 pm samples

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were taken for statistical evaluation. From 30 normally-fed bulls, 8–23 measurements were available throughout periods of 22–78 days. Out of these, one value was randomly chosen and used for analysis. To avoid bleeding stress, a vinyl catheter was inserted under epidural anaesthesia into either the vena cava caudalis or the aorta abdominalis of 13 bulls as described by Walters et al. (1984). Concentrations of hormones were compared with those of all other animals, in which blood was taken after a puncture of the vena jugularis. Blood was withdrawn into 10-ml syringes prepared with EDTA and heparin. After cooling in an ice water bath for 10 min, samples were centrifuged at 4 °C with 5 000 rpm. Plasma was stored < − 18 °C until analysis. Procedures for the collection of blood and its storage were as described by Walters et al. (1984).

Hormone determination

Hormone concentrations were determined by earlier-described and validated radioimmuno-assays (Schams et al. 1991b) having a lower limit of detection of 0.5 ng/ml for GH and 30 ng/ml for IGF-1. The intra-assay coefficient of variation was less than 9% for both hormones, the inter-assay coefficient of variation was 8.8 ± 2.5% for IGF-1 (n = 6) and 11.7 ± 3.2% for GH (n = 11), respectively.

Genotyping of bulls

Using the polymerase chain reaction, primers BGH1 (5′-GCTGCTCCTGAGGGCCCTTCG-3′) and BGH2 (5′-GCGGCGG CACTTCATGACCCT-3′) amplified a 223-bp fragment spanning intron IV and exon V of the GH gene. DNA was amplified in a total volume of 100 µl (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 100 µM of each dNTP, 2 U *Taq* DNA polymerase, 20 pMol of primers BGH1 and BGH2) for 30 cycles at the following temperatures: denaturation 94 °C, 60 s; primer annealing 60 °C, 60 s; primer extension 72 °C, 60 s. Restriction endonuclease digestion with 15 units of *Alu*I for 3 hours and electrophoretic separation on a 3% agarose gel led to fragments of 171 + 52 bp (LL), 223 + 171 + 52 bp (LV) and 223 bp (VV).

Statistical analysis

As the age of bulls influences the hormone concentration, statistical analyses were performed separately for young (< 18 months) and mature (> 18 months) bulls based on the following model:

$$Y_{ikl} = \mu + \text{genotype}_i + \text{group}_j + \text{bull}_{ijk} + e_{ijkl},$$

where group contains the factors breed, AI station, and month of sampling. When animals of a single breed are analysed, group stands for AI station and month of sampling. Bulls are nested within genotype and group. Three values per day and bull were included. The young Black and White bulls were located in one AI station, where sampling was done in one period and one blood sample per day was collected out of which only GH was determined. Therefore, for this group only genotype and bull effects can be discerned and the latter serve as error terms. IGF-1 values were available for mature Black and White bulls of one AI station. Interactions between genotype and group were not significant and were not incorporated into the model.

Results

The determination of the three growth hormone genotypes LL, LV and VV (Fig. 1) permitted an estimation of the frequencies for alleles L and V: German Black and White 0.80, 0.20 (n = 23), Bavarian and Tyrolean Brown 0.90, 0.10 (n = 20) and Simmental 0.71, 0.29 (n = 41), respectively. The genotype distributions indicated

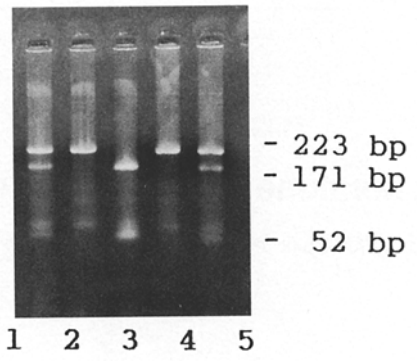

Fig. 1 Identification of growth hormone genetic variants using PCR and *Alu*I RFLP. *Lane 1*: leucine, valine (LV); *lane 2*: valine, valine (VV); *lane 3*: leucine, leucine (LL); *lane 4*: valine, valine (VV); *lane 5*: leucine, valine (LV)

Hardy-Weinberg equilibrium. Three-days severe feed restriction after normal feeding tended to depress concentrations of GH and IGF-1, while realimentation seemed to increase the level of both hormones. A comparison between the two more frequent genotypes showed that in 10 of the 13 cases (age, breed, physiological state), genotype LL was associated with higher *GH* concentrations than genotype LV (Table 1). Significance ($P < 0.05$) is reached in mature Black and Whites and is approached ($P < 0.06$) in young bulls of this breed. The difference is reversed in mature Brown bulls (normal feeding, realimentation) and in mature fasted Simmentals, but the LL-LV difference is considerable with values of + 1.15 to + 1.93 ng/ml in a total of five cases. Due to the low frequency of allele V, only four VV homozygote carriers could be identified in Simmental bulls which had slightly higher IGF-1 concentrations than LL and LV genotypes. Since they where distributed in three different groups an estimation of the effect of genotype VV on both plasma GH and IGF-1 concentrations is not possible. A comparison of IGF-1 concentrations between the two frequent GH genotypes showed higher values for LV than for LL in 11 of the 12 cases considered with only one exception (young, realimented Simmentals). The difference approaches significance ($P < 0.09$) in realimented, mature Simmental bulls. Large differences of − 305 to − 101 ng/ml occurred in seven cases (Table 2). Concentrations of both GH and IGF-1 were also influenced by group (AI station, month of sampling, breed) and bull effects within genotypes and groups. Bulls with an LL genotype usually revealed higher GH and lower IGF-1 concentrations than those with an LV genotype.

Discussion

In all three breeds investigated, allele L was found in a higher gene frequency than V, which agrees with the data of US Holstein Friesians (Lucy et al. 1991) and of Holsteins, Herefords, Aberdeen Angus from Japan but

Table 1 Differences in the GH hormone concentrations in GH genotypes of bulls

Type	Physiological status[a]	Number of animals (LL/LV)	Number of samples (LL/LV)	Mean value ng/ml	Difference (LL – LV) ng/ml
Young black and white	N	6/3	6/3	2.20	$1.15 \pm 0.58^{[*]}$
Mature black and white	N	8/6	12/12	3.05	$1.70 \pm 0.35^{(*)}$
	F	2/3	6/9	2.79	0.54 ± 0.41
	R	2/3	6/9	3.73	0.37 ± 0.79
Mature brown	N	12/3	36/9	2.64	-1.23 ± 0.73
	F	12/3	36/9	2.59	0.08 ± 0.58
	R	12/3	36/9	2.82	-0.53 ± 0.56
Young simmental	N	4/5	12/15	8.24	1.83 ± 1.42
	F	4/5	12/15	7.92	0.24 ± 2.63
	R	4/5	12/15	5.21	1.93 ± 2.03
Mature simmental	N	17/11	51/33	4.67	0.14 ± 0.64
	F	17/11	51/33	4.17	-0.35 ± 0.66
	R	17/11	51/33	4.25	1.44 ± 0.92

[a] N, normal feeding; F, fasting; R, realimentation
$^{[*]}$ $P < 0.06$
$^{(*)}$ $P < 0.05$

Table 2 Differences in the IGF-1 hormone concentrations in GH genotypes of bulls

Type	Physiological status[a]	Number of animals (LL/LV)	Number of samples (LL/LV)	Mean value ng/ml	Difference (LL – LV) ng/ml
Mature black and white	N	2/3	6/9	847	-280 ± 78
	F	2/3	6/9	750	-149 ± 45
	R	2/3	6/9	687	-201 ± 58
Mature brown	N	12/3	36/9	1437	-41 ± 51
	F	12/3	36/9	1383	-62 ± 45
	R	12/3	36/9	1248	-101 ± 52
Young simmental	N	4/5	12/15	1126	-149 ± 41
	F	4/5	12/15	833	-58 ± 30
	R	3/5	9/15	920	94 ± 52
Mature simmental	N	17/11	51/33	1166	-98 ± 44
	F	17/11	51/33	1106	-287 ± 41
	R	17/11	51/33	1140	$-305 \pm 39^{[*]}$

[a] N, normal feeding; F, fasting; R, realimentation
$^{[*]}$ $P < 0.09$

not of Japanese Blacks (Chikuni et al. 1991). We can offer no explanation why allele L is predominant. Animals with LL genotypes generally showed higher concentrations of growth hormone than those with LV in all groups, breeds and physiological states, and significance was achieved in the mature Black and White bulls. The results indicate that genotype LL is associated with higher levels of GH than genotype LV. Moreover, recombinant-derived GH with valine at position 127 elicited a greater milk response than did the leucine variant (Eppard et al. 1992). The genetic polymorphism in cattle at amino acid position 127 reflects a single-base mutation. Further linked polymorphisms possibly exist in regions that are involved in gene regulation. In mice, divergent selection for body weight revealed a distinct growth hormone haplotype in lines selected for higher body weight and higher growth rate. Four polymorphic loci were identified where variations mainly appeared in the 5′ flanking region, with one polymorphism in the structural gene (Salmon et al. 1988). A similar case of quantitatively different gene products was found in milk protein genotypes where β-lactoglobulin allele B produces less β-lactoglobulin than allele A (Graml et al. 1989). The observed inverse relationship of insulin-like growth factor I, i.e., higher concentrations in genotype LV than in LL, seems to reflect the antagonism of GH and IGF-1 also found in lactating dairy cows (Ronge et al. 1988; Schams et al. 1991a) and can be considered as another effect associated with the GH polymorphism. Ronge et al. (1988) pointed out both the positive correlation of IGF-1 to energy and protein balance and the negative correlation to milk yield. IGF-1 was positively related to daily gain in male calves (Groenewegen et al. 1990) and to body weight in heifers (Davis and Bishop 1991). It has not yet been established whether the LL, LV and VV GH genotypes have different effects on milk or beef production. However, there are indications that genotype LV is associated with a higher breeding value for daily gain than either LL or VV in Simmental bulls (Schlee and Graml, unpublished).

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