Neuroendocrine control of growth hormone release in sheep: Effect of dose and route of administration of growth hormone releasing factor on plasma growth hormone levels.

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ABSTRACT

There has been considerable interest recently in the use of growth hormone (*somatotrophin*) to enhance growth, carcass leanness and milk yield in a number of species. Despite the similarity of bovine and ovine growth hormone, the administration of exogenous somatotrophin to lambs has produced disappointing results. Conceivably, stimulation of endogenous GH secretion may be more beneficial, and in this communication we present our preliminary results on the effective doses and rates of administration of growth hormone releasing factor in a study of the neuroendocrine regulation of growth hormone secretion in sheep. Sheep were housed indoors on a complete pelleted diet and were fitted with jugular vein catheters. Some sheep were also fitted with cannulae placed in a lateral ventricle of the brain. Bovine GRF was given at different doses to the sheep either intravenously (*iv*), intracerebroventricularly (*icv*) or intramuscularly (*im*) and plasma GH levels monitored. There was a dose-dependent increase in GH levels when the GRF was given *iv* with both 30 and 3 μg being effective, while im administration was only effective at 30 μg. Central administration (*icv*) was also effective at both doses but did not stimulate GH release at 300 ng. Further studies are required to elucidate the mechanisms underlying GRF stimulation of GH release, but the efficacy of GRF as a growth promoter can be investigated using intramuscular administration.

Keywords Sheep, GRF, GH, intravenous, intramuscular, intracerebral.

INTRODUCTION

Growth hormone releasing factor (GRF) has been isolated and sequenced from a number of species (see Della-Fera *et al.*, 1986). It occurs naturally as a 40 or 44 amino-acid form and appears to be similar across species; in particular, the 1-29 sequence (the part containing the active moiety) is very highly conserved.

Recently, the availability of large amounts of recombinantly produced bovine somatotrophin (BST) has generated considerable interest and research effort into the potential of BST to enhance growth, carcass leanness and milk yields in a number of species. However, despite the similarity of the bovine and ovine growth hormones, the result of BST administration to sheep has produced disappointing results (see Spencer, 1986). Possibly sheep have very sensitive feedback control mechanisms; or perhaps the subtle differences between bovine and ovine GH (3 amino-acid residues in 191) are particularly important, as indeed are the 2-3 amino-acid differences between ovine and bovine prolactin (Wallis, 1989). Alternatively, as BST may show up to 10% difference in composition from endogenous bovine growth hormone, this may be of particular importance when changing species, especially when the changes occur in the N-terminal sequence (Wallis, 1989). It seemed possible that stimulation of endogenous GH production may provide a better way of enhancing the GH mediated growth responses in sheep and a clearer understanding of the neuroendocrine control of GH is necessary to be able to exploit this potential.

The studies reported here are part of a programme of research investigating neuroendocrine regulation of GH in sheep and in this communication we present our preliminary studies on the effective doses and routes of administration of GRF in lambs.

MATERIALS AND METHODS

Forty five sheep (30-38 kg liveweight) were individually housed in crates indoors and fed a complete pelleted diet. After adaptation to these conditions the animals

were placed on a restricted ration of 450g/d (approximately 1.5% body weight). This amount is a maintenance ration and has previously proved sufficient to elevate basal GH levels slightly without producing malnutrition, stress or any other effects that may interfere with the study.

On the days of study, the sheep were blood sampled via jugular vein catheters at 15 min intervals for 1 h prior to treatment and for 90 min following GRF administration at various doses via one of three routes of administration: intravenously (*iv*), intramuscularly (*im*) or intracerebroventricularly (*icv*). Blood samples were collected at 15 min intervals for 90 min after GRF administration into heparinised tubes, centrifuged and plasma stored for GH estimations using radioimmunoassay (Spencer *et al.*, 1990).

INTRAVENOUS GRF

Eleven sheep were given GRF (bovine growth hormone releasing factor 1-29NH^2; Sigma Chemical Co.) intravenously in 100 µl saline and flushed in with 5 ml saline. Seven sheep received 30 µg GRF (approximately 1 µg/kg; 0.3 nmol/kg); four sheep received 3 µg (0.03 nmol/kg) GRF.

INTRAMUSCULAR GRF

Eight sheep were used in this part of the trial; four animals receiving 30 µg im in the shoulder and four getting 3 µg.

INTRACEREBROVENTRICULAR GRF

26 sheep were fitted with both jugular vein catheters and with indwelling intracerebroventricular cannulae positioned in a lateral ventricle (for details see Spencer *et al*, 1990). Eight sheep received 300 ng GRF in 100µl saline directly into a lateral ventricle; five received 3 µg, seven received 30 µg in 100 µl saline and six saline alone.

Statistical analysis was made using paired t-test to assess the differences between mean pre-treatment and the post-treatment levels of GH.

RESULTS

Intravenous administration of GRF resulted in a dose-dependent increase in plasma GH levels by 15 min after treatment (Figure 1). The elevation in GH was not only greater, but also more prolonged following 30 µg compared with the response to 3 µg. Evenso, 3 µg produced a significant elevation in GH ($P<0.05$) and a doubling in the mean post-treatment GH levels compared with the mean pre-treatment levels. In the case of 30 µg there was almost a ten-fold increase in the mean GH levels (Table 1).

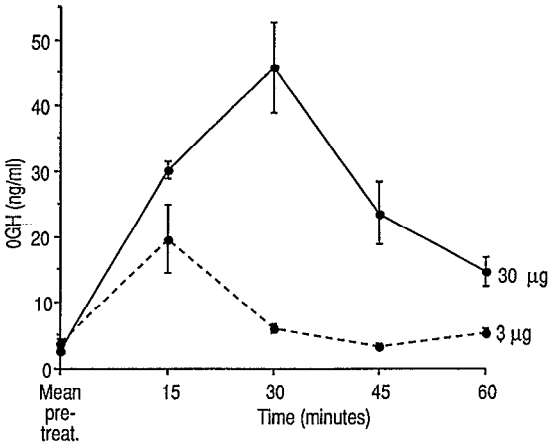

FIG 1 Change in ovine growth hormone following intravenous administration of bovine GRF 1-29 at a dose of 3 µg (0.1µg/kg) or 30 µg (1 µg/kg).

TABLE 1 Mean plasma growth hormone levels (ng/ml) in sheep in the 1 hour immediately preceding treatment and the one hour after treatment with bovine GRF 1-29 either intravenously (*iv*), intramuscularly (*im*) or intracerebroventricularly (*icv*). Values are mean ± sem; *=$P<0.05$, **= $P<0.01$ by paired t-test.

Treatment	N	Mean basal level	Mean Post-treatment level
3 µg *iv*	4	3.77 ± 0.88	7.00 ± 1.18 *
30 µg *iv*	7	2.53 ± 0.39	23.70 ± 5.10 **
3 µg *im*	4	2.72 ± 0.70	2.65 ± 0.50
30 µg *im*	4	2.00 ± 0.11	4.77 ± 2.20 *
0.3 µg *icv*	8	1.65 ± 0.21	2.14 ± 0.36
3 µg *icv*	5	3.16 ± 0.84	6.45 ± 2.02 *
30 µg *icv*	6	2.44 ± 0.22	6.25 ± 1.23 *

Following intramuscular administration of GRF

there was a small but significant elevation in plasma GH concentration with 30 μg, at 15 min and a doubling in mean post-treatment levels ($P<0.05$), but there was no discernible change in GH levels with 3 μg given via this route (Figure 2).

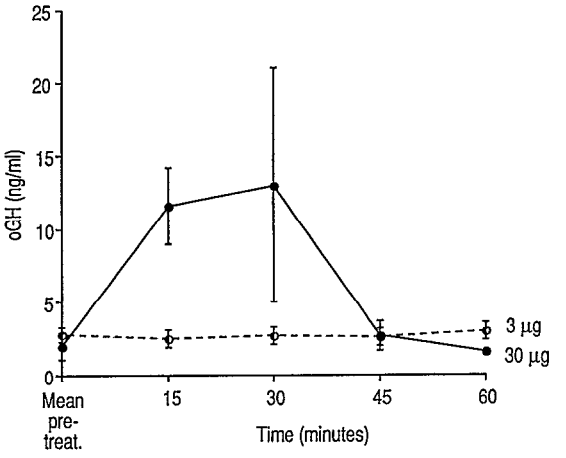

FIG 2 Change in ovine growth hormone following intramuscular administration of bovine GRF 1-29 at a dose of 3 ug (0.1μg/kg) or 30 ug (1 μg/kg).

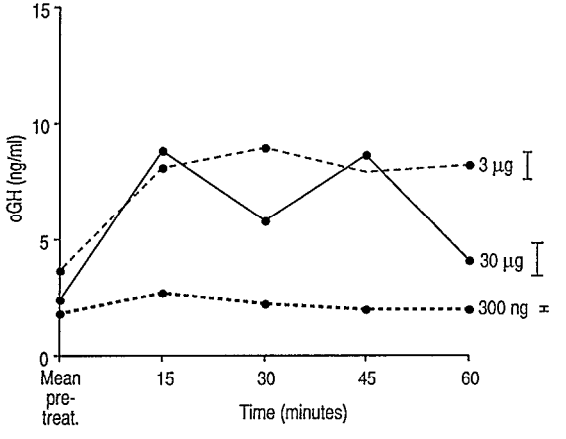

FIG 3 Change in ovine growth hormone following intracerebroventricular administration of bovine GRF 1-29 at 300 ng, 3 ug or 30 ug. The mean standard errors for each treatment are shown at the right on each line.

Intracerebroventricular administration of GRF produced a result intermediate between *iv* and *im* administration (Table 1). In contrast to the response to *im* administration of GRF, a dose of 3 μg given *icv* stimulated an increase in plasma GH levels. However the response was not as large as that obtained with a similar dose given *iv*. At the higher dose (30 μg) there was no greater increase in circulating GH levels than with 3 μg given by this route (Figure 3). Administration of saline or 300 ng GRF resulted in no significant alteration of plasma GH levels.

DISCUSSION

The results of these studies show that the 1-29 sequence of bovine GRF is capable of stimulating GH release in a dose-dependent fashion when given intravenously to sheep. The 1-29 GRF was able to produce a significant stimulation of GH secretion at 0.03 nmol/kg in these sheep. Using bGRF 1-40 sequence, Della-Fera *et al.* (1986) were unable to demonstrate an effect at a similar (gravimetric) dose, although human pancreatic GRF 1-40 did stimulate a GH increase in the same study.

Human pancreatic GRF1-40 was found to have a similar effective minimum dose whether given *iv* or subcutaneously (Della-Fera *et al.*, 1986), although the magnitude of the response was greater with *iv* administration. In the present trials, 0.03nmol/kg given *im* was ineffective in stimulating GH release, although the same dose was clearly effective *iv*. This demonstrates that *im* administration of GRF is an effective route for stimulating GH release in sheep, but suggest that higher doses may be needed than might be indicated from *iv* studies to date.

It is interesting that the *icv* administration was less effective than either im or iv routes. It might have been expected that concentrating the GRF close to the hypothalamus would allow a much greater concentration to reach the pituitary. Thus it might be expected that lower doses of GRF may be effective when given this way. This is clearly not the case, as the stimulation of GH secretion by 30 ug GRF was less *icv* than via the other routes, and 3 μg was less effective when given *icv* than *iv*. A ten-fold lower dose was completely ineffective. For some other peptides it has also been shown that icv administration is not as effective as expected, but most of those studies have been undertaken in the rat

(Lumpkin *et al.*, 1980; Murikama *et al.*, 1987). It would appear that elevated plasma levels of GRF are needed to induce an elevation of GH levels, but central levels of GRF may be unimportant.

In conclusion, it seems that intramuscular administration of the 1-29 sequence of bovine GRF is effective in stimulating GH release in sheep, but whether this would prove to be a potentially useful way of enhancing growth remains to be demonstrated.

ACKNOWLEDGEMENTS

We would like to thank P. Dobbie, P. Fowke and R. Lasenby for help with the preparation and sampling of the animals; S. Johnston and C. Berry for help with the GH assay, and C. Harris and M. Holmes for animal care.

REFERENCES

Della-Fera, M.A.; Buonomo F.C.; Baile, C.A.; 1986 Growth hormone releasing factors and secretion of growth hormone in sheep, calves and pigs. *Domestic Animal Endocrinology 3*: 165-176.

Lumpkin, M.D.; Negro-Vilar, A.; McCann, S.M.; 1980 Paradoxical elevation of growth hormone by intraventricular somatostatin: possible ultrashort-loop feedback. *Science 211*: 1072-1074.

Murakami, Y.; Kato, Y.; Kabayama, Y.; Inoue, T.; Koshiyama, H.; Imura, H.; 1987. Involvement of hypothalamic growth hormone (GH)- releasing factor in GH secretion induced by intracerebroventricular injection of somatostatin. *Endocrinology 120*: 311-316.

Spencer, G.S.G. 1986. Biotechnology in the potential practical application of somatotrophic hormones for improving animal performance. *Reproduction Nutrition, Development 27(2B)*: 581-589.

Spencer, G.S.G.; Bass, J.J.; Hodgkinson, S.C.; Edgely, W.H.; Moore, L.G.; 1990 Effect of intracerebroventricular injection of IGF-1 on circulating growth hormone levels in the sheep. *Journal of Endocrinology* (submitted for publication)

Wallis, M. 1989. Species specificity and structure-function relationships of growth hormone. In: *Biotechnology in Growth Regulation.* Eds. R.B. Heap, C.G. Prosser, G.E. Lamming. Butterworths. London. pp3-14.