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Response of growth hormone to various doses of growth hormone releasing factor and thyrotropin releasing hormone administered separately and in combination to dairy calves

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Løvendahl, P., Woolliams, J. A. and Sinnett-Smith, P. A. 1991. **Response of growth hormone to various doses of growth hormone releasing factor and thyrotropin releasing hormone administered separately and in combination to dairy calves.** Can. J. Anim. Sci. **71**: 1045–1052. Doses of growth hormone releasing factor (GRF) and thyrotropin releasing hormone (TRH) and combinations of these were administered by intravenous injection to six calves aged 155 ± 3 days and weighing 136 ± 16 kg. Injections were at 09:00, 12:00 and 15:00 h on 4 days, and doses were 0, 15, 30 and 60 pmol GRF kg^{-1} and 0, 275, 550 and 1100 pmol TRH kg^{-1}, with GRF plus TRH at all combinations of these doses. Response of serum growth hormone (GH) was measured as the mean at 5, 10, 15 and 20 min following injection (PEAK) and the area under the curve during 0–60 min (AUC). The correlation between PEAK and AUC was 0.98. The variation in PEAK was related to GH prior to injection and to PEAK 3 h earlier. Separate multiplicative effects for each secretagogue were fitted, with the effects related to the logarithm of dose. Doubling the dose increased PEAK by 1.46-fold following GRF ($P < 0.05$) and 1.25-fold following TRH ($P < 0.05$). There was no evidence that the results for either secretagogue were affected by the presence or absence of the other. This multiplicative model provides a description of the synergy between these secretagogues.

Key words: GH-release, GRF, TRH, calves, dose response

Løvendahl, P., Woolliams, J. A. et Sinnett-Smith, P. A. 1991. **Réponse de l'hormone de croissance (GH) à diverses doses de somatocrinine (GRF) et de thyrolibérine (TRH) administrées séparément ou en association à des veaux laitiers.** Can. J. Anim. Sci. **71**: 1045–1052. Diverses doses, simples et combinées, de GRF hypothalamique et de TRH ont été administrées par voie intraveineuse à six veaux de 155 ± 3 j et pesant 136 ± 16 kg. Les injections étaient faites à 09:00, 12:00 et 15:00 h pendant 4 jours de suite, aux doses de 0, 15, 30 et 60 pmol kg^{-1} pour la GRF et de 0, 275, 550 et 1100 pmol kg^{-1} pour la TRH, en plus de toutes les combinaisons de doses des deux hormones. Pour mesurer la réponse de l'hormone de croissance (GH), on a pris la moyenne des réactions à 5, 10, 15 et 20 minutes après l'injection (PIC), ainsi que la surface comprise sous la courbe de 0 à 60 minutes (AUC). La corrélation entre les valeurs PIC et les valeurs AUC était de 0,98. On a établi les relations entre les variations des valeurs PIC et les valeurs pré-injection, ainsi que les valeurs PIC de l'injection précédente (3 h plus tôt). Les effets multiplicatifs séparés des deux sécrétagogues ont été ajustés en regard du logarithme de la dose. Le doublement de la dose a eu pour effet d'accroître les valeurs PIC de 1,46 fois après injection de GRF ($P < 0,05$) et de 1,25 fois après injection de TRH ($P < 0,05$). Rien n'indique que les résultats observés pour chaque hormone aient été affectés par la présence ou l'absence de l'autre. Ce modèle multiplicatif fournit une description du synergisme des deux sécrétagogues.

Mots clés: Décharge de GH, GRF (somatocrinine), TRH (thyrolibérine), veaux, réponse à la dose

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Growth hormone (GH) has important regulatory roles during growth, pregnancy and lactation, but the mechanisms controlling somatotrophs in the pituitary are poorly understood. The release of GH from the anterior lobe of the pituitary is promoted by growth hormone releasing factor (GRF) which in its native form in cattle is a 44 amino acid peptide hormone (Esch et al. 1983; Baile and Buonomo 1987). The sequence of the bovine and the human form shows only one amino acid difference within the first 29 amino acids (Baile and Buonomo 1987). A synthetic peptide containing these 29 amino acids has a GH-releasing potency equal to that of the full-length peptide (Petitclerc et al. 1987).

GH release is also induced in cattle by thyrotropin releasing hormone (TRH) (Johke 1978). Further, a synergistic effect between GRF and TRH on GH release has been reported in cultured bovine pituitary cells (Ingram and Bicknell 1986) and in vivo in lactating cows (Lapierre et al. 1987a) and calves (Hodate et al. 1985), although its physiological importance is unknown. Although this synergy is not affected by photoperiod, it is not evident if GRF and TRH are administered in darkness (Lapierre et al. 1987b).

Dose–response relationships for GRF have been demonstrated in calves (Johke et al. 1984; Della-Fera et al. 1986; Enright et al. 1987), and only limited information is available on the dose response to TRH (Johke 1978; Hedlund et al. 1977). Experiments testing synergy between GRF and TRH have used only single doses of each hormone, thus although the synergistic effect has been established, there is no information on the comparative dose–response relationships of GH release with GRF and TRH in the presence or absence of the synergy. This study investigated such relationships with the long-term objective of obtaining greater understanding of the mechanisms that control the release of GH.

MATERIALS AND METHODS

Treatments and Procedures

ANIMALS. Six British Friesian calves, five females and one male, aged 155 ± 3 days and weighing 136 ± 16 kg were penned individually in a well-ventilated barn with fluorescent tube lights during experimental days. The calves consumed $3-4$ kg d^{-1} concentrate (g kg^{-1}: barley 749, soya-bean meal 125, molasses 100, salt and mineral mixture 26) to appetite. Water and hay were available ad libitum throughout.

Synthetic human pancreatic growth hormone releasing factor fragment (1–29) NH_2 (peptide purity > 98%; Bachem, Saffron-Walden, Essex, United Kingdom) was used (henceforth abbreviated to GRF) at doses of 0, 15, 30 and 60 pmol kg^{-1} body weight (denoted G_0, G_1, G_2 and G_3, respectively). Thyrotropin releasing hormone (TRH, Sigma Chemical Company, Poole, Dorset, United Kingdom) was used at doses of 0, 275, 550 and 1100 pmol kg^{-1} body weight (denoted T_0, T_1, T_2 and T_3, respectively). Treatments of GRF plus TRH were at all combinations of these doses. GRF and TRH were dissolved in sterile physiological saline. Saline was also used as vehicle and placebo in a volume of 5 mL per intravenous injection. Twelve of the 16 treatments were allocated to each of the six animals as shown in Table 1.

In the absence of any direct experimental evidence, the decision to give multiple injections to only a few calves was made because previous studies with another pituitary releasing hormone (LH–RH) had shown large variation but with a repeatability of 0.5 (Land 1981). The treatments were given in random order with three injections daily, at 09:00, 12:00 and 15:00 h on four consecutive days.

An indwelling jugular cannula was placed in each animal 1 day prior to the start of the treatments, through which all treatments were given as bolus injections, and blood samples were taken. Samples were taken at -15, -5, 5, 10, 15, 20, 30, 60, 90 and 120 min relative to each injection. The cannulae were kept patent with sterile Na-citrate (4.5% wt vol^{-1}). After centrifugation ($2000 \times g$, 4°C, 20 min), serum was harvested and stored at -20°C until assayed.

Table 1. Allocation of calves to treatments

Calf	Treatment[z]
1	$(G_0, G_1, G_2, G_3) \times (T_0, T_1, T_2)$
2	$(G_0, G_1, G_2, G_3) \times (T_0, T_1, T_3)$
3	$(G_0, G_1, G_2, G_3) \times (T_0, T_2, T_3)$
4	$(G_0, G_1, G_2) \quad \times (T_0, T_1, T_2, T_3)$
5	$(G_0, G_1, G_3) \quad \times (T_0, T_1, T_2, T_3)$
6	$(G_0, G_2, G_3) \quad \times (T_0, T_1, T_2, T_3)$

[z] $(...) \times (...)$ denotes all combinations, e.g., $(G_0, G_1) \times (T_0, T_1) = (G_0T_0, G_0T_1, G_1T_0, G_1T_1)$. G_0, G_1, G_2 and G_3 denote GRF at 0, 15, 30 and 60 pmol kg^{-1} body weight. T_0, T_1, T_2 and T_3 denote TRH at 0, 275, 550 and 1100 pmol kg^{-1} body weight.

Assay

GH was assayed using a double antibody radio-immunoassay based on that of Hart et al. 1975 but modified as follows: recombinant DNA-derived bovine GH (American Cyanamid Company, Princeton, NJ) was used for radiolabelling (Iodogen method, Pierce Chemicals, Cambridge, United Kingdom) and standards. Antisera used were guinea-pig anti-bGH and donkey anti-guinea-pig gammaglobulin bound to cellulose (SAC–CEL, Immuno Diagnostic Systems, Washington, Tyne and Wear, United Kingdom).

A 0.05 M borate buffer of pH 7.8 containing 0.2% wt vol^{-1} bovine serum albumin and 0.1% wt vol^{-1} Na azide was used. Serum sample, ^{125}I-bGH and first antibody was incubated 24 h at 4°C. Bound GH was precipitated with SAC–CEL by centrifugation, and ^{125}I-GH bound determined in a gamma counter. At the final dilution of 1 : 40 000 of the first AB in the tubes, binding in the absence of unlabelled hormone was 39%, and the nonspecific binding was 5%. The lowest detectable concentration of GH was between 0.4 and 0.8 ng mL^{-1} standard. Serial dilution of calf serum with buffer paralleled the standard curve. Cross-reactivity with FSH, LH and prolactin was not detectable at concentrations up to 10 μg mL^{-1}. Recovery of known amounts of GH added to serum was 94%. Inter- and intra-assay coefficients of variation were 13 and 6%, respectively (three assays).

Statistical Analyses

The GH concentrations in the single serum samples were combined into the mean of samples taken at -15 and -5 min (PRIOR) and the mean of samples taken at 5, 10, 15, and 20 min after injection (PEAK). Also, the area under the curve of GH concentration plotted against time during the first 60 min after injection (AUC) was calculated. Transformations to natural logarithms were made to obtain approximate normality for all three variables.

Linear mixed models were fitted using routines of GENSTAT (Genstat 5 Committee 1987). Treatments effects were modelled in two analyses: first, a full 4 × 4 factorial (15 df); and second, a reduced model excluding nonlinear effects of the logarithm of dose and their interactions and including factors for GRF given or not (G_1, G_2, G_3) or (G_0); 1 df), for TRH given or not (T_1, T_2, T_3) or (T_0), 1 df) and their interactions (1 df), linear effects of the logarithm of dose of GRF in the absence of TRH (1 df) or in its presence (1 df) and linear effects of the logarithm of dose of TRH in the absence of GRF (1 df) or in its presence (1 df). The between-animal variation was modelled as a

blocking factor. Further adjustments were made for days (3 df) and time of injection (2 df). The effect of GH concentration prior to injection was included as a covariate.

From examining the results of the analyses, two further effects required attention. First, despite the logarithmic transformation, considerable heterogeneity in the size of the residual variance was found. One option considered was further transformation, but this would have removed the simple interpretation offered by logarithms. Therefore the heterogeneity was accomodated by application of restricted maximum likelihood (Patterson and Thompson 1971; Davidian and Carroll 1987) and estimating separate residual variances for each of the four subsets of data (G_0,T_0), (G_0) × (T_1, T_2, T_3), (G_1, G_2, G_3) × (T_0) and (G_1, G_2, G_3) × (T_1, T_2, T_3).

Analyses also revealed that carry-over effects were apparent between injections 3 h apart. The validity of the results therefore required that these be adequately modelled. Examination of the residuals showed a strong negative, linear relationship between the residual error and the response obtained 3 h previously (Fig. 1). Therefore the effect was modelled by including the deviation from the mean of the previous response as a covariate. For injections at time 09:00 h this covariate was made equal to zero.

RESULTS

An indication of the time course of GH concentration following intravenous injections is shown in Fig. 2 for the average of the treatments for placebo (G_0) × (T_0), GRF alone (G_1, G_2, G_3) × (T_0), TRH alone (G_0) × (T_1, T_2, T_3) and GRF plus TRH (G_1, G_2, G_3) × (T_1, T_2, T_3). Peak response was observed within 15 min and then declined towards pre-injection concentrations rapidly before 60 min and then more slowly in the next 60 min. The correlation between PEAK over the first 20 min and AUC was high ($r = 0.98$; 70 df; $P < 0.001$), and results will be presented only for PEAK.

The estimated means for each of the 16 treatments after adjustment for the effects described previously are shown in Fig. 3. The difference between the full and reduced models for treatments (see Statistical Analyses) was tested by a likelihood test and was found not to be significant ($P > 0.1$), i.e., the information contained by the data was adequately described by
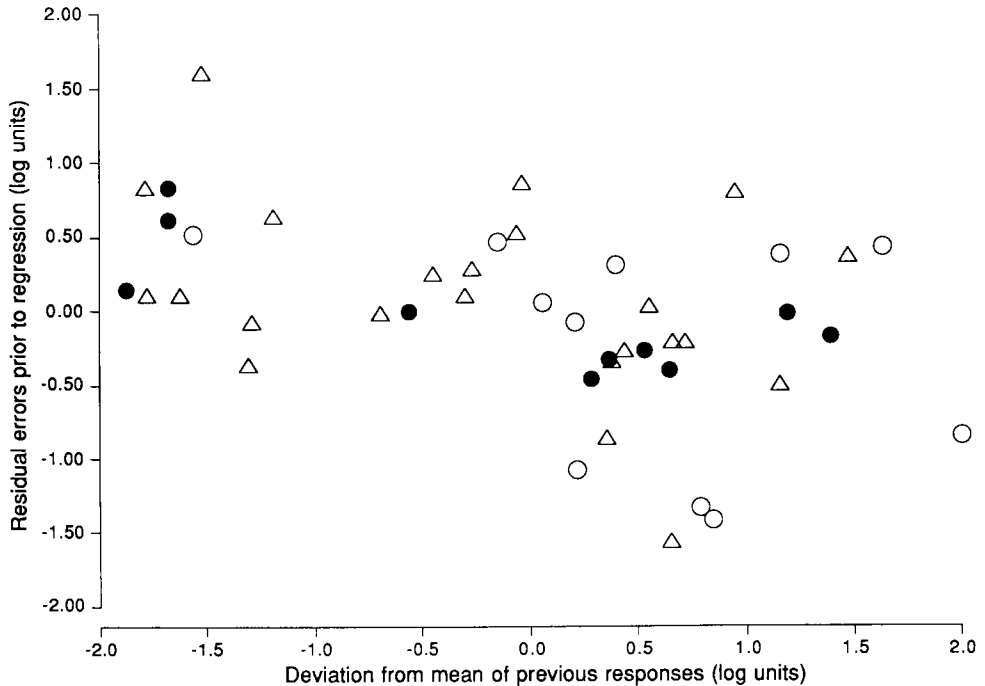
Fig. 1. The effect of magnitude of response 3 h previously on the response following administration of GRF, TRH or GRF plus TRH. (● , TRH alone; ○ , GRF alone; △ , GRF plus TRH).

the reduced model, involving regressions on the logarithm of doses of each secretagogue.

Using the reduced model, there was no evidence that dose–response relationships for either TRH or GRF differed according to whether each secretagogue was given separately or combined with the other, but the variation associated with administering GRF alone made this dose response the least clear (see Fig. 3). The pooled dose responses were significant for both TRH and GRF over the ranges considered, with mean concentration increasing 1.25-fold ($P < 0.05$) for a doubling of the dose of TRH and 1.46-fold ($P < 0.01$) for a doubling of the dose of GRF.

For GRF alone the lowest dose of 15 pmol kg^{-1} body weight was sufficient to give a detectable response (i..e, significantly greater than saline, with $P < 0.05$) compared with 550 pmol kg^{-1} body weight in TRH alone.

Since, on the logarithmic scale of analysis, the responses were linearly related to the logarithm of dose (interaction terms over and above the reduced model were not significant), it is possible to obtain further information on the relationship between the secretagogues by comparing the average of the treatment means for GRF alone (G_1, G_2, G_3) $\times$ (T_0), TRH alone (G_0) $\times$ (T_1, T_2, T_3,) and GRF plus TRH (G_1, G_2, G_3) $\times$ (T_1, T_2, T_3) with saline (G_0, T_0). Following injection of GRF alone PEAK was 4.44-fold greater ($P < 0.001$) than that following saline. Following TRH alone, PEAK was only 1.55-fold greater ($P < 0.001$). PEAK following GRF plus TRH was 9.22-fold greater ($P < 0.001$) than following saline. On the scale of analysis there was no evidence of synergism ($P > 0.1$), since the multiplicative effect was consistent with the product of separate contributions from GRF and TRH (i.e., $9.22/(4.44 \times 1.55) = 1.34$, and the estimate of 1.34 was not significantly different from 1).
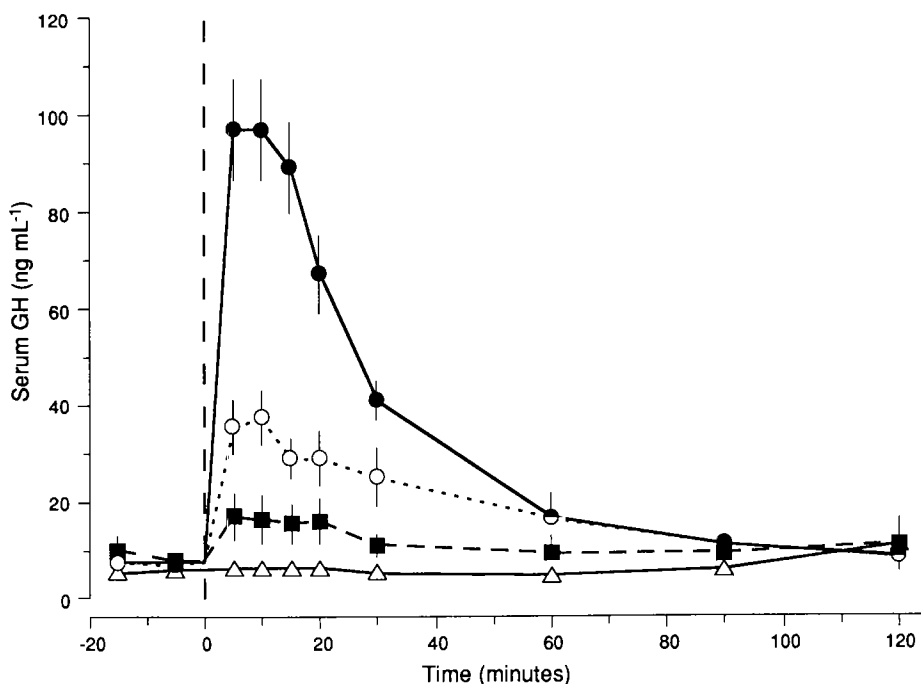
Fig. 2. Serum GH response in calves following administration of saline ($\triangle$, $n = 6$ trials), GRF pooled over doses of 15, 30 and 60 pmol kg^{-1} body weight ($\circ$, $n = 15$ trials), TRH pooled over doses of 275, 550 and 1100 pmol kg^{-1} body weight ($\blacksquare$, $n = 15$ trials) and combined administration of GRF and TRH pooled over the same doses ($\bullet$, $n = 36$ trials). Values are mean $\pm$ SE.

PRIOR was not affected by any of the experimental factors. However, PEAK was associated with PRIOR, and the partial regression coefficient of PEAK on PRIOR was 0.78 ± 0.18 ($P < 0.01$). Since this was on logarithmic scales, it suggests a doubling of PRIOR would be associated with a 1.72-fold increase in PEAK. This relationship did not differ according to whether GRF, or TRH or both were administered.

The relationship between response and the response to a treatment 3 h previously is shown in Fig. 1. The relationship was modelled by linear regression, with a coefficient of -0.31 ± 0.07 ($P < 0.01$).

Variation unexplained by the between-animal variation or the fixed effects was large, and heterogeneity was marked even after transformation onto the logarithmic scale. After adjusting for the models described, the residual variances ranked "saline," treatments

involving "TRH alone," treatments involving "GRF plus TRH" and treatments involving "GRF alone," with associated coefficients of variation on the observed scale of 33, 38, 69 and 83%, respectively. Thus administration of GRF resulted in greater coefficients of variation. This variation was a major contributor to the difficulty encountered in establishing a clear dose response for GRF when administered on its own.

DISCUSSION

To study GH responsiveness in dairy calves it is necessary to have clear information on dose–response curves of potentially important secretagogues such as GRF and TRH, including minimum doses, the position of the curve most sensitive to dose. Further, in the absence of prior evidence on the physiological importance of synergy between GRF and TRH, information is needed on the two-dimensional joint response curve for TRH and GRF.
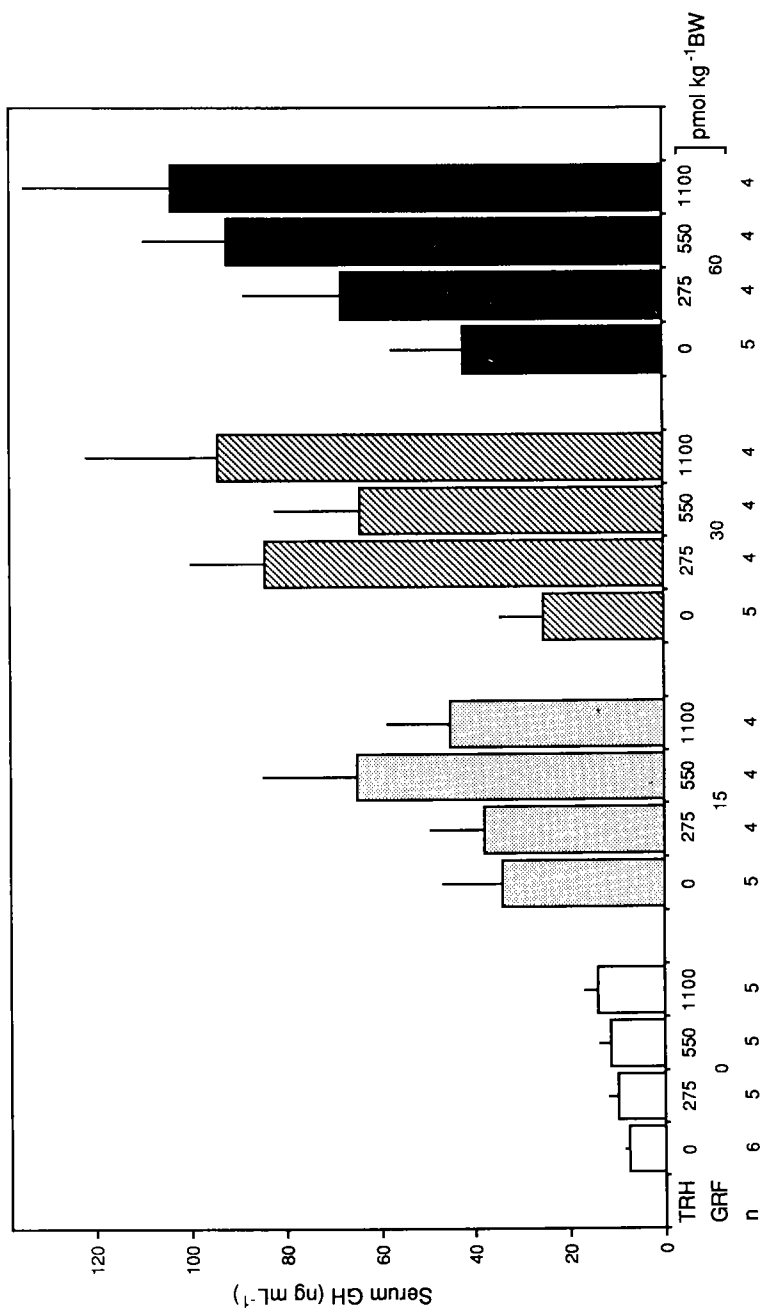
Fig. 3. Mean concentration of GH for 20 min following the intravenous injection of GRF (0, 15, 30 and 60 pmol kg^{-1} BW) and TRH (0, 275, 550 and 1100 pmol kg^{-1} BW), and all combinations of GRF and TRH (unshaded, (T_0, T_1, T_2, T_3); shaded, (T_0, T_1, T_2, T_3) × (G_1); diagonally shaded, (T_0, T_1, T_2, T_3) × (G_2); and solid, (T_0, T_1, T_2, T_3) × (G_3). Values are mean ± SE.

This trial has provided information on these aspects beyond that previously published and has clearly identified factors important to the precision of subsequent trials.

The relationship between response and GH concentration prior to injection for both GRF and TRH and the increased coefficient of within-animal variation when GRF was injected were both important in determining precision and may both be related to the synchrony of endogenous GH-pulsing and exogenous stimuli. Tannenbaum and Ling (1984) showed in rats that GH response to GRF was very small during an endogenous GH trough compared to when GRF was given during an endogenous GH peak. For both secretagogues, size of response may depend in part on the size of the releasable GH pool in the pituitary, and this would be affected by the time since the most recent peak. Further, the response to GRF may also be more profoundly affected by the endogenous somatostatin. Thus regression on GH concentrations prior to injection gave an improvement in precision, but variation in response to GRF remained large.

A further factor influencing response, and hence precision, was found to be the carryover effect from injections 3 h previously. This is despite two studies in calves (Plouzek et al. 1983; Hodate et al. 1985) which have used repeated injections at 3-h intervals and have explicitly reported no such effects and other studies using slightly longer intervals (e.g., Enright et al. 1987) in which carry-over effects were not described. Nevertheless, the finding in this trial was clear, and a negative linear relationship was found between the magnitudes of responses 3 h apart. Although it was possible to model the carry-over effect, longer time intervals between injections should be used to avoid such a problem altogether.

The present trial showed that the threshold dose for an observable GH release in calves was less than 15 pmol GRF kg^{-1} body weight. Upper bounds to this dose have been variously estimated with different fragments as 6.5 pmol kg^{-1} (GRF(1–40) Della-Fera et al. 1986), 36 pmol kg^{-1} (GFR(1–44), Moseley et al. 1984), 90 pmol kg^{-1} (GRF(1–40), Enright et al. 1987) and 67 pmol kg^{-1} (GRF(1−29) and GRF(1–40), Petitclerc et al. 1987). The generality of these results relies on the equipotency of fragments shown by Petitclerc et al. (1987). The result of this trial places a firm emphasis on the lowest estimate of 6.5 pmol kg^{-1}. The variation in response and failure to cope with heterogeneity of data are probable causes of the high estimates in some of the studies mentioned in which lower doses were deemed not to have elicited responses.

Dose responsiveness for GRF was observed between 15 and 60 pmol kg^{-1}, although the variation made this difficult to establish, and taken together with the dose–response studies mentioned previously, suggests the range of doses used in this trial contains the most sensitive region of dose response.

For TRH, the threshold derived from this trial lies between 275 and 550 pmol kg^{-1} bodyweight. Previous estimates were less than 300 pmol kg^{-1} in cows (Johke 1978) and less than 325 pmol kg^{-1} in calves (Tucker and Wettemann 1976). These results are consistent and suggest that the lowest dose used here is very near to the threshold. Hedlund et al. (1977) gave TRH to calves at doses greater than those used here and showed that compared with GRF the maximal response is much smaller and that little responsiveness is observed above 1375 pmol kg^{-1}. Thus a tentative conclusion is that the highest dose used in this study, 1100 pmol kg^{-1}, is close to the ED_{50}.

In the regions of the dose-response curve examined, effects of TRH and GRF appeared multiplicative and so exhibited little interdependence on a logarithmic scale. There was no evidence of different regressions of the logarithm of response on the logarithm of dose for one secretagogue in the presence or absence of the other, and the comparison of treatments with GRF alone, TRH alone and GRF plus TRH also suggested this. Thus in the absence of precise mechanisms and dynamic equations to describe the interaction, the synergy reported on the observed scale (Hodate et al. 1985) may be reduced by considering the effects on a logarithmic scale.

In conclusion, the results, in characterising more fully the two-dimensional dose-response surface for TRH and GRF and in identifying factors influencing the large variation in response, will allow better design and more informed interpretation of experiments to understand the pituitary responsiveness to GH secretagogues in calves.

ACKNOWLEDGMENTS

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