GH-releasing peptide-6 overcomes refractoriness of somatotropes to GHRH after feeding

C D McMahon, L T Chapin, R P Radcliff, K J Lookingland¹
and H A Tucker

Department of Animal Science, Michigan State University, East Lansing, Michigan 48824-1225, USA

¹Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan 48824-1317, USA

(Requests for offprints should be addressed to C D McMahon who is now at Dairy Science Building, AgResearch, Ruakura Agricultural Centre, P.B. 3123, Hamilton 2001, New Zealand; Email: chris.mcmahon@agresearch.co.nz)

Abstract

After a meal, somatotropes are temporarily refractory to growth hormone-releasing hormone (GHRH), the principal hormone that stimulates secretion of growth hormone (GH). Refractoriness is particularly evident when free access to feed is restricted to a 2-h period each day. GH-releasing peptide-6 (GHRP-6), a synthetic peptide, also stimulates secretion of GH from somatotropes. Because GHRH and GHRP-6 act via different receptors, we hypothesized that GHRP-6 would increase GHRH-induced secretion of GH after feeding. Initially, we determined that intravenous injection of GHRP-6 at 1, 3 and 10 µg/kg body weight (BW) stimulated secretion of GH in a dose-dependent manner. Next, we determined that GHRP-6- and GHRH-induced secretion of GH was lower 1 h after feeding (22.5 and 20 ng/ml respectively) than 1 h before feeding (53.5 and 64.5 ng/ml respectively; pooled s.e.m. = 8.5). However, a combination of GHRP-6

at 3 µg/kg BW and GHRH at 0.2 µg/kg BW synergistically induced an equal and massive release of GH before and after feeding that was fivefold greater than GHRH-induced release of GH after feeding. Furthermore, the combination of GHRP-6 and GHRH synergistically increased release of GH from somatotropes cultured *in vitro*. However, it was not clear if GHRP-6 acted only on somatotropes or also acted at the hypothalamus. Therefore, we wanted to determine if GHRP-6 stimulated secretion of GHRH or inhibited secretion of somatostatin, or both. GHRP-6 stimulated secretion of GHRH from bovine hypothalamic slices, but did not alter secretion of somatostatin. We conclude that GHRP-6 acts at the hypothalamus to stimulate secretion of GHRH, and at somatotropes to restore and enhance the responsiveness of somatotropes to GHRH.

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Introduction

Two neuropeptides principally regulate secretion of growth hormone (GH): growth hormone-releasing hormone (GHRH) stimulates secretion of GH, and somatostatin inhibits secretion of GH (Tannenbaum & Ling 1984, Plotsky & Vale 1985). After feeding, basal and GHRH-induced secretion of GH decrease, but the cause is unknown (Moseley *et al.* 1988). However, decreased basal and GHRH-induced secretion after feeding are not due to increased activity of somatostatin neurons (McMahon *et al.* 2000). Therefore, factors other than GHRH and somatostatin must contribute to decreased secretion of GH after feeding.

Growth hormone-releasing peptide-6 (GHRP-6) is a synthetic peptide that stimulates secretion of GH and, when combined with GHRH, is either additive or synergistic in stimulating secretion of GH (Cheng *et al.* 1989, Blake & Smith 1991, Bowers *et al.* 1991, Wu *et al.* 1996).

GHRH and GHRP-6 induce secretion of GH via different receptors and pathways of signal transduction (Mau *et al.* 1995, Chen *et al.* 1996, Howard *et al.* 1996, Wu *et al.* 1997). Therefore, it is possible that somatotropes are refractory to GHRH, but not GHRP-6 after feeding. In addition, it is not clear if GHRP-6 acts only at somatotropes or if it increases secretion of GHRH and/or decreases secretion of somatostatin to stimulate secretion of GH.

Our objectives were to determine: (1) the minimal effective concentration of GHRP-6 required to stimulate secretion of GH *in vivo*; (2) if GHRP-6 and GHRH injected together stimulate greater secretion of GH before and after feeding than either treatment alone; (3) if GHRP-6 and GHRH stimulate greater secretion of GH from perfused somatotropes than either treatment alone; and (4) if GHRP-6 stimulates secretion of GHRH or inhibits secretion of somatostatin from perfused hypothalamic slices.

Materials and Methods

Animals and maintenance

Eight male Holstein calves born at Michigan State University's Dairy Cattle Teaching and Research Center were castrated at 1 week of age, fed whole milk until 8 weeks of age, then fed a diet containing 18% crude protein and 19.6% acid detergent fiber (Land O'Lakes, Indianapolis, IN, USA). Steers were moved into individual stalls in rooms (four steers per room) where food was available *ad libitum* from 1000 to 1200 h daily and water was available *ad libitum*. Lights were on for 18 h each day and temperature was maintained at $20 \pm 1^\circ\text{C}$ (mean $\pm$ s.e.m.) in the rooms. At the start of experiments, steers were 24 ± 1 weeks old and weighed 171 ± 8 kg. The Michigan State University All University Committee on Animal Use and Care approved this experiment.

Experiment 1: Dose-response of GHRP-6 on secretion of GH in vivo

Steers were randomly allocated to two groups of four. A jugular vein of each steer was cannulated 24 h before the experiment. Patency of each cannula was maintained with sterile 3.5% sodium citrate. Blood samples (6 ml) were collected at 20-min intervals from 20 min before to 60 min after i.v. injection of vehicle (sterile water) or GHRP-6 (Bachem, Torrance, CA, USA) at 1, 3 and 10 $\mu\text{g/kg}$ body weight (BW). Blood was allowed to clot at 20°C for 2 h, stored at 4°C for 22 h and then centrifuged. Harvested serum was stored at -20°C until required for assay for GH.

Experiment 2: Effect of GHRP-6 and GHRH on secretion of GH 1 h before feeding compared with 1 h after feeding

Steers were randomly allocated to two groups of four. Each steer was injected i.v. with vehicle, a suboptimal dose of GHRP-6 (3 $\mu\text{g/kg}$ BW), GHRH ([Leu²⁷, Hse⁴⁵] bovine GHRH₍₁₋₄₅₎ lactone (Pharmacia & Upjohn, Kalamazoo, MI, USA; 0.2 $\mu\text{g/kg}$ BW; McMahon *et al.* 2000), and GHRP-6 (3 $\mu\text{g/kg}$ BW) together with GHRH (0.2 $\mu\text{g/kg}$ BW) in a Latin-square order of treatments. One group was injected at 0900 h and the other group was injected at 1300 h on each treatment day. One to two days separated each replicate in the Latin square for each group.

Experiment 3: Effect of GHRP-6 and GHRH on secretion of GH from perfused dispersed anterior pituitary cells

Pituitaries were collected from a local abattoir (Bellingar Packing, Ashley, MI, USA) and isolated anterior pituitary cells were prepared and perfused as previously described (Gaynor *et al.* 1996). Anterior pituitary cells

($n=8$ perfusion chambers per treatment) were treated with vehicle (minimal essential medium alpha, MEM α), GHRP-6 (10^{-8} M), GHRH (10^{-8} M), GHRP-6 (10^{-8} M) together with GHRH (10^{-8} M), a GHRH receptor antagonist (GHRHra) ([Ac-Tyr₁, D-Arg₂]-GHRH₍₁₋₂₉₎; 10^{-6} M; Bachem), or GHRH (10^{-6} M) together with GHRP-6 (10^{-8} M).

Experiment 4: Effect of GHRP-6 on secretion of GHRH and somatostatin from perfused hypothalamic slices

Bovine hypothalami were collected from a local abattoir (Bellingar Packing) within 10–15 min of death, bisected mid-sagittally and a single 1-mm thick slice was taken from and including the third ventricle from each hemi-hypothalamus using custom built Plexiglass tissue slicer as described elsewhere (McMahon *et al.* 2001). Each sagittal hemi-hypothalamic slice had its hypothalamic stalk attached.

Single slices were placed in barrels of 5 ml syringes, which served as perfusion chambers containing MEM α saturated in 100% oxygen (West *et al.* 1997a). These perfusion chambers were transported to the laboratory at 4°C .

In the laboratory, chambers were connected to a peristaltic pump and perfused at 37°C with MEM α saturated with 95% O₂–5% CO₂. Perfusion was performed as previously described (West *et al.* 1997a). Slices ($n=6$ per treatment) were treated with vehicle (MEM α), or GHRP-6 for 20 min at 10^{-10} M, 10^{-8} M, and 10^{-6} M by turning 3-way stopcocks to gain access to treatments held in 50 ml siliconized conical tubes.

Collected fractions were stored at -20°C until required for assay for GHRH and somatostatin.

Growth hormone assay

GH was measured using a radioimmunoassay (Gaynor *et al.* 1995) in which the intra-assay and interassay coefficients of variation were 12.1 and 14.9% respectively.

GHRH and somatostatin assays

GHRH and somatostatin were measured using radioimmunoassays as previously described (McMahon *et al.* 2001). Intra-assay coefficients of variation were 4.4% and 7.9% respectively.

Statistical analyses

Net areas under GH, GHRH and somatostatin curves were calculated as follows. Total areas under the curves were calculated using the trapezoid method after injection of vehicle or treatment, from 0 to 40 min for Experiments 1 and 2, from 20 to 140 min for Experiment 3 and from 40 to 160 min for Experiment 4. The area of the rectangle

calculated from mean concentrations of GH, GHRH and somatostatin at time 0 and projected to 40 min for Experiments 1 and 2 or from 40 (sample before treatment) to 160 min for Experiment 3 and from 20 (sample before treatment) to 140 min for Experiment 4 were used to estimate baseline, which was subtracted from the total areas under the curve to yield net areas in each experiment.

Net GH peak was calculated as the greatest concentration from 0 to 60 min minus the concentration at 0 min.

Data were log-transformed where appropriate, to stabilize the variance. Experiments 1, 3 and 4 were subjected to ANOVA using the generalized linear models procedure in SAS (1990) with factors of treatment (all experiments), replicate (first or second replicate on the same day; Experiments 3 and 4), and period (successive experiments over different days; Experiment 4) included in the model statement. Data in Experiment 2 were subjected to ANOVA using the Mixed Procedure in SAS with factors of treatment, time (before or after feeding), replicate of the Latin square (replicate), replicate $\times$ treatment and treatment $\times$ time as fixed variables, and animal nested with time as a random variable in the model statement. Differences between means, when the *F*-test was significant ($P < 0.05$), were evaluated using the PDIF option of SAS and in Experiment 3 adjusted for multiple comparisons using the method of Tukey (Sokal & Rohlf 1995). Data are reported as least squares means $\pm$ pooled standard error of the mean (S.E.M.).

Results

Experiment 1: Dose-response of GHRP-6 on secretion of GH in vivo

GHRP-6 stimulated secretion of GH in a dose-dependent manner compared with vehicle-injected controls (Fig. 1).

Experiment 2: Effect of GHRP-6 and GHRH on secretion of GH 1 h before feeding compared with 1 h after feeding

Mean basal concentrations of GH were greater ($P < 0.01$) before (5.2 ± 0.7 ng/ml) than after feeding (1.4 ± 0.8 ng/ml).

As shown in Fig. 2, the net area under the GH curve was greater ($P < 0.05$) for GHRH given before feeding (1207 ng/ml per min; 0900 h) than for GHRH given after feeding (422 ng/ml per min; 1300 h) (pooled S.E.M. = 210). GHRP-6 induced greater ($P < 0.05$) net GH peak before (53.5 ng/ml) than after feeding (22.5 ng/ml) (pooled S.E.M. = 8.5). However, net areas under the GH curves were not different ($P = 0.44$) for GHRP-6 given before (713 ng/ml per min) or after feeding (472 ng/ml per min) (pooled S.E.M. = 210). GHRH and GHRP-6 given together stimulated the greatest area under the GH

Figure 1 Effect of i.v. injection of vehicle (sterile water, ●) and GHRP-6 at 1 µg/kg (○), 3 µg/kg (▼), and 10 µg/kg body weight (▽) to Holstein steers on concentrations of GH in serum. Arrow indicates time of i.v. injection. Pooled S.E.M. = 11.2 ng/ml.

curve (2315 ng/ml per min) compared with either GHRH (815 ng/ml per min) or GHRP-6 alone (592 ng/ml per min) (pooled S.E.M. = 148; $P < 0.001$). There were no differences between net GH peaks ($P = 0.23$) or net

Figure 2 Effect of i.v. injection of vehicle (sterile water, ●), GHRH (0.2 µg/kg, ○), GHRP-6 (3 µg/kg, ▼) and GHRH together with GHRP-6 (▽), 1 h before feeding (0900 h), or 1 h after feeding (1300 h), on concentrations of GH in serum in Holstein steers. Arrows indicate time of i.v. injection. Pooled S.E.M. = 13 ng/ml.

Figure 3 Effect of vehicle (MEM α , ●), GHRH (10^{-8} M, ○), GHRP-6 (10^{-8} M, ▼), GHRH together with GHRP-6 (10^{-8} M each, ▽), GHRH receptor antagonist (GHRHra; 10^{-6} M, ■) and GHRHra with GHRP-6 (□) on concentrations of GH in perisates from dispersed bovine anterior pituitary cells. Solid bar indicates when vehicle, GHRH, GHRP-6, GHRH with GHRP-6, and GHRHra were perfused for 20 min. Open bar indicates 60-min period during which GHRHra (10^{-6} M) was perfused alone from 21 to 40 min, together with GHRP-6 (10^{-8} M) from 41 to 60 min, and alone from 61 to 80 min. Potassium chloride (60 mM) was perfused between 181 and 200 min (cross-hatched bar) to stimulate secretion of GH, to verify that somatotropes were viable. Pooled S.E.M. = 20.2 ng/ml.

areas under the GH curve ($P=0.59$) for combined treatment of GHRH with GHRP-6 before and after feeding.

Experiment 3: Effect of GHRP-6 and GHRH on secretion of GH from perfused dispersed anterior pituitary cells

GHRH increased net area under the GH curve (3392 ng/ml per min) compared with controls (276 ng/ml per min) (pooled S.E.M. = 847; $P<0.05$; Fig. 3). In contrast, GHRP-6 did not alter net GH peak ($P=0.6$) or net area under the GH curve ($P=0.7$) compared with controls. Combined treatment of GHRH with GHRP-6 stimulated a greater area under the GH curve (9113 ng/ml per min) compared with either GHRH ($P<0.01$) or controls ($P<0.001$). The net areas under GH curves were not different among GHRHra-treated (404 ng/ml per min), GHRP-6 and GHRHra-treated (617 ng/ml per min) and control-treated somatotropes (276 ng/ml per min; pooled S.E.M. = 847; $P=1.0$).

Experiment 4: Effect of GHRP-6 on secretion of GHRH and somatostatin from perfused hypothalamic slices

Net area under the GHRH curve was greater for hypothalamic slices treated with GHRP-6 at 10^{-6} M (4773 ng/ml per min) than for controls (1704 ng/ml per

Figure 4 Effect of vehicle (MEM α , ●), GHRP-6 at 10^{-10} M (○), 10^{-8} M (▼), or 10^{-6} M (▽) on concentrations of GHRH (top) and somatostatin (SS; bottom) in perisates from 1 mm slices of bovine hemi-hypothalami. Solid bar indicates when vehicle or GHRP-6 were perfused for 20 min. Cross-hatched bar indicates a 20-min period when 60 mM KCl was perfused to stimulate secretion of GHRH and somatostatin, to verify that tissues were viable. Pooled S.E.M. values are 19.2 pg/ml for GHRH and 19.5 pg/ml for somatostatin.

min, pooled S.E.M. = 819, $P=0.07$; Fig. 4). GHRP-6 did not alter concentrations of somatostatin compared with controls ($P=0.42$).

Discussion

Decreased basal and GHRH-induced secretion of GH after feeding is a common phenomenon in ruminants (Moseley *et al.* 1988, Plouzek *et al.* 1988, Trenkle 1989). Furthermore, reduced secretion of GH from somatotropes after feeding is not limited to that induced by GHRH, because α_2 -adrenergic-induced secretion of GH is also reduced after feeding (Gaynor *et al.* 1993). How and why somatotropes become refractory to GHRH after feeding is not known. However, given that the combination of GHRH with GHRP-6 induced rapid and massive release of GH before and after feeding, it seems likely that releasable pools of GH are not reduced and that receptors

to GHRH and GHRP-6 are not down-regulated. Rather, it is likely that there is a change in receptor signaling after feeding that is overcome by stimulating GHRH and GHRP-6 receptors together, while remaining refractory to either peptide alone.

Somatostatin is the principal hormone inhibiting secretion of GH and it is reasonable to expect increased activity of somatostatin neurons after feeding. However, previous investigations in our laboratory have demonstrated that reduced basal and GHRH-induced secretion of GH are not the result of increased activity (determined using the presence of Fos as an immunoreactive marker of neuronal activity) of somatostatin neurons, decreased activity of GHRH neurons, or both (McMahon *et al.* 2000). Therefore, factors other than somatostatin bestow on somatotropes a refractoriness to GHRH after feeding, and GHRP-6 overcomes such factors for at least 1 h after feeding.

GHRP-6 does not act solely at somatotropes, and the brain is considered to be a major target. The present data demonstrate that GHRP-6 stimulates secretion of GHRH without altering secretion of somatostatin *in vitro*. Our data extend those of others who have reported that GHRP-6 stimulates secretion of GHRH and increases the frequency of pulses of GHRH without altering secretion of somatostatin *in vivo* (Guillaume *et al.* 1994, Fletcher *et al.* 1996). In addition, GHRP-6 increases expression of *c-fos*, an immediate-early gene, in the arcuate nucleus, where GHRH neurons are located (Merchenthaler *et al.* 1984), suggesting direct action of GHRP-6 on GHRH neurons (Dickson *et al.* 1995). In contrast, Fairhall *et al.* (1995) reported that GHRP-6 inhibited secretion of somatostatin, thereby enhancing GHRH-induced secretion of GH. Furthermore, somatostatin was found to inhibit GHRP-6-induced secretion of GH *in vitro* (Cheng *et al.* 1989, Wu *et al.* 1996). Therefore, although the current study and that of Guillaume *et al.* (1994) do not support a decrease in secretion of somatostatin into hypophysial-portal blood, this does not discount the possibility that GHRP-6 blocks the somatostatin inhibition of GHRH neurons in the arcuate nucleus (Fairhall *et al.* 1995). It is possible, as Fairhall *et al.* (1995) suggested, that GHRP-6 blocks somatostatin receptors, which, in turn, enables secretion of GHRH. Blockade of somatostatin receptors on GHRH neurons, rather than directly stimulating GHRH neurons does not account for increased expression of *c-fos* in the arcuate nucleus unless GHRP-6 activates neurons other than GHRH, which, in turn, stimulate secretion of GHRH. This concept is not without foundation as it is believed that GHRP-6 acts in conjunction with another or other neurotransmitters that are, as yet, unknown (termed U-factor), to stimulate secretion of GH (Bowers 1999).

One disadvantage of perfusing slices of hypothalami is that pharmacological doses are often required to induce secretion of GHRH and somatostatin (West *et al.* 1997a,b).

Indeed, in the current study 10^{-6} M GRHP-6 induced secretion of GHRH, whereas lower molar concentrations had no effect. Slices of hypothalami perfused in the current study were 1 mm thick and, therefore, receptors for GHRP-6, somatostatin and GHRH may not be as readily accessible as they would be, for example, on dispersed somatotrope cells. Therefore, after diffusing into tissue, 10^{-6} M GRHP-6 in media may be diluted to a lower molar concentration at the receptors.

A clear role for GHRP-6 in regulating the secretion of GH has yet to be established. The current data show that GHRP-6 stimulates secretion of GHRH, but GHRP-6 also has independent and direct actions on somatotropes. For example, GHRP-6 stimulated secretion of GH from somatotropes *in vitro* (Wu *et al.* 1996) and stimulated secretion of GH *in vivo* after hypophysial-portal vessels – those transporting GHRH to somatotropes – had been removed (Fletcher *et al.* 1994). Yet, it is not clear if GHRP-6 stimulates secretion of GH via specific receptors only or also acts via GHRH receptors on somatotropes. For example, blockade of GHRH receptors reduced GHRP-6-induced secretion of GH *in vivo* and blocked GHRP-2 (another GHRP)-induced secretion of GH *in vitro* (Wu *et al.* 1994, Pandya *et al.* 1998). Receptors for GHRP-6-like peptide are located in the anterior pituitary gland and hypothalamus, which lends support to their having direct actions in the hypothalamus and anterior pituitary gland (Codd *et al.* 1989, Sethumadhavan *et al.* 1991, Howard *et al.* 1996). In the present investigation, GHRP-6 did not stimulate secretion of GH *in vitro* and, therefore, blockade of GHRH receptors was also without effect. Thus the present data support the concept of synergy between GHRP-6 and GHRH to stimulate secretion of GH *in vitro* (Cheng *et al.* 1989). These data contrast with other findings in which GHRP-6 was additive, rather than synergistic, with GHRH to stimulate secretion of GH *in vitro* (Blake & Smith 1991, Bowers *et al.* 1991, Wu *et al.* 1996, Bowers 1999).

In the current study, combined treatments of GHRP-6 and GHRH were additive before feeding, but synergistic after feeding. Typically, *in vivo*, GHRP-6 and GHRH are synergistic, especially when sub-maximal doses of GHRP-6 are used (Bowers 1999). However, not only does meal-feeding synchronize secretion of GH, but also somatotropes are more responsive to either GHRH or GHRP-6 alone before than after feeding. Importantly, the combined treatments of GHRP-6 and GHRH given before and after feeding were equal and massive in magnitude. We are unaware of any treatment able to stimulate greater secretion of GH in cattle and believe we achieved maximal secretion of the available pools of GH. Therefore, although sub-maximal doses of GHRP-6 combined with GHRH typically elicit synergistic secretion of GH *in vivo* (Bowers 1999), we speculate that the releasable pools of GH are depleted before synergistic actions are observed before feeding.

In conclusion, GHRP-6 restores and enhances GHRH-induced secretion of GH after feeding via independently increasing the secretion of GHRH from the hypothalamus and acting in synergy with GHRH at somatotropes.

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References

- Blake AD & Smith RG 1991 Desensitization studies using perfused rat pituitary cells show that growth hormone-releasing hormone and His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂ stimulate growth hormone release through distinct receptor sites. *Journal of Endocrinology* **129** 11–19.
- Bowers CY 1999 Growth hormone-releasing peptides. In *Handbook of Physiology*, Section 7: The endocrine system, Volume V: Hormonal control of growth. pp 267–297. Eds JL Kostyo & HM Goodman. Oxford: Oxford University Press.
- Bowers CY, Sartor AO, Reynolds GA & Badger TM 1991 On the action of the growth hormone-releasing hexapeptide, GHRP. *Endocrinology* **128** 2027–2035.
- Chen C, Wu D & Clarke IJ 1996 Signal transduction systems employed by synthetic GH-releasing peptides in somatotrophs. *Journal of Endocrinology* **148** 381–386.
- Cheng K, Chan WW, Barreto A Jr, Convey EM & Smith RG 1989 The synergistic effects of His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂ on growth hormone (GH)-releasing factor-stimulated GH release and intracellular adenosine 3',5'-monophosphate accumulation in rat primary pituitary cell culture. *Endocrinology* **124** 2791–2798.
- Codd EE, Shu AYL & Walker RF 1989 Binding of a growth hormone releasing hexapeptide to specific hypothalamic and pituitary binding sites. *Neuropharmacology* **28** 1139–1144.
- Dickson SL, Leng G, Dyball REJ & Smith RG 1995 Central actions of peptide and non-peptide growth hormone secretagogues in the rat. *Neuroendocrinology* **61** 36–43.
- Fairhall KM, Mynett A, Robinson ICAF 1995 Central effects of growth hormone-releasing hexapeptide (GHRP-6) on growth hormone release are inhibited by central somatostatin action. *Journal of Endocrinology* **144** 555–560.
- Fletcher TP, Thomas GB, Willoughby JO & Clarke IJ 1994 Constitutive growth hormone secretion in sheep after hypothalamo-pituitary disconnection and the direct *in vivo* pituitary effect of growth hormone releasing peptide 6. *Neuroendocrinology* **60** 76–86.
- Fletcher TP, Thomas GB & Clarke IJ 1996 Growth hormone-releasing hormone and somatostatin concentrations in the hypophyseal portal blood of conscious sheep during the infusion of growth hormone-releasing peptide-6. *Domestic Animal Endocrinology* **13** 251–258.
- Gaynor PJ, Chapin LT, Lookingland KJ & Tucker HA 1993 α_2 -Adrenergic receptor-mediated regulation of growth hormone secretion in meal-fed Holstein steers. *Proceedings of the Society for Experimental Biology and Medicine* **204** 318–322.
- Gaynor PJ, Lookingland KJ & Tucker HA 1995 5-Hydroxytryptaminergic receptor-stimulated growth hormone secretion occurs independently of changes in peripheral somatostatin concentration. *Proceedings of the Society for Experimental Biology and Medicine* **209** 79–85.
- Gaynor PJ, Simmons CR, Lookingland KJ & Tucker HA 1996 5-Hydroxytryptaminergic receptor-mediated regulation of growth hormone secretion in Holstein steers occurs via α_2 -adrenergic-dependent and -independent mechanisms. *Proceedings of the Society for Experimental Biology and Medicine* **212** 355–361.
- Guillaume V, Magnan E, Cataldi M, Dutour A, Sauze N, Renard M, Razafindraibe H, Conte-Devolx B, Deghenghi R, Lenaerts V & Oliver C 1994 Growth hormone (GH)-releasing hormone secretion is stimulated by a new GH-releasing hexapeptide in sheep. *Endocrinology* **135** 1073–1076.
- Howard AD, Feighner SD, Cully DF, Arena JP, Liberatore PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Parese PS, Diaz C, Chou M, Liu KK, McKee KK, Pong S-S, Chaung L-Y, Elbrecht A, Dashkevich M, Heavens R, Rigby M, Sirinathsinghji DJS, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG & Van der Ploeg LHT 1996 A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* **273** 974–977.
- McMahon CD, Chapin LT, Lookingland KJ, Radcliff RP & Tucker HA 2000 Feeding reduces activity of growth hormone-releasing hormone and somatostatin neurons. *Proceedings of the Society for Experimental Biology and Medicine* **223** 210–217.
- McMahon CD, Chapin LT, Radcliff RP, Lookingland KJ & Tucker HA 2001 Somatostatin inhibits alpha-2-adrenergic-induced secretion of growth hormone-releasing hormone. *Neuroendocrinology* **73** (In Press).
- Mau SE, Witt MR, Bjerrum OJ, Saermark T & Vilhardt H 1995 Growth hormone releasing hexapeptide (GHRP-6) activates the inositol (1,4,5)-triphosphate/diacylglycerol pathway in rat anterior pituitary cells. *Journal of Receptor and Signal Transduction Research* **15** 311–323.
- Merchenthaler I, Vigh S, Schally AV, Petrusz P 1984 Immunocytochemical localization of growth hormone-releasing factor in the rat hypothalamus. *Endocrinology* **114** 1082–1085.
- Moseley WM, Alaniz GR, Claffin WH & Krabill LF 1988 Food intake alters the serum growth hormone response to bovine growth hormone-releasing factor in meal-fed Holstein steers. *Journal of Endocrinology* **117** 253–259.
- Pandya N, DeMott-Friberg R, Bowers CY, Barkan AL & Jaffe CA 1998 Growth hormone (GH)-releasing peptide-6 requires endogenous hypothalamic GH-releasing hormone for maximal GH stimulation. *Journal of Clinical Endocrinology and Metabolism* **83** 1186–1189.
- Plotsky PM & Vale W 1985 Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophyseal-portal circulation of the rat. *Science* **230** 461–463.
- Plouzek CA, Molina JR, Hard DL, Vale WW, Rivier J, Trenkle A & Anderson LL 1988 Effects of growth hormone-releasing factor and somatostatin on growth hormone secretion in hypophyseal stalk-transected beef calves. *Proceedings of the Society of Experimental Biology and Medicine* **189** 158–167.
- SAS 1990 *SAS/STAT User's Guide*, Version 6, edn 4, pp 891–996. Cary, NC: SAS Institute Inc.
- Sethumadhavan K, Veeraragavan K & Bowers CY 1991 Demonstration and characterization of the specific binding of growth hormone-releasing peptide to rat anterior pituitary and hypothalamic membranes. *Biochemical and Biophysical Research Communications* **178** 31–37.

- Sokal RR & Rohlf FJ 1995 *Biometry: the Principles and Practice of Statistics in Biological Research*, edn 3, pp 392–450. New York: WH Freeman and Company.
- Tannenbaum GS & Ling N 1984 The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. *Endocrinology* **115** 1952–1957.
- Trenkle A 1989 Influence of feeding on growth hormone secretion and response to growth hormone-releasing factor in sheep. *Journal of Nutrition* **119** 61–65.
- West CR, Gaynor PJ, Lookingland KJ & Tucker HA 1997a Regulation of growth hormone-releasing hormone and somatostatin from perfused, bovine hypothalamic slices. I. α_2 -Adrenergic receptor regulation. *Domestic Animal Endocrinology* **14** 334–348.
- West CR, Lookingland KJ & Tucker HA 1997b Regulation of growth hormone-releasing hormone and somatostatin from perfused, bovine hypothalamic slices. II. Dopamine receptor regulation. *Domestic Animal Endocrinology* **14** 349–357.
- Wu D, Chen C, Katoh K, Zhang J & Clarke IJ 1994 The effect of GH-releasing peptide-2 (GHRP-2 or KP 102) on GH secretion from primary cultured ovine pituitary cells can be abolished by a specific GH-releasing factor (GRF) receptor antagonist. *Journal of Endocrinology* **140** R9–R13.
- Wu D, Chen C, Zhang J, Bowers CY & Clarke IJ 1996 The effects of GH-releasing peptide-6 (GHRP-6) and GHRP-2 on intracellular adenosine 3',5'-monophosphate (cAMP) levels and GH secretion in ovine and rat somatotrophs. *Journal of Endocrinology* **148** 197–205.
- Wu D, Clarke IJ & Chen C 1997 The role of protein kinase C in GH secretion induced by GH-releasing factor and GH-releasing peptides in cultured ovine somatotrophs. *Journal of Endocrinology* **154** 219–230.

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