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Effects of Novel Growth Hormone Secretagogues on Growth Hormone Secretion in Farm Animals

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Abstract
The requirement for growth hormone (GH) secretion by the anterior pituitary gland in beef calves is demonstrated by a complete lack of long bone-growth and muscle accretion after hypophysectomy (surgical removal of the pituitary gland). When the connecting link (hypophyseal stalk) to the basal region (hypothalamus) of the brain is surgically severed, long bone growth and body weight gain are greatly limited compared with sham-operated controls. This limited growth results from obliteration of episodic GH secretion and reduced basal blood concentration of the hormone compared with sham-operated controls. Thus, the hypophyseal stalk-transected (HST) calf provides an appropriate model to determine mechanisms by which hypothalamic neuropeptides from the brain regulate GH secretion, and thereby growth in the young calf. Neuropeptides have been isolated and characterized in bovine hypothalamus that stimulate GH secretion (GH-releasing hormone [GHRH]) or factor [GHRF] and inhibit GH secretion (GH release-inhibiting hormone [GHRIH] or somatostatin [SRIH]). A dose of .067 micrograms of GHRF per kilogram of body weight injected intravenously in HST calves abruptly increased plasma GH concentration to 55 nanograms per milliliter from the control period mean of 5 nanograms per milliliter. HST calves then were infused intravenously with .033 and .067 microgram somatostatin per kilogram of body weight, during which a pulse injection of .067 microgram of GHRF was administered. GH increase was limited to 9 and 5 micrograms per kilogram body weight during the .033- and .067 microgram SRIH infusions after GHRF; no GH rebound was observed after the SRIH was discontinued. GHRF from humans contains 40 to 44 amino acids. Rat hypothalamic GHRF analogs containing 29 to 32 amino acids elicited dose-dependent GH peak release in these HST calves. In 1977, Bowers and Monomy isolated novel GH releasing peptides consisting of only six amino acids; they caused GH release by isolated pituitary cells in culture and acute GH release when administered intravenously. We recently have utilized a novel nonpeptidyl GH secretagogue of low molecular weight in the pig to determine its mechanisms of action within the central nervous system.

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Summary

The requirement for growth hormone (GH) secretion by the anterior pituitary gland in beef calves is demonstrated by a complete lack of long bone-growth and muscle accretion after hypophysectomy (surgical removal of the pituitary gland). When the connecting link (hypophyseal stalk) to the basal region (hypothalamus) of the brain is surgically severed, long bone growth and body weight gain are greatly limited compared with sham-operated controls. This limited growth results from obliteration of episodic GH secretion and reduced basal blood concentration of the hormone compared with sham-operated controls.

Thus, the hypophyseal stalk-transected (HST) calf provides an appropriate model to determine mechanisms by which hypothalamic neuropeptides from the brain regulate GH secretion, and thereby growth in the young calf. Neuropeptides have been isolated and characterized in bovine hypothalamus that stimulate GH secretion (GH-releasing hormone [GHRH]) or factor [GHRF] and inhibit GH secretion (GH release-inhibiting hormone [GHRIH] or somatostatin [SRIH]). A dose of .067 micrograms of GHRF per kilogram of body weight injected intravenously in HST calves abruptly increased plasma GH concentration to 55 nanograms per milliliter from the control period mean of 5 nanograms per milliliter. HST calves then were infused intravenously with .033 and .067 microgram somatostatin per kilogram of body weight, during which a pulse injection of .067 microgram of GHRF was administered. GH increase was limited to 9 and 5 micrograms per kilogram body weight during the .033- and .067 microgram SRIH infusions after GHRF; no GH rebound was observed after the SRIH was discontinued. GHRF from humans contains 40 to 44 amino acids. Rat hypothalamic GHRF analogs containing 29 to 32 amino acids elicited dose-dependent GH peak release in these HST calves.

In 1977, Bowers and Monomy isolated novel GH releasing peptides consisting of only six amino acids; they caused GH release by isolated pituitary cells in culture and acute GH release when administered intravenously. We recently have utilized a novel nonpeptidyl GH secretagogue of low molecular weight in the pig to determine its mechanisms of action within the central nervous system.

Introduction

Hypothalamic regulation of GH secretion is mediated by a stimulatory factor, GH-releasing factor (GHRF), and an inhibitory factor, GH-release-inhibitory hormone, somatostatin (SRIH). Intravenous administration of GHRF causes a pulse release of GH into the circulating blood, similar to that occurring during endogenous GH secretion. It has been suggested that the pattern of GH secretion, frequency and amplitude of GH spikes, is important in the regulation of growth in the laboratory rat but may not be important for other species such as cattle. The objective of this study was to determine the effect of GHRF analogs on the secretion of GH in prepuberal calves and a novel GH secretagogue on GH secretion in the prepuberal pig. The overall goal is to gain a better understanding of the mechanisms by which these neuropeptides effect central nervous system regulation of GH secretion in farm animals.

Materials and Methods

Animals

Thirteen Aberdeen Angus calves from 6 to 10 months old, weighing 105-232 kilograms, were subjected to hypophysectomy (n = 5), hypophyseal stalk transection (n = 4), or cranial sham operation (n = 4). Body weight change and the histology of endocrine gland development were determined. Crossbred beef heifers (4 months old, 111 ± 8 kilograms body weight [mean ± standard error]) were subjected to hypophyseal stalk transection (n = 5) or sham operation control (n = 2). The calves were fitted with a jugular cannula for repeat blood sampling and hormone administration. The effects of intravenous injection of human pancreatic GHRF, [hpGHRF], analogs of rat hypothalamic GHRF, [Nle^{27}]rhGHRF(1-29)NH_2 and rhGHRF(1-32)OH, thyrotropin releasing hormone (TRH), and somatostatin (SRIH) on plasma GH concentrations were determined. Finally, the effects of a nonpeptidyl GH secretagogue, L-692,585, on GH secretion were determined in hypophyseal stalk transected (n = 3) and sham operated (n = 3) castrated male Yorkshire pigs (approximately 32 kilograms body weight). The pigs were fitted with a jugular cannula for repeat blood sampling.

Hormone Radioimmunoassays

Validated radioimmunoassays for GH, adrenocorticotropic hormone (ACTH), cortisol, and prolactin were carried out in duplicate plasma samples.

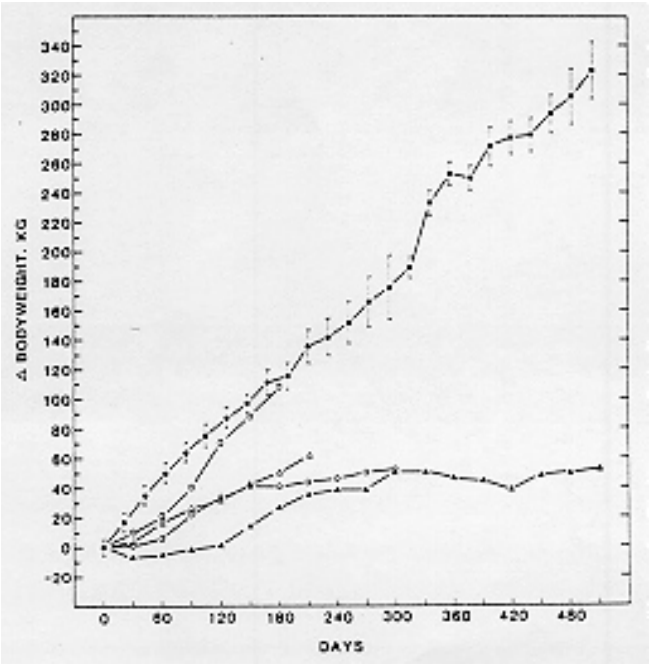
Statistical Analysis

The experimental units were the individual calves or pigs. Dose-response data were analyzed using a Latin square design; other data were analyzed using Student's *t* test for comparison among treatment groups.

Results and Discussion

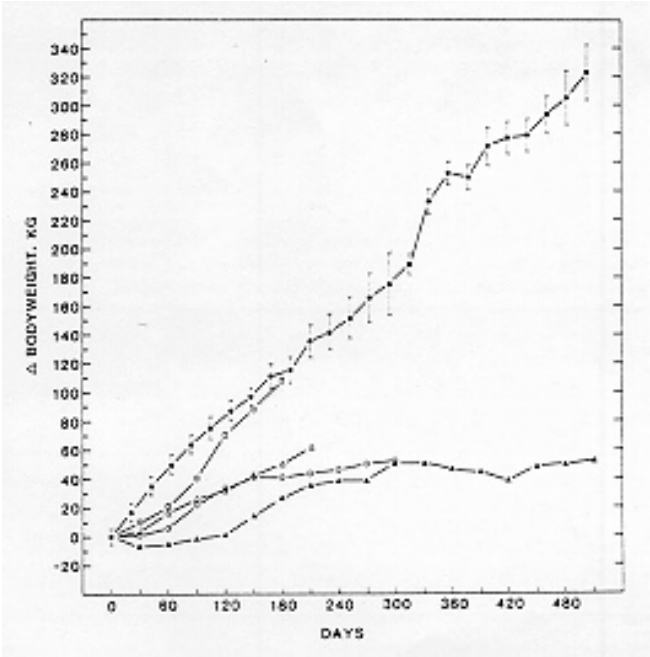
After hypophyseal stalk transection, the weight of the pituitary gland decreased in calves ($p < .005$) compared with sham-operated controls (.19 ± .01 versus .57 ± .07 grams per 100 kilograms body weight; mean ± standard error). Histological examination of the pituitary revealed large infarcts in the *pars distalis* and normal-appearing *pars nervosa* and *pars intermedia*. Growth was inhibited in calves after HST or hypophysectomy. Body-weight increase in calves after hypophyseal stalk transection (.089 kilograms per day) or hypophysectomy (-.061 kilograms per day) was reduced markedly compared with that found in sham-operated calves (.467 kilograms per day) or in unoperated calves (.594 kilograms per day) during a period of 90 days. Growth during a prolonged period after hypophysectomy (*calves 14* and *16*) or after hypo-physeal stalk transection (*calf 89*) was limited severely ($p < .01$) as compared with that found in four sham-operated calves and nine unoperated controls (Figure 1).

Figure 1. Growth rates in Aberdeen Angus calves during prolonged periods after hypophysectomy in *bull calf 16* (Ÿ, .099 kilograms per day) and in *heifer calf 14* (_, .288 kilograms per day), after hypophysial stalk transection of *heifer calf 89* (°, .167 kilograms per day) as compared with those found in sham-operated calves (o, .600 kilograms per day) and in unoperated calves (l, .641 kilograms per day). (Anderson, *Am. J. Physiol.* 232:E497-E503, 1977.)



In initial studies with GH-releasing factor or hormone (GHRF or GHRH), we established a dose-dependent acute release of GH into peripheral blood of intact crossbred beef heifer calves (four months old; 111 kilograms body weight). Animals received an intravenous injection of human pancreatic GH-releasing factor [hpGHRF(1,40)OH] and a GHRH analog, Nle^{27} rat hypothalamic GHRF [Nle^{27}rGHRF(1,29)NH_2] dissolved in 0.1% acetic acid and then diluted with a sterile buffer solution. The buffer was physiological saline (0.818%) containing 0.01 molar Na_2PO_4/H_2O, pH 7.0;0.01% ascorbic acid; and 1% bovine serum albumin. An indwelling jugular vein cannula was inserted for repeat blood sampling and hormone injection. Plasma GH increased in a dose-dependent manner, with a peak of GH occurring between 5 and 15 minutes after injection of hpGHRF (Figure 2).

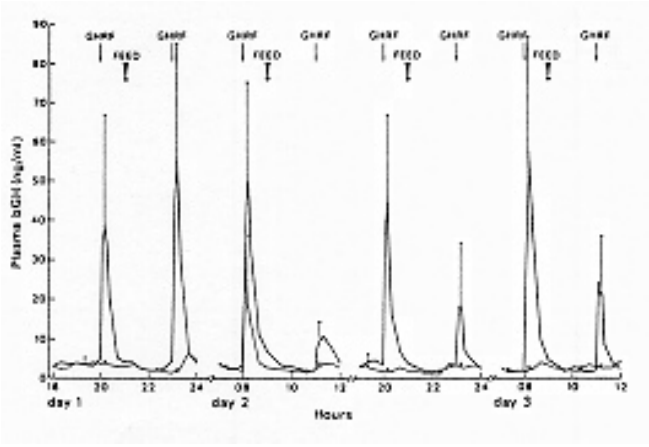
Figure 2. Mean concentrations of plasma GH in crossbred beef heifers in response to iv injections of 0, .01, .033, .067 and .1 mg hpGHRF (1,40)OH/kg body wt (n=5). The dose of hpGHRF is indicated above the arrow at the time of administration. To indicate representative SE at baseline and peak concentrations, the samples 10 min before and after injection of hpGHRF have SE marked by the bars. (Plouzek et al., *Proc. Soc. Exp. Biol. Med.* 188:198-205, 1988.)



GH levels during the 20-minute period after injection of 0.067 ($p < .05$) and 0.1 microgram hpGHRF per kilogram body weight ($p < .025$) were greater than controls, whereas 0.01 and 0.033 micrograms hpGHRF per kilogram body weight were similar to controls. Five-month-old heifers receiving GHRF every 3 hours for 42 hours responded to 50% of the GHRF injections (Figure 3), whereas a greater degree of responses (77%) was observed in three-and-a-half-month-old bull calves given GHRF every 4 hours for 10 days. The amplitude of the GH response to GHRF may have been related to feeding.

The GH response to GHRF was greater before feeding than after feeding (Figure 3).

Figure 3. The average plasma GH concentrations of crossbred beef heifers receiving 0 or .067 mg hpGHRF(1,40)OH/kg body wt at 3-h intervals for 42 h. The thick line illustrates the response of animals receiving hpGHRF (n=5), and the thin line illustrates the plasma response of animals receiving buffer without hpGHRF (n=5). To represent SE before hpGHRF injection and during the peak response of GH after hpGHRF injection, SE are shown by the bars for samples 40 min before hpGHRF injection and 10 min after injection. Times of feeding and hpGHRF administration are indicated by the arrows. (Plouzek et al., *Proc. Soc. Exp. Biol. Med.* 188:198-205, 1988.)



Effects of multiple injections of Nle^{27} rGHRF(1,29)NH_2 for 10 days in bull calves are shown in Table 1.

The mean GH response in animals given rGHRF was 25 nanograms per milliliter ($p < .05$), compared with 7 nanograms per milliliter plasma in the controls. Somatomedin-C (insulin-like growth factor-I [IGF-I]), insulin, triiodothyronine, thyroxine, and cortisol concentrations were not changed with administration of rGHRF. Thus, the overall results indicate that intact prepubertal bull and heifer calves were responsive to GHRF administered as either discrete or multiple intravenous injections.

The neurohypophyseal link between the hypothalamus and the pituitary is essential for conveying these releasing and inhibiting hormones. After surgical hypophyseal stalk transection (HST), normal episodic secretion of GH is abolished in cattle and pigs, and these animals have depressed growth rates. HST calves and pigs serve as useful models to study the isolated effects of releasing and inhibiting substances on pituitary hormone secretions. Seven crossbred beef heifers (four months old, 111 ± 8 kilograms body weight (± standard error) were fitted with an indwelling jugular cannula and subjected to either HST or cranial sham operation control (SOC). After the hypophyseal stalk was severed, a nylon disc (8.0 millimeters diameter and .45 millimeter thickness) was inserted between the severed ends of the tubular stalk to prevent regeneration between the hypothalamus and pituitary gland. In seven calves subjected to either HST or SOC, the plasma GH levels during surgery and immediately in the postoperative recovery period are depicted in Figure 4.

Table 1. Effect of multiple injections of Nle^{27} rGHRF (1,29)NH_2 on plasma hormone means over 10 days in bull calves.

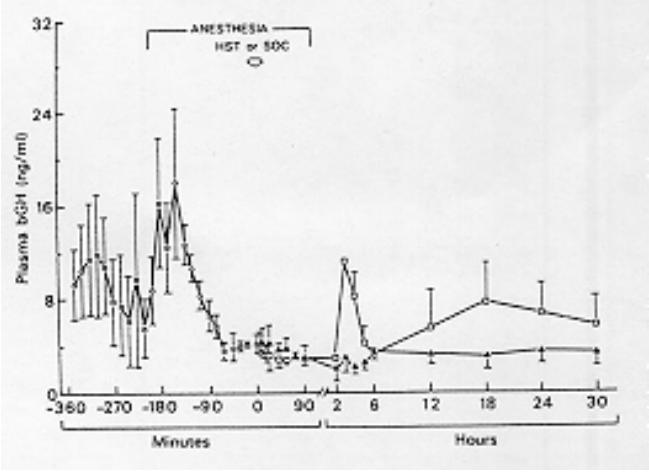
	rGHRF	Control	SE
GH (ng/ml)[a]	6.5	8.7	1.5
GH (ng/ml)[b]	25.1[c]	7.0	8.0
GH (ng/ml)[d]	9.8	7.8	1.8
Sm-C (nmole/liter)	8.1	6.6	2.4
Insulin (ng/ml)	.54	.51	.12
Triiodothyronine (ng/dl)	1.31	1.34	.22
Thyroxine (mg/dl)	7.7	7.4	1.1
Cortisol (mg/dl)	2.2	1.8	.3

[a]Mean of 20, 10 and 1 min before rGHRF injection.
[b]Mean of 5, 10, 15, and 20 min after rGHRF injection.
[c]Mean significantly different ($p < .05$) from corresponding control mean.
[d]Mean of 40 and 60 min after rGHRF injection.
(Plouzek et al., *Proc. Soc. Exp. Biol. Med.* 188:198-205, 1988.)

GH secretion during the preanesthesia period, 9 ± 3.9 nanograms per milliliter (mean values from -345 to -195 minutes), was variable but tended to decrease just preceding anesthesia. GH returned to presurgery baseline concentrations at 3-4 hours in the SOC calves after anesthesia was removed; however, HST calves did not resume GH concentrations similar to those observed before surgery (SOC, 10 ± 1.8 versus HST 3 ± 1.0 nanograms per milliliter; $p < .05$). The HST calves responded to .067 and .133 micrograms hpGHRF per kilogram body weight with a rapid increase in plasma GH, which peaked within 10-20 minutes and then declined to preinjection concentrations within 60 minutes (Figure 5). After HST, all calves responded to 100% of the hpGHRF injections. This was similar to their responses before surgery in which the calves responded to 80% of hpGHRF challenges. Infusion of 2 and 4 micrograms SRIH (kilogram body weight per hour) or equivalent to .033 and .067 microgram SRIH per kilogram body weight per minute depressed GH release in response to an administered bolus of .067 micrograms hpGHRF per kilogram body weight (Figure 6). GH levels 20 minutes after hpGHRF were 9 ± 1.6 nanograms per milliliter

during infusion of .0333 microgram SRIH and 5 ± .4 nanograms per milliliter during infusion of .067 microgram SRIH. The changes in plasma GH during the infusion of either dose of SRIH were not significantly different in any part of the experiment or between doses of SRIH. After the infusion of SRIH were stopped, GH concentrations remained stable.

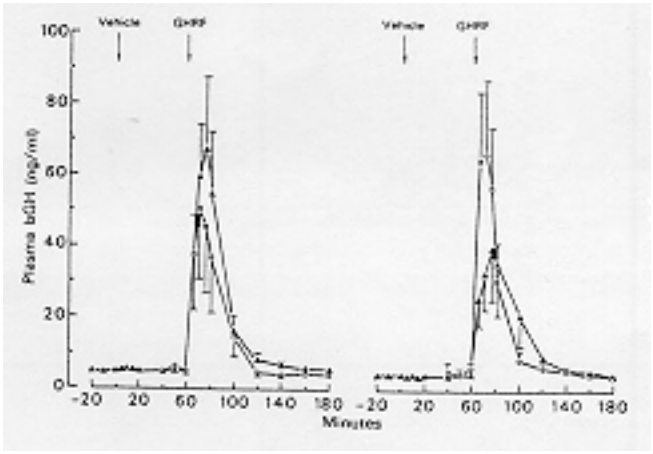
Figure 4. Plasma GH concentrations in beef calves before and after surgery are illustrated. The GH response indicated by the open circles illustrates the period before surgical intervention, which is indicated by the open arrow (n=7). Plasma GH after HST is illustrated by closed triangles (n=5), and GH after SOC is indicated by squares (n=2). Values are means ± SE. SE bars for samples taken 2 and 3 h after surgery are within the symbol. (Plouzek et al., *Proc. Soc. Exp. Biol. Med.* 189:158-167, 1988.)



Our results indicate that intravenous injection of the brain neuropeptide GHRH causes acute peak release of GH into the peripheral blood of intact bull and heifer calves. After hypophysial stalk transection, the calves remain acutely responsive to GHRF challenge, releasing similar amounts of GH into peripheral blood within a few minutes. In contrast, constant infusion of somatostatin-14 (SRIH) during a 2-hour period in HST calves greatly inhibits GH release during a GHRH challenge. Thus, the HST calf provides a unique model to abolish episodic GH release and to show that GHRF administration can directly stimulate GH release by the pituitary gland.

In 1977, the first peptides of the GH-releasing peptide (GHRP) series were discovered. They were derivatives of the pentapeptide Met-enkephalin and had the structures Tyr-DTrp-Gly-Phe-Met-NH_2 or Tyr-DPhe-Gly-Phe-Met-NH_2. Several analogs with ability to specifically cause GH release both *in vivo* and *in vitro* have been synthesized. A particularly potent hexapeptide, His-DTrp-Ala-Trp-DPhe-Lys-NH_2, causes GH release and hypothalamic binding activity 1000 times greater than the original GHRPs. With a burgeoning interest in this family of GHRPs, novel nonpeptidyl GH secretagogues recently have been developed consisting of substituted benzolactams with high biological potency to cause GH-release. We have used one of these potent benzolactam analogs, L-692,585, to determine central mechanisms of its GH releasing action in the young castrate male pig subjected to hypophyseal stalk transection or sham operation control. Before cranial surgery, GH concentration averaged 5 or 6 nanograms per milliliter plasma (Table 2).

Figure 5. Plasma GH concentrations in HST calves in response to 0, .067, and .133 mg(1-40)-OH hpGHRF/kg body wt are illustrated by the closed triangles (n=5). For comparison, the response to .067 and .133 mg(1-40)-OH hpGHRF/kg body wt before surgery in these animals is indicated by the open circles in the left and right panels, respectively. The GH response to .067 mg hpGHRF is shown in the left panel, and the right panel indicates the effect of the more concentrated dose of hpGHRF. Arrows indicate the time of vehicle or hpGHRF administration. Values are means ± SE. (Plouzek et al., *Proc. Soc. Exp. Biol. Med.* 189:158-167, 1988.)



Intravenous injection of L-692,585 acutely increased GH levels 15-fold, whereas intravenous injection of hGHRF caused only a 2- to 5-fold increase in GH plasma levels. The combined treatment of L-692,585 with hGHRF caused a 28-fold increase in GH concentration compared with the control period. After HST, L-692,585 alone or combined with hGHRF caused a similar immediate increase in plasma GH concentration. Basal cortisol concentrations were reduced in the HST pigs relative to the SOC group. GHRF did not significantly increase cortisol levels when administered alone or further increase L-692,585-induced response.Overall, the results indicate that coadministration of GHRF with L-692,585 allows the isolated pituitary gland to release GH maximally. Transection of the hypothalamo-pituitary stalk markedly decreased the GH and cortisol response to

L-692,585, but did not reduce the synergistic GH response to L-692,585 plus hGHRF coadministration.

Table 2. Effect of hypothalamo-pituitary stalk transection in the pig on GH secretory activity of L-692,585.

GH in peripheral plasma (ng/ml)			
Item	SOC	HST	Probability
Yorkshire barrows, n	3	3	
Presurgery (d -7 to -3)			
Control period	6.4	5.2	
L-692,585*	101	71	
hGHRF**	13	25	p < .01
L-692,585 + hGHRF	171	174	
Postsurgery (d +3 to +9)			
Control period	6.7	5.8	
L-692,585*	79	14	p < .005
hGHRF**	13	27	p = .05
L-692,585 + hGHRF	115	94	

***.1 mg/kg body weight**

****.02 mg/kg body weight**
(Hickey et al. *Proceedings of 76th Annu. Mtg. of the Endocrine Society,* Anaheim, Calif., 1994, Abstract 661.)

Implications

Episodic and basal secretion of growth hormone by the pituitary gland in calves and pigs is essential for normal growth and development; pituitary gland removal abruptly limits growth in these species. When the neurohypophyseal link between the pituitary gland and hypothalamus at the base of the brain is severed, episodic GH secretion is abolished and growth impaired. The administration of brain neuropeptides that stimulate (i.e., GHRFs, GHRPs, and nonpeptidyl GH secretagogues) and inhibit (i.e., somatostatin, SRIH, and related analogs) GH secretion in HST and intact calves and pigs provide novel models to further our understanding of central nervous system regulation of growth and development in farm animals.

Figure 6. Mean concentrations of plasma GH in HST calves during iv infusion of 2 and 4 mg somatostatin(SS)-14 (SRIH)/kg body wt per h) or equivalent to .033 and .067 mg SRIH/kg body wt per min and injected with .067 mg(1-40)-OH hpGHRF/kg body wt are illustrated by closed triangles (n=2). The same animals' response to these hormone treatments before surgical intervention is shown by the open circles. Arrows indicate the time of hpGHRF injections, and the hatched areas illustrate the period of SRIH infusion. (Plouzek et al., *Proc. Soc. Exp. Biol. Med.* 189:158-167, 1988.)