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Effects of Growth Hormone-Releasing Factor and Somatostatin on Growth Hormone Secretion in Hypophysial Stalk-Transected Beef Calves[1] (42792)

CATHIE A. PLOUZEK,[2] J. R. MOLINA,[3] D. L. HARD,[4] W. W. VALE, J. RIVIER, A. TRENKLE, AND L. L. ANDERSON[5]

Department of Animal Science, Iowa State University, Ames, Iowa 50011, and The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, San Diego, California 92138

Abstract. The effects of growth hormone-releasing factor (GHRF) on growth hormone (GH) secretion were studied in beef calves after hypophysial stalk transection (HST). Peripheral GH concentration during surgery was elevated for 60 min after the initiation of anesthesia to 15 ng/ml, which was greater than plasma levels after HST and during the recovery period (0–30 hr mean, 3 ng/ml; $P < 0.05$). Episodic GH secretion normally seen in sham-operated controls (SOC) was abolished after HST. Before HST, calves responded to 80% of the GHRF challenges, whereas after HST calves responded to every challenge of GHRF with an increase in plasma GH. A dose of 0.067 μg human pancreatic (hp) hpGHRF(1–40)OH/kg body wt 3 days after HST increased plasma GH to 55 ng/ml from a control period mean of 5 ng/ml ($P < 0.04$). On Day 8, HST calves received two injections of 0.067 μg hpGHRF/kg body wt at 3-hr intervals, with feeding 70 min after the first injection. During two preinjection control periods, basal GH averaged <4 ng/ml and increased to 17 ($P < 0.02$) and 9 ($P < 0.04$) ng/ml immediately after the first and second injection of hpGHRF, but the response declined over the 8-day period after surgery. On Days 19 and 20, the HST calves were infused iv with 0.033 and 0.067 μg somatostatin(SS)-14 (SRIH)/kg body wt, during which a pulse injection of 0.067 μg hpGHRF/kg body wt was administered. GH increased to 9 and 5 ng/ml during the 0.033- and 0.067-μg SRIH infusions after GHRF; no somatotropic rebound was observed after the SRIH was discontinued as was seen in the animals while the hypothalamic–hypophysial connections were intact. Five and six months after HST the responses to two analogs of rat hypothalamic GHRF were similar to those in SOC calves. These results indicate that HST calves responded to exogenous GHRF with an abrupt increase in plasma GH, but GH response to GHRF during SRIH infusion was greatly inhibited.

The hypothalamus regulates episodic growth hormone (GH) secretion from the pituitary by its endogenous release of GH-releasing factor (GHRF) and GH-release-inhibiting hormone, somatostatin (SRIH). The neurohypophysial link between the hypothalamus and the pituitary is essential for conveying these releasing and inhibiting hormones. After surgical hypophysial stalk transection (HST), normal episodic secretion of GH is abolished in cattle and pigs (1, 2), and these animals have depressed growth rates (3). Unlike hypophysectomized animals, HST animals can serve as a model to study the isolated effects of releasing and inhibiting substances on pituitary hormone secretions. It is known that GHRF can stimulate an endogenous GH release in man, rats, pigs,

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[2] Present address: Section on Cellular Differentiation, Human Genetics Branch, NICHHD, NIH, Building 10, Room 10N-321, Bethesda, MD 20892.

[3] Present address: Department of Animal Science, Universidad de Costa Rica, Ciudid Universitaria "Rodrigo Facio," Costa Rica, Central America.

[4] Present address: Animal Sciences Division, Monsanto Co., 700 Chesterfield Village Parkway, St. Louis, MO 63198.

[5] To whom reprint requests should be addressed at Department of Animal Science, Iowa State University, 11 Kildee Hall, Ames, IA 50011.

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sheep, and cattle (4–6). The effects of GHRF on GH release in HST cattle, however, have not been reported. This study was designed to investigate the effects of HST surgery on GH secretion and of GHRF on GH release as a single-pulse dose or as a multiple-pulse dose as influenced by feeding, to verify that the pituitary would respond to other exogenous stimuli after HST, and to examine the interactions of GHRF and SRIH on GH release in prepubertal HST calves.

Materials and Methods. *Animals.* Seven crossbred beef heifers (4 months old, 111 ± 8 kg body wt (±SE)) were individually penned with straw bedding in an environmentally controlled room at 18°C with 12-hr photoperiods. They were fed at 12-hr intervals a diet consisting of 49% cracked corn, 39% dehydrated alfalfa, 5.6% solvent-extracted soybean meal, 5.6% cane molasses, 0.5% dicalcium phosphate, 0.2% iodized salt, 0.02% trace mineral, and 0.1% vitamin A (5.2 million IU/kg). Animals were fed in the morning, and experiments were conducted in the afternoon. An indwelling catheter (Tygon microbore tubing, 1.27-mm i.d., Fisher Scientific, Pittsburgh, PA) was inserted in a jugular vein before surgery and maintained for all experiments. The catheters were filled with sterile saline containing 100 U heparin/ml between experiments and with sterile saline with 40 U heparin/ml when blood samples were being obtained. Blood samples were treated with heparin (4 U/ml), and the plasma was stored at −20°C until radioimmunoassay for GH (7). The GH assay had intra- and interassay coefficients of variation of 1.7 and 9.0%, respectively.

Peptides. Animals received bolus iv injections of human pancreatic (hp) hpGHRF (1–40)OH (8), rat hypothalamic (rh) rhGHRF(1–32)OH, rat hypothalamic (rh) [Nle^{27}]rhGHRF(1–29)NH_2 (9), and thyrotropin-releasing hormone (TRH, Sigma Chemical Co., St. Louis, MO). Somatostatin(SS)-14 (SRIH) was administered iv by continuous-infusion pumps (Harvard Model 1201, Harvard Apparatus, Millis, MA). The GHRF, TRH, and SRIH were dissolved in 0.1% acetic acid (1 μg/μl) and then diluted with a sterile buffer solution. The buffer was physiological saline (0.818% NaCl) containing 0.01 *M* $NaH_2PO_4 \cdot H_2O$, pH 7.0, 0.01% ascorbic acid, and 1% bovine serum albumin. Solutions for injection and infusion were prepared the day of experimentation.

Surgery. Commencing 3 hr before anesthesia and surgery and continuing throughout surgical intervention, blood was sampled at 15-min intervals. Subsequently, blood was sampled hourly for 5 hr, then at 6-hr intervals for 30 hr. Hypophysial stalk transection (HST) was performed in five calves by a supraorbital approach described previously (10, 11). Two sham-operated controls (SOC) were subjected to the same surgical procedures, with the exception that the hypophysial stalk was not severed. Anesthesia was induced by iv injection of thiamylal sodium (0.5–1.0 g, Surital, Parke–Davis, Morris Planes, NJ) and maintained by a closed-circuit system of halothane (1–4%, Ayerst Laboratories, New York, NY) and O_2 (400–800 ml/min). After the hypophysial stalk was severed, a nylon disk (8.0-mm diameter and 0.45-mm thickness) was inserted between the severed ends of the tubular stalk to prevent regeneration between the hypothalamus and the pituitary gland. Water and food intake returned to normal 6–24 hr after surgery. Postmortem examination of each heifer confirmed the completeness of stalk transection. The nylon disk was in the proper location and had prevented vascular regeneration of the stalk in each calf. The pituitary gland was cut transversely and fixed in Susa's solution for histological evaluation. The glands were sectioned at 6 μm and stained with performic acid–Alcian blue–periodic acid–Schiff–orange G by the method of Heath (12), whereas other sections were stained with hematoxylin and eosin.

hpGHRF dose response. Three and four days after surgery, the HST calves (170 kg) received 0.067 and 0.133 μg hpGHRF/kg body wt, respectively, to test the GH secretory response. Plasma samples were collected 20, 10, and 1 min before the saline control period; 5, 10, 15, 20, 40, and 60 min during the control period; and 5, 10, 15, 20, 40, 60, 80, 100, and 120 min after GHRF injection.

TRH response. Five days after surgery, the HST calves were injected iv with 100 μg TRH to determine whether the isolated pituitary gland would respond to other exogenous stimuli at a pharmacological dosage

known to release GH in intact calves. Plasma sampling was the same as that described in the first experiment.

Multiple injections of hpGHRF. Eight days after surgery, the HST calves received two iv injections of 0.067 μg hpGHRF/kg body wt at 3-hr intervals, with feeding 70 min after the first hpGHRF injection. Plasma was sampled at 20-min intervals before the first hpGHRF injection, at 5-min intervals for the first 20 min after hpGHRF injection, and at 20-min intervals thereafter.

SRIH and hpGHRF on GH secretion. Nineteen days after surgery, two HST calves (183 kg) were infused with 0.033 or 0.067 μg SRIH/(kg body wt * min) for 75 min in a crossover design. After 30 min of SRIH infusion, 0.067 μg hpGHRF/kg body wt was administered iv as a bolus injection. Plasma was sampled 30, 15, and 1 min before SRIH infusion; 15, 29, 35, 40, 45, 50, and 70 min during SRIH infusion and hpGHRF injection; and 90, 110, 130, and 150 min after SRIH infusion.

rhGHRF dose response. Five months after surgery, the GH response to [Nle^{27}]rhGHRF (1–29)NH_2 was compared in three HST calves (245 kg body wt) and two SOC calves (304 kg body wt). The doses tested were 0, 0.0083, 0.0165, 0.033, 0.067, and 0.133 μg rhGHRF/kg body wt. Plasma was collected 20, 10, and 1 min before injection and 5, 10, 15, 20, 40, 60, 80, 100, and 120 min after injection. The experiment was repeated at 6 months after surgery using the same dosages of another analog, rhGHRF(1–32)OH.

Statistical analyses. The experimental units in this study were the individual calves. Dose–response data were analyzed by using a Latin square design while the other data were analyzed by using Student's *t* test for comparisons among treatment groups (13, 14).

Results. *Surgery.* In the seven calves subjected to either HST or SOC, the plasma GH levels during surgery and immediately in the postoperative recovery period are depicted in Fig. 1. GH secretion during the preanesthesia period, 9 ± 3.9 ng/ml (mean values from −345 to −195 min), was variable, but tended to decrease just preceding anesthesia. The first 60 min after the initiation of anesthesia (−180 to −135 min), GH increased to 15 ± 4.6 ng/ml, a concentration greater than that in SOC calves during the first 90 min after time zero (3 ± 0.7, $P < 0.05$) and all the periods ($P < 0.05$) following time zero in the HST calves (0–90 min, 4 ± 0.8 ng/ml; 2–5 hr, 2.5 ± 0.8 ng/ml; 6–30 hr, 3 ± 0.8 ng/ml).

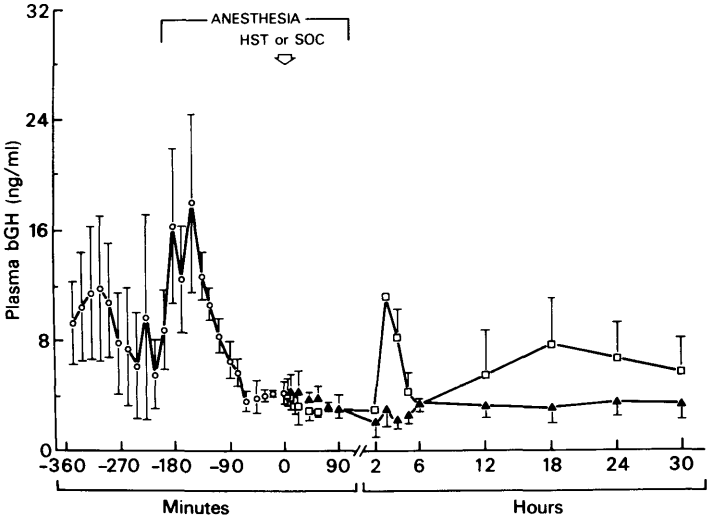

FIG. 1. Plasma GH concentrations in beef calves before and after surgery are illustrated. The GH response indicated by the open circles illustrates the period before surgical intervention, which is indicated by the open arrow ($n = 7$). Plasma GH after HST is illustrated by closed triangles ($n = 5$), and GH after SOC is indicated by squares ($n = 2$). Values are means ± SE. SE bars for samples taken 2 and 3 hr after surgery are within the symbol.

Growth hormone returned to presurgery baseline concentrations at 3–4 hr 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 ng/ml; HST, 3 ± 1.0 ng/ml; $P < 0.05$).

Histology. Histological examination of pituitary glands from HST calves indicated the persistence of secretory cells in the same areas of the adenohypophysis as those in SOC calves (Fig. 2). In sections from HST and SOC calves stained with performic acid–Alcian blue–periodic acid–Schiff–orange G, acidophils, basophils, and chromophobes were present. Pituitary gland weight was 35% less ($P < 0.01$) in HST (0.91 ± 0.05 g; 0.2 ± 0.01 g/100 kg body wt) than in SOC (2.59 ± 0.20 g; 0.6 ± 0.03 g/100 kg body wt) calves.

hpGHRF dose response. The HST calves responded to 0.067 and 0.133 μg hpGHRF/kg body wt with a rapid increase in plasma GH, which peaked within 10–20 min and then declined to preinjection concentrations within 60 min (Fig. 3). After HST, all animals 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. During the control period, mean GH (5–20 min after injection of saline) was 5 ± 0.3 ng/ml, which was less than 55 ± 16 ng/ml ($P < 0.04$) during the 0.067-μg hpGHRF period (5–20 min after hpGHRF). The mean of the control period during the 0.133-μg hpGHRF trial was 4 ± 0.4 ng/ml, which contrasts with the hpGHRF period mean of 33 ± 11 ng/ml ($P < 0.06$). The GH response between the two hpGHRF doses was not statistically different.

TRH response. GH secretion in response to TRH was variable in HST calves. Only two of the five HST calves responded to TRH injections by an increase in GH similar to that evoked by hpGHRF; GH levels were slightly less during vehicle injections and the response to TRH was modest in the other three animals (Fig. 4). Although only 40% of them responded to a TRH challenge, all HST calves released GH in subsequent treatments with hpGHRF and rhGHRF.

Multiple injections of hpGHRF. Eight days after surgery, all HST calves responded to the two injections of hpGHRF (Fig. 5). Plasma concentrations of GH were the same before each injection of hpGHRF (3.7 and 3.6 ng/ml) and significantly lower than those after hpGHRF ($P < 0.05$). The second injection of hpGHRF, which occurred after feeding of the calves before surgery and after HST, did not increase plasma GH to the same extent as the first injection (9 vs 17 ng/ml, $P < 0.09$).

SRIH and hpGHRF on GH secretion. Infusion of 2 and 4 μg SRIH (kg body wt * hr) or equivalent to 0.033 and 0.067 μg SRIH * kg body wt * min depressed GH release in response to an administered bolus of 0.067 μg hpGHRF/kg body wt (Fig. 6). GH levels 20 min after hpGHRF were 9 ± 1.6 ng/ml during infusion of 0.033 μg SRIH and 5 ± 0.4 ng/ml during infusion of 0.067 μg 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 infusions of SRIH were stopped, GH concentrations remained stable.

rhGHRF dose response. Plasma GH was stimulated in a dose-dependent manner by two analogs of rat hypothalamic GHRF in HST and SOC calves (Tables I and II). All animals responded to either analog of rhGHRF when the dosage was 0.0165 μg rhGHRF/kg body wt or greater. No differences in plasma GH response between HST and SOC calves were observed.

Discussion. This was the first demonstration that plasma GH concentrations were acutely altered by the surgical procedures of HST and SOC in prepubertal calves. When the anesthesia was administered, GH increased abruptly for 60 min. After the anesthesia was discontinued, GH returned to presurgery concentrations in the SOC calves, whereas it dropped to low basal levels in HST calves. The return of episodic GH secretion in SOC calves and its absence in HST calves were similar to results reported by Anderson *et al.* (1, 15). In contrast, basal GH secretion in HST pigs remained greater than that in SOC animals (2, 16). Although plasma prolactin levels were not altered by anesthesia in these calves, HST caused consistently greater prolactin secretion than that in SOC animals (17, 18).

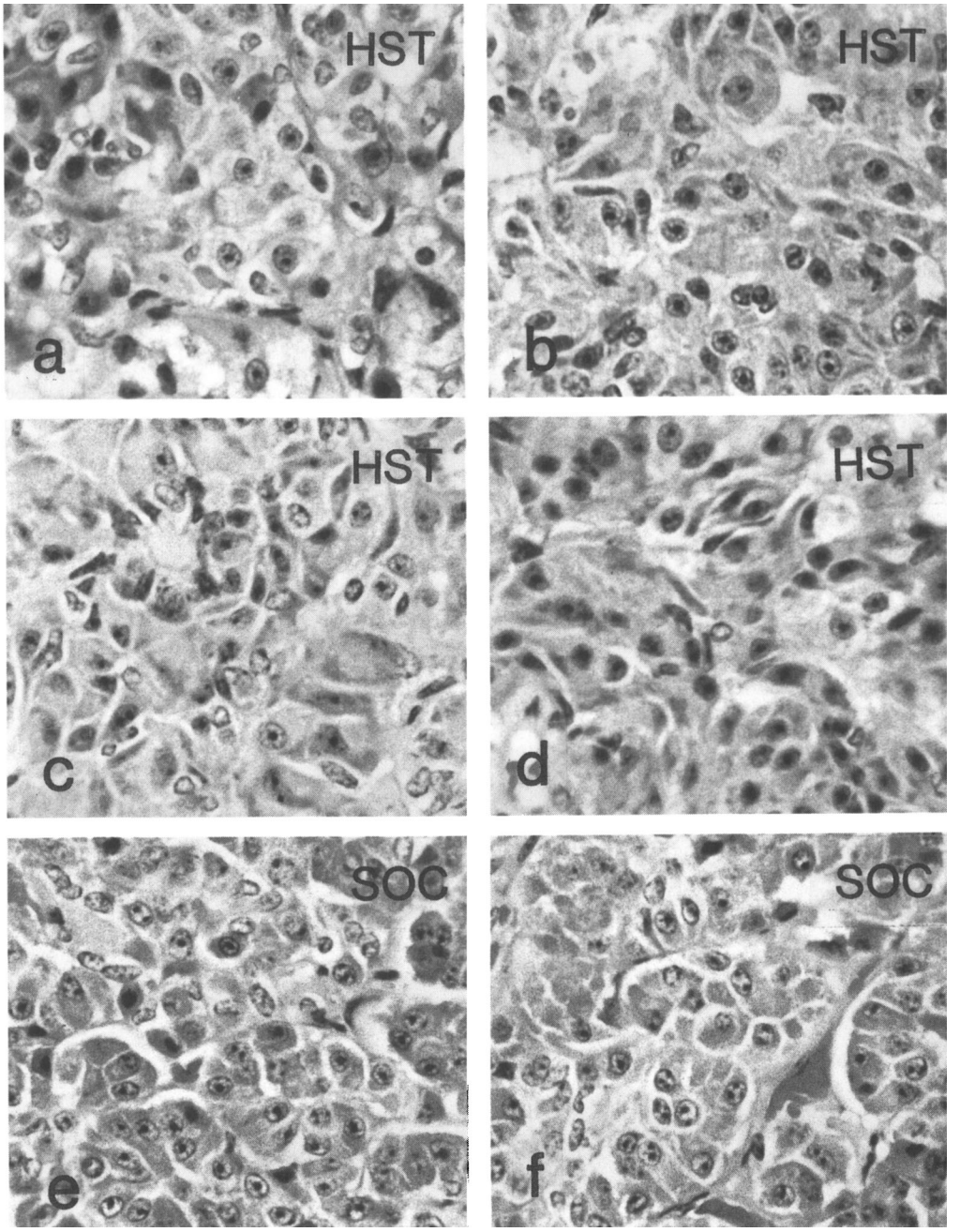

FIG. 2. Photomicrographs of adenohypophysis of four HST (a, b, c, and d) and two SOC (e and f) calves. Histological cross sections are from the middle one-third of the anteromedial part of the adenohypophysis. Acidophils with cytoplasm were dispersed in anteromedial regions of the adenohypophysis in both groups of HST and SOC calves. Acidophils are associated with somatotrophs, lactotrophs, and adrenocorticotrophs. Chromophobes were evident throughout the adenohypophysis in HST and SOC calves. Histological sections indicate survival of adenohypophysial cells in HST calves (×360).

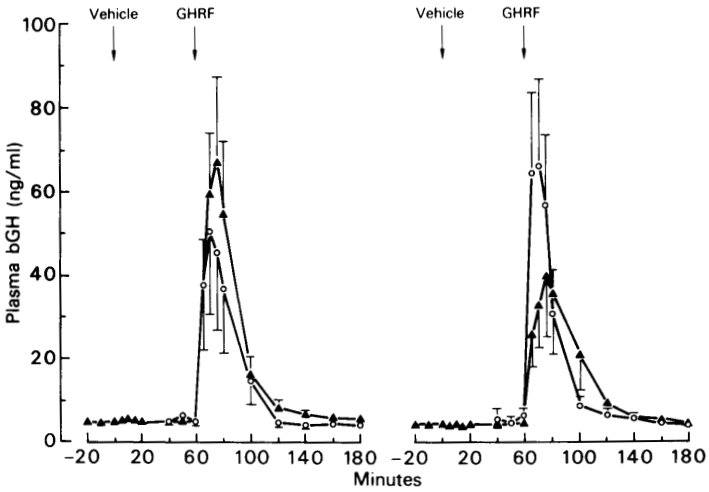

FIG. 3. Plasma GH concentrations in HST calves in response to 0, 0.067, and 0.133 μg (1–40)-OH hpGHRF/kg body wt are illustrated by the closed triangles (n = 5). For comparison, the response to 0.067 and 0.133 μg (1–40)-OH hpGHRF/kg body wt before surgery in these animals is indicated by the open circles in the left and right panels, respectively. The GH response to 0.067 μg 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.

The pattern of abrupt GH release in response to GHRF in HST calves was similar to that seen before surgery and in SOC animals. The high amplitude of the GH response to the first injection of hpGHRF at 3 days after surgery could not be replicated by

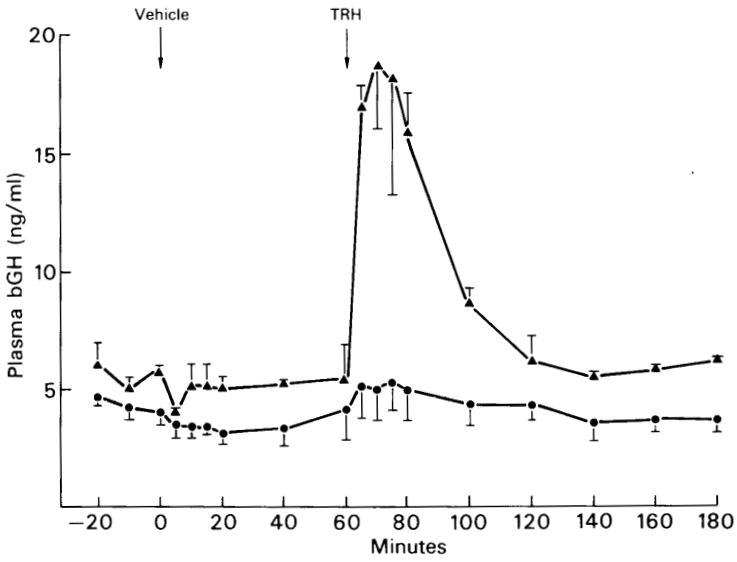

FIG. 4. The GH responses of HST calves receiving 0 and 100 μg TRH are illustrated (n = 5). The time of vehicle and TRH injection is indicated by the arrows. TRH caused an abrupt rise in GH secretion in two HST calves (▲), whereas in the other three calves (●) TRH caused a modest effect on GH secretion. Values are means ± SE.

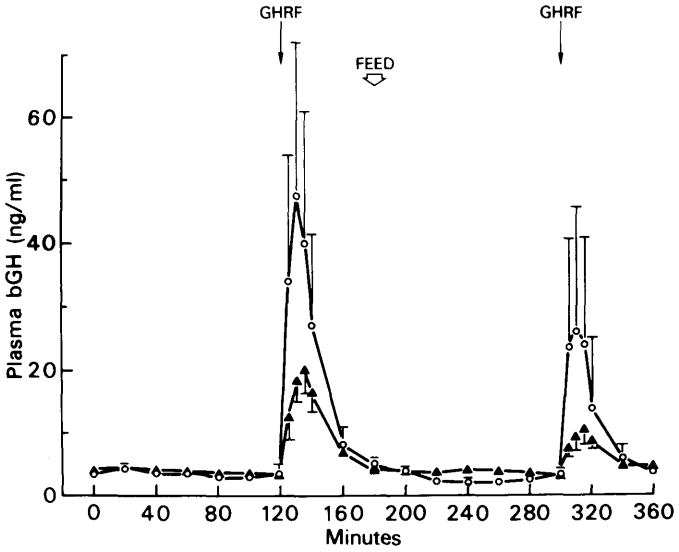

FIG. 5. Plasma GH in HST calves receiving 0.067 μg hpGHRF/kg body wt at 3-hr intervals over 6 hr are indicated by the closed triangles (n = 5). The response to the same treatment before HST is shown by the open circles. The time of feeding and hpGHRF injections are illustrated by arrows. Values are means ± SE.

later challenges. This may have been the result of a large stored GH pool in the pituitary gland which had been stimulated by endogenous GHRF before surgical manipulation. Because endogenous GHRF could not affect the pituitary gland after HST, the stored GH pool in the pituitary likely diminished. There was a dose dependency to rhGHRF in HST calves several months after surgery, which is similar to the GH response to hpGHRF or

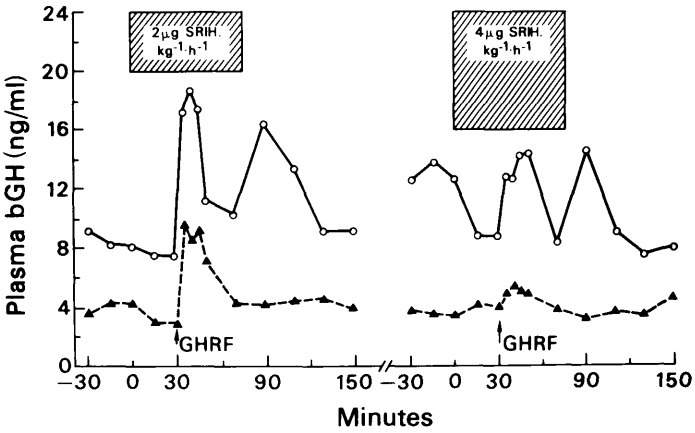

FIG. 6. Mean concentration of plasma GH in HST calves during iv infusion of 2 and 4 μg somatostatin(SS)-14 (SRIH)/(kg body wt * hr) or equivalent to 0.033 and 0.067 μg SRIH/kg body wt * min and injected with 0.067 μg (1–40)-OH hpGHRF/kg body wt are illustrated by closed triangles (n = 2). The same animals' 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.

TABLE I. EFFECT OF RAT HYPOTHALAMIC GHRF, [Nle^{27}]rhGHRF(1-29)NH_2, ON PLASMA GH LEVELS IN HST AND SOC CALVES 5 MONTHS AFTER SURGERY

Dose of [Nle^{27}]rhGHRF(1-29)NH_2 given iv (μg/kg body wt)	Plasma GH concentration[a] (ng/ml)	
	HST calves	SOC calves
0.0000[b]	3.0 ± 0.6	5.6 ± 1.2
0.0083	6.7 ± 2.1	9.4 ± 0.9
0.0165	12.2 ± 3.7	10.9 ± 3.4
0.033	12.8 ± 5.4	14.7 ± 2.0
0.067	16.5 ± 5.8	14.4 ± 5.6
0.133	24.6 ± 7.4	8.5 ± 1.8

[a] Values are means ± SE.
[b] Vehicle consisting of 0.01 *M* $NaH_2PO_4 \cdot H_2O$, 0.01% ascorbic acid, and 1% bovine serum albumin.

rhGHRF seen in HST pigs (16). Furthermore, both analogs of rat hypothalamic GHRF were effective in causing GH release in HST as well as SOC calves.

The pituitary in HST calves was capable of releasing GH in response to TRH, but only 40% of the animals responded to the TRH injections, whereas 100% of them responded to hpGHRF and rhGHRF. This variable response to TRH has also been observed in intact cattle (19). Szabo (20) demonstrated that GHRF and TRH act through different mechanisms in the pituitary to release GH. The release of GH by GHRF is cAMP- and Ca^{2+}-mediated, whereas TRH mediates GH release by a cAMP-independent, Ca^{2+}-dependent process. The different mechanisms for mediation of GH release by the peptides may account for the variation in the ability to respond to the stimuli.

When HST calves were subjected to hpGHRF at 3-hr intervals, they responded to the first hormone injection with a GH release greater than that to the second hpGHRF injection, which occurred after feeding. The depression in GH response to the same dose of hpGHRF after 3 hr may be related to effects of feeding, number of GH-release challenges since surgery, or down regulation of the pituitary. The calves were fed between GH-release challenges during the presurgery period. These results are similar to the animals' response before surgical manipulation; however, the magnitude of the GH response to 0.067 μg hpGHRF before and after feeding was enhanced after surgery. In studies with sheep, feeding was observed to depress the GH response to GHRF (21, 22).

During SRIH infusion, GH release in response to hpGHRF was diminished in HST calves compared with that seen in the same animals before surgical intervention. After SRIH infusion, plasma GH was not altered. This contrasts with intact calves in which a somatotropic rebound was observed after SRIH withdrawal (23–26). Because the pituitary was no longer under endogenous SRIH and GHRF regulation after HST, it may not need to compensate for the GH-release suppression during SRIH infusion periods in these calves. For example, when these same treatment regimens of GHRF and SRIH were conducted before surgical manipulation, the variation of GH secretion was much greater than that after HST. The mean of all experimental coefficients of variation before surgery was 61%, whereas after HST it was reduced to 17% ($P < 0.001$). The reduced variation of GH secretion after HST may be explained by the lack of episodic secretion of GH that has been observed previously in HST calves (1, 15).

TABLE II. EFFECT OF RAT HYPOTHALAMIC GHRF, rhGHRF(1-32)OH, ON PLASMA GH LEVELS IN HST AND SOC CALVES 6 MONTHS AFTER SURGERY

Dose of rhGHRF(1-32)OH given iv (μg/kg body wt)	Plasma GH concentration[a] (ng/ml)	
	HST calves	SOC calves
0.0000[b]	1.7 ± 0.2	2.1 ± 0.2
0.0083	3.1 ± 0.8	3.2 ± 0.8
0.0165	3.4 ± 0.5	4.2 ± 0.6
0.033	5.9 ± 1.9	7.3 ± 1.5
0.067	7.4 ± 2.9	19.0 ± 6.5
0.133	17.4 ± 9.9	26.0 ± 3.6

[a] Values are means ± SE.
[b] Vehicle consisting of 0.01 *M* $NaH_2PO_4 \cdot H_2O$, 0.01% ascorbic acid, and 1% bovine serum albumin.

In conclusion, this is the first demonstration of a GH response to hpGHRF and rhGHRF injections in HST calves. Without the hypophysial stalk connection, the calves always responded to GHRF challenges. This contrasts with the SOC calves as well as the calves before HST in that they responded to 80% of the GHRF challenges. After HST, the pituitary remains capable of a positive response to other exogenous substances such as TRH, as well as of secreting amounts of prolactin greater than those found in SOC calves (17, 18). Finally, the somatotropic rebound observed in intact calves after SRIH withdrawal is not observed after HST. After HST, the pituitary was able to respond to a variety of stimuli; however, desensitization of the pituitary may occur during the period immediately after surgery, but the calves remain responsive to GHRF challenges several months later. Furthermore, the HST calves provide an appropriate *in vivo* model to study the effects of releasing and inhibiting factors which act on the pituitary to regulate GH secretion. Because normal episodic GH secretion is eliminated after HST, different releasing and inhibiting substances can be tested to determine the physiological regulation of GH secretion.

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