PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 172, 508–513 (1983)

Growth Hormone Secretion after Hypophysial Stalk Transection in Pigs[1] (41596)

JOHN KLINDT, J. J. FORD, J. G. BERARDINELLI,[2] AND L. L. ANDERSON[3]

Department of Animal Science, Iowa State University, Ames, Iowa 50011, and Roman L. Hruska U.S. Meat Animal Research Center, U.S. Department of Agriculture, Science and Education Administration, Agricultural Research Service, Clay Center, Nebraska 68933

Abstract. The level and pattern of growth hormone (GH) secretion were investigated in mature ovariectomized gilts after hypophysial stalk transection and sham operation. A nylon disk was inserted between severed ends of the stalk to prevent vascular regeneration. Blood samples were collected via indwelling jugular catheters at 15-min intervals for 3 hr, 2 days before surgery (Day −2) and 2 days after surgery (Day +2), and at 4-hr intervals from Day +3 to Day +8. Mean overall serum concentrations of GH after hypophysial stalk transection remained similar ($P > 0.05$) to presurgical levels. These mean concentrations also were similar to those in sham-operated and unoperated controls. However, hypophysial stalk transection significantly dampened ($P < 0.05$) the episodic secretion of GH and resulted in elevated basal blood concentrations of the hormone as compared with either presurgical levels or those in the two control groups. These results indicate that synthesis and secretion of GH continue in the absence of hypothalamic control in hypophysial stalk-transected gilts. Thus, the hypothalamus is required for regulation of both episodic release and the tonic inhibition of basal secretion of growth hormone in the pig.

Evidence resulting from several experimental approaches indicates that secretion of growth hormone (GH) by the adenohypophysis is under the influence of a dual system of hypothalamic control, inhibitory and stimulatory (1). Extrahypothalamic peptides, such as gastrointestinal and pancreatic hormones, also can stimulate and inhibit GH secretion but their influence on neurophysiological control of GH secretion is unclear (1, 2). The minute-to-minute regulation of GH secretion depends upon neural influences that are largely independent of metabolic events (1). Metabolic changes during prolonged periods of rapid growth, glucose intolerance, protein depletion, and psychogenic disorders may affect both basal and episodic GH secretion (1). Deafferentation studies indicate that the stimulatory regulation of GH occurs in the anterior portion of the medial basal hypothalamus whereas the caudal portion of this region is primarily inhibitory (3, 4). This control of hypophysial GH secretion seems to be mediated through a hypothalamic releasing hormone and a hypothalamic release-inhibiting hormone (1). GH release-inhibiting hormone, somatostatin (SRIH), from ovine and porcine hypothalami and GH-releasing hormone (GHRH) have been purified and characterized from porcine hypothalami and human pancreatic tumor, but their physiological roles are unknown (5–8). The objective of the present study was to determine the role of the hypothalamus in the regulation of GH secretion and the effects of hypophysial stalk transection on sequential profiles of GH secretion in sexually mature gilts.

Materials and Methods. *Animals.* Yorkshire gilts [211 ± 5 (mean and SE) days of age, 122 ± 7 kg body weight] were used in this study. Each gilt was ovariectomized and implanted with a chronic jugular catheter (9) 3

[1] This study was supported in part by the U.S. Department of Agriculture, ARS, CSRS, Cooperative Agreement No. 58-519B-9-863. Journal Paper J-10712 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA; Projects 2443 and 2444. Mention of trade names, or companies does not constitute an implied warranty by the U.S. Department of Agriculture, Iowa State University, or the authors. This investigation was presented in part at the 74th Annual Meeting of the American Society of Animal Science, Guelph, Ontario, 1982.

[2] Present Address: Department of Animal and Range Sciences, Montana State University, Bozeman, Montana 59715.

[3] Address reprint requests to: Dr. L. L. Anderson, Department of Animal Science, 11 Kildee Hall, Iowa State University, Ames, Iowa 50011.

0037-9727/83 $1.50

days before hypophysial stalk transection (HST). During the afternoon, 2 days before HST (Day −2), blood samples were collected at 15-min intervals for a 3-hr period. Serum was harvested and frozen 18 to 24 hr after blood collection. On the day of cranial surgery (Day 0) the gilts were assigned randomly to three treatment groups: UC, unoperated control group ($N = 6$); SOC, sham-operated control group ($N = 6$); and HST, hypophysial stalk transection group ($N = 8$). The HST and sham surgeries were performed as described by Anderson *et al.* (10, 11). Two days after surgery (Day +2) blood samples were collected as on Day −2. Blood samples also were collected at 4-hr intervals for a 120-hr period beginning Day +3. Nine days after HST the gilts were killed and completeness of stalk transection was confirmed. The pituitary gland was cut transversely and fixed in Susa's solution for subsequent histological evaluation. The glands were sectioned at 6 μm and stained with performic acid–Alcian blue–periodic acid–Schiff–orange G, whereas other sections were stained with hematoxylin and eosin.

Radioimmunoassay. Serum concentrations of porcine GH (pGH) were measured by using a double-antibody homologous radioimmunoassay (12) following modification. Radioiodinated pGH was prepared by a lactoperoxidase method (13). Fifty microliters 0.5 *M* phosphate buffer, pH 7.5; 0.5 mCi Na ^{125}I in 12.5 μl 0.5 *M* phosphate buffer; 5 μl lactoperoxidase (1 mg/ml 0.5 *M* phosphate buffer); and 10 μl 0.001% hydrogen peroxide was added to 2 μg GH (USDA pGH-B1). The reaction was allowed to proceed for 5 min before addition of 0.5 ml cold 0.01 *M* phosphate-buffered saline (PBS), pH 7.4. The reaction mixture was transferred to a 1.5 × 50-cm column of Sephadex G-100. The column was eluted with PBS, pH 7.4, and 2-ml fractions were collected into 0.2 ml PBS–5% bovine serum albumin (BSA). The fractions and an aliquot of the reaction mixture were checked for total and tricholoracetic acid (TCA) precipitable radioactivity. Fractions considered to contain monomeric pGH were pooled. The TCA precipitability of the reaction mixture exceeded 50%, and that of the pooled fractions was 75% or greater. Radioiodinated pGH was 30–35% precipitable by the antiserum at a 1:42,000 dilution in the assay.

Each assay tube contained 600 μl buffer (PBS–1% BSA, pH 7.4) and sample (up to 300 μl), 200 μl antiserum at 1:42,000 dilution in 1:400 normal rabbit serum in PBS–50 m*M* EDTA, pH 7.4, and 100 μl goat anti-guinea pig gamma globulin diluted in PBS. The reaction was incubated for 24 hr at 4°C before addition of ^{125}I-pGH, an additional 24 hr before addition of the second antibody, and for 72 hr before termination of the assay by addition of 2 ml PBS. Antibody-bound and free ^{125}I-pGH were separated by centrifugation. Assay parameters and pGH concentrations were calculated utilizing a weighted logit-log regression equation (14). The reference preparation was USDA pGH-B1 which has biological potency of 5.4 IU/mg by the rat tibia assay. Addition of pLH, pFSH, and pPRL in doses up to 100 ng/tube did not inhibit binding of ^{125}I-pGH. Dilutions of adult and fetal serum and a porcine pituitary extract inhibited ^{125}I-pGH binding in a dose-dependent manner parallel to the purified pGH preparation. Sensitivity of the assay was 0.1 ng/tube. Interassay and intrassay coefficients (mean ± SE) of variation were 18.7 ± 3.8% ($N = 4$/sample, three samples) and 6.4 ± 2.5% ($N = 6$/sample, four samples).

The mean of all observations within each sampling period for each animal is the overall mean concentration or level of secretion. Basal concentrations of pGH were defined as the mean of all observations at the three lowest values within each individual sampling period for each animal. The number of observations comprising secretory spikes was determined. A secretory spike was defined as hormone values which exceeded the mean by two standard deviations. Means of the parameters of the secretory patterns across groups were tested by Student's *t* test. Means within treatment groups were tested by the paired Student's *t* test.

Results. Postmortem examination of each HST gilt indicated that the hypophysial stalk had been completely severed. The nylon disk was in the proper location and had prevented stalk regeneration in each of the eight gilts. Although pituitary gland weight in HST gilts averaged less than SOC animals (249 mg vs 362 mg), histological examination of them from HST gilts indicated peristence of at least some secretory cells in the same areas of the adenohypophysis as SOC animals (11).

TABLE I. MEAN PERIPHERAL SERUM CONCENTRATIONS OF GROWTH HORMONE BEFORE AND AFTER SURGERY IN THE PIG

Surgical group[a]	No. of gilts	Day −2	Day +2	Ratio Day +2/Day −2	Day +3 to Day +8
		Overall mean concentration[b]			
		ng/ml	ng/ml		ng/ml
UC[a]	6	1.2 ± 0.2	1.3 ± 0.2	1.11 ± 0.15 (0.70–1.69)[c]	1.5 ± 0.3
SOC	6	1.6 ± 0.2	1.5 ± 0.2	1.09 ± 0.21 (0.39–2.0)	1.3 ± 0.1
HST	8	1.4 ± 0.2	1.5 ± 0.2	1.10 ± 0.09 (0.67–1.40)	1.3 ± 0.1
		Mean basal concentration[b]			
		ng/ml	ng/ml		ng/ml
UC	6	0.9 ± 0.40	1.0 ± 0.1	1.09 ± 0.05 (0.93–1.32)	0.8 ± 0.1
SOC	6	1.1 ± 0.13	1.1 ± 0.1	0.98 ± 0.07 (0.80–1.33)	0.8 ± 0.1
HST[d]	8	1.1 ± 0.07	1.3 ± 0.1	1.31 ± 0.10 (0.97–1.87)	1.0 ± 0.1

[a] UC = unoperated control, SOC = sham-operated control, HST = hypophysial stalk transection.
[b] Values are means ± SE. Means within columns were not significantly different ($P > 0.05$).
[c] Range of individual animal values.
[d] Day −2 less than Day +2 ($P < 0.05$).

TABLE II. MEAN ESTIMATES OF VARIATION OF GROWTH HORMONE IN PERIPHERAL SERUM OF PIG

Surgical group[a]	No. of gilts	Day −2	Day +2	Ratio Day +2/Day −2	Day +3 to Day +8
		Within animal coefficients of variation[b]			
		(%)	(%)		(%)
UC	6	32 ± 8	38 ± 6[f]	1.77 ± 0.50 (0.27–2.72)[c]	60 ± 14[h]
SOC	6	36 ± 7	44 ± 11[g]	1.98 ± 0.81 (0.23–5.96)	37 ± 16[i]
HST[e]	8	33 ± 6	13 ± 2[f,g]	0.47 ± 0.07 (0.12–0.74)	17 ± 2[h,i]
		No. observations greater than twice standard deviation[b]			
UC	6	0.7 ± 0.2	0.7 ± 0.3[j]	—[d]	1.7 ± 0.2
SOC	6	1.0 ± 0.0	0.8 ± 0.3[k]	—	1.2 ± 0.4
HST[e]	8	0.8 ± 0.2	0.0 ± 0.0[j,k]	—	0.6 ± 0.2

[a] UC = unoperated control, SOC = sham-operated control, HST = hypophysial stalk transection.
[b] Values are means ± SE.
[c] Range of individual animal values.
[d] Not calculable because some denominators are zero.
[e] Day −2 greater than Day +2 ($P < 0.05$).
[f–k] Means within columns which are significantly different ($P < 0.05$) are denoted by the same superscript.

Means and estimates of variation for the various parameters of the GH secretory patterns are presented in Tables I and II. It is evident from the peripheral GH concentrations presented in Fig. 1 and the means presented in Table I, that surgical treatment and day of sampling had no effect ($P > 0.05$) on the level of GH secretion as reflected by the overall mean concentrations. The peripheral GH concentrations as well as the coefficients of variation presented in Table II demonstrate that episodic GH secretion is significantly decreased by severing the connections between the pituitary and hypothalamus.

The peripheral concentrations of pGH on Days −2 and +2 in representative animals from each treatment group are presented in Fig. 1. The results from control sampling periods, indicate that pGH concentration in the peripheral circulation fluctuates in a manner indicative of episodic secretion. Figure 2 and Table II present the peripheral serum patterns and mean values of pGH for the Days +3 to +8 sampling period. The results from this relatively infrequent, yet extensive sampling period confirm those observed from the intensive samplings and indicate that HST decreases episodic pGH secretion with minimal or no effect on overall level of hormone secretion.

Discussion. The level of GH remained relatively constant but its pattern of secretion was significantly altered after hypophysial stalk transection in the pig. Peripheral concentrations of GH fluctuated in a manner indicative of episodic secretion in blood obtained before HST, before and after SOC as well as in unoperated controls in this study as has been observed in other species (15–17). After hypophysial stalk transection in these gilts this episodic pattern of GH secretion was abolished. This decrease with no change in overall mean concentration of GH is indicative of a higher level of basal secretion in the HST gilts, and supported by mean basal GH concentrations that were 30–35% greater in HST animals as compared with controls. An elevated level of circulating GH after HST suggests that it is regulated in part by tonic hypothalamic inhibition, possibly through SRIH, which depresses basal GH secretion in the pig.

The presence of acidophilic somatotrophs in the pituitary 9 days after HST indicated viability of the gland in each of these gilts.

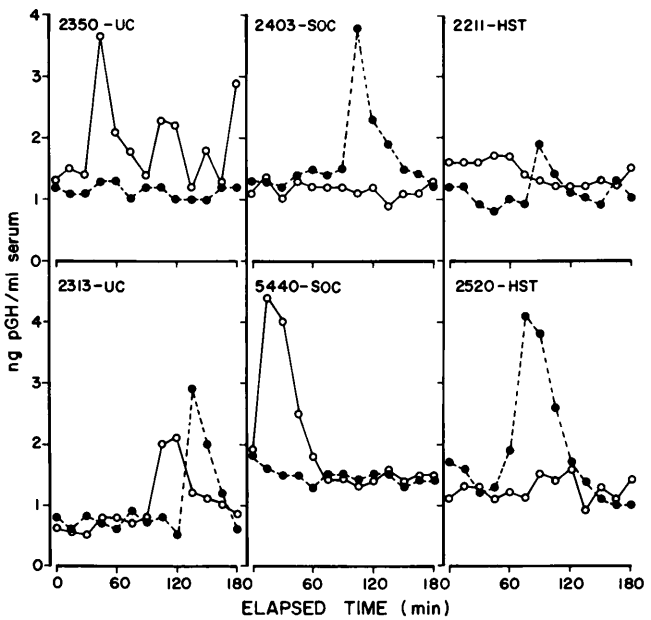

FIG. 1. Sequential profiles of peripheral serum concentrations of GH in two gilts from each of the treatment groups (UC, unoperated control; SOC, sham operated control; HST, hypophysial stalk transection) on Day −2 (●) and Day +2 (○). Hypophysial stalk transection or sham operation was performed on Day 0. Four-digit numbers designate individual gilts.

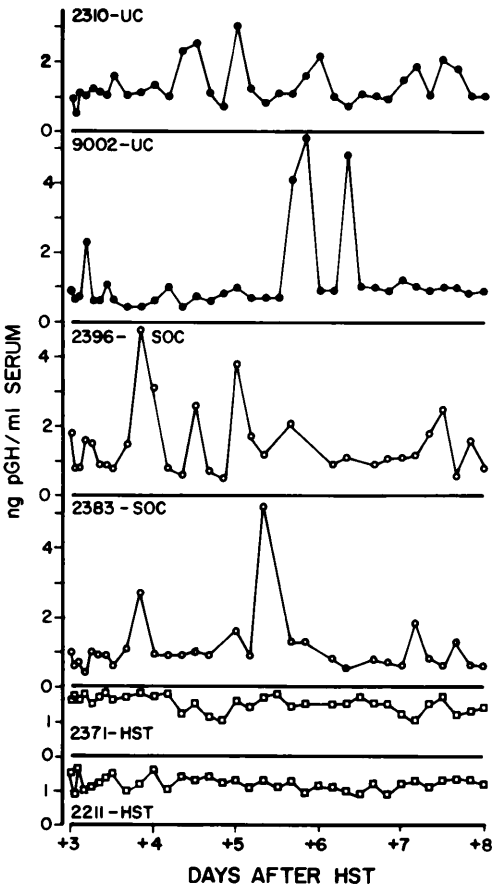

FIG. 2. Sequential profiles of peripheral serum concentrations of GH in two gilts from each treatment group (UC, unoperated control; SOC, sham operated control; HST, hypophysial stalk transection) during a 120-hr period from Day +3 to Day +8. HST or SOC was performed on Day 0. Four-digit numbers designate individual gilts. Symbols indicate unoperated control (●), sham-operated control (○), and hypophysial stalk-transected (□) animals.

Viable somatotrophs after HST suggest that the absence of episodic GH secretory activity resulted from lack of stimulatory influences. PRL concentrations in peripheral serum are twofold greater in these HST gilts than in sham-operated controls (11). This observation is consistent with the hypothesis that the major hypothalamic influence on PRL secretion is inhibitory and provides further evidence of the viability of adenohypophysial cells in HST gilts. In contrast after HST of beef calves and pregnant beef heifers, both GH and PRL concentrations in peripheral serum decrease as compared with sham-operated controls (18–20). GH and PRL secretion are markedly influenced by seasonal changes in this species. PRL secretion remains positively correlated whereas GH secretion is unaffected by seasonal changes after HST of calves and pregnant heifers (18, 20). Histological examination of pituitary glands from these HST calves and heifers indicated acidophils in same areas as in controls.

The episodic release of porcine GH is abolished after hypophysial stalk transection, but no significant changes in the overall secretion level of this hormone are observed in this species. This is consistent with the view that hypothalamic regulation of porcine GH secretion is regulated by inhibitory and stimulatory mechanisms. The increased basal level of circulating GH after HST indicates that GH secretion is under tonic inhibitory influences in the pig. The results from this study clearly indicate the dependency of the pituitary gland upon the hypothalamus for the episodic release of GH in this species.

We thank M. E. Shell, C. R. Bohnker, W. G. McDonald, D. S. Keeney, J. R. Molina, A. Alcivar, C Van Orden, and D. Taubenheim for excellent technical assistance, and Dr. R. M. Melampy and Dr. N. G. Ghoshal for advice. Our gratitude is extended to Dr. D. N. Marple for the generous gift of the pGH antiserum and to the USDA Hormone Program and Dr. D. J. Bolt for the purified pGH.

1. Martin JB. Brain regulation of growth hormone secretion. In: Martini L, Ganong WF, eds. Frontiers in Neuroendocrinology. New York, Raven Press, Vol 4:pp129–168, 1976.
2. Said SI. Peptides common to the nervous system and the gastrointestinal tract. In: Martini L, Ganong WF, eds. Frontiers in Neuroendocrinology. New York, Raven Press, Vol 6:pp293–331, 1980.
3. Krey LC, Lu KH, Butler WR, Hotchkiss J, Piva F, Knobil E. Surgical disconnection of the medial basal hypothalamus and pituitary function in the rhesus monkey. II. GH and cortisol secretion. Endocrinology **96**:1088–1093, 1975.
4. Wroblewska B, Domanski E. Role of the medial-basal hypothalamus in the secretion of growth hormone during pregnancy and lactation in ewes. J Endocrinol **89**:349–354, 1981.
5. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science **197**:77–79, 1973.
6. Schally AV, Dupont A, Arimura A, Redding TW,

Nishi N, Linthicum GL, Schlesinger DH. Isolation and structure of somatostatin from porcine hypothalami. Biochemistry **15**:509–514, 1976.

7. Brazeau P, Bohlen P, Ling N, Esch F, Benoit R, Guillemin R. Purification and partial characterization of the hypothalamic growth-hormone releasing hormone. Endocrinology **108**:A837, 1981.
8. Guillemin R, Brazeau P, Bohlen P, Esch F, Ling N, Wehrenberg WB. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science **218**:585–587, 1982.
9. Ford JJ, Maurer RR. Simple technique for chronic venous catheterization of swine. Lab Anim Sci **28**:615–618, 1978.
10. Anderson LL, Dyck GW, Mori H, Henricks DM, Melampy RM. Ovarian function in pigs following hypophysial stalk transection or hypophysectomy. Amer J Physiol **212**:1188–1194, 1967.
11. Anderson LL, Berardinelli JG, Malven PV, Ford JJ. Prolactin secretion after hypophysial stalk transection in pigs. Endocrinology **111**:380–384, 1982.
12. Marple DN, Aberle ED. Porcine plasma growth hormone levels: radioimmunoassay technique and its application. J Anim Sci **34**:261–265, 1972.
13. Thorell JI, Johansson BG. Enzymatic iodination of polypeptides with ^{125}I to high specific activity. Biochim Biophys Acta **251**:363–369, 1971.
14. Duddleson WG, Midgley AR, Niswender GD. Computer program sequence for analysis and summary of radioimmunoassay data. Comput Biomed Res **5**:205–217, 1972.
15. Anfinson MS, Davis SL, Christian E, Everson DO. Episodic secretion of growth hormone in steers and bulls: An analysis of frequency and magnitude of secretion spikes occurring in a 24-hour period. Proc West Sect Amer Soc Anim Sci **26**:175–177, 1975.
16. Davis SL, Ohlson DL, Klindt J, Everson DO. Estimates of repeatability in the temporal patterns of secretion of growth hormone, prolactin and thyrotropin in sheep. J Anim Sci **49**:724–728, 1979.
17. Saunders A, Terry LC, Audet J, Brazeau P, Martin JB. Dynamic studies of growth hormone and prolactin secretion in the female rat. Neuroendocrinology **21**:193–203, 1976.
18. Anderson LL, Hard DL, Carpenter LS, Awotwi EK, Diekman MA. Neuroendocrine regulation of prolactin and thyrotrophin secretion after hypophysial stalk transection in beef calves. Program of the 62nd Annual Meeting of the Endocrine Society, Washington, DC, Abstr 689, 1980.
19. Anderson LL, Hard DL, Carpenter LS, Awotwi EK, Diekman MA. Prolactin secretion in hypophysial stalk-transectioned beef heifers during pregnancy and lactation. Proceedings 72nd Annual Meeting of the American Society of Animal Science, Ithaca, N.Y. Abstr 364, 1980.
20. Anderson LL, Hard DL, Awotwi EK, Trenkle AH. Neuroendocrine regulation of growth hormone secretion after hypophysial stalk transection in beef calves. Program of the 63rd Annual Meeting of the Endocrine Society, Cincinnati, Ohio, Abstr 979, 1981.

Received July 29, 1982. P.S.E.B.M. 1983, Vol. 172.