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Growth Hormone and Prolactin Secretion after Hypothalamic Deafferentation in Pigs[1] (42400)

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Abstract. Control of growth hormone (GH) and prolactin (PRL) secretion was investigated in ovariectomized, prepuberal Yorkshire gilts by comparing the effects of anterior (AHD), complete (CHD), and posterior (PHD) hypothalamic deafferentation with sham-operated controls (SOC). Blood samples were collected sequentially via an indwelling jugular catheter at 20-min intervals during surgery and recovery from anesthesia (Day 0) and Days 1 and 2 after cranial surgery. Mean serum concentrations of GH after AHD, CHD, and PHD were reduced ($P < 0.01$) when compared with SOC gilts. Furthermore, episodic GH release evident in SOC animals was obliterated after hypothalamic deafferentation. PRL concentrations in peripheral serum of hypothalamic deafferentated gilts remained similar ($P > 0.05$) to those of SOC animals. These results indicate that anterior and posterior hypothalamic neural pathways play a minor role in the control of PRL secretion in the pig in as much as PRL levels remained unchanged after hypothalamic deafferentation. These findings may be interpreted to suggest that the hypothalamus by itself seems able to maintain tonic inhibition of PRL release. In contrast, the maintenance of episodic GH secretion depends upon its neural connections traversing the anterior and posterior aspects of the hypothalamus in the pig.

It is well recognized that growth hormone (GH) is regulated by at least two hypothalamic hormones, GH-releasing hormone (GHRH), and GH-releasing-inhibiting hormone, somatostatin (SRIH, 1). The ventromedial-arcuate region of the hypothalamus has been implicated as an important neural locus for GH regulation because electrical stimulation of it causes a rise in plasma GH in the rat (2, 3), and lesions result in growth retardation (4, 5) accompanied by marked suppression in spontaneous GH surges (6, 7). Deafferentation studies in the monkey and ewe indicate that stimulation of GH release occurs in the anterior portion of the medial basal hypothalamus, whereas the caudal portion is primarily inhibitory (8, 9). After hypophysial stalk transection (HST) of gilts, basal serum levels of GH are elevated, but the episodic release of the hormone is abolished (10).

Prolactin (PRL) secretion probably is regulated by both PRL-release-inhibiting factors (PIF) and PRL-releasing factors of hypothalamic origin (11, 12). Deafferentation of the neural pathways between the suprachiasmatic–preoptic region and the medial basal hypothalamus produced no change in PRL secretion in the ewe (13). Serum PRL concentrations remained low after complete hypothalamic deafferentation in female rats (14). PRL concentrations in peripheral circulation are consistently elevated for prolonged periods after HST in gilts (15). These results indicate that basal secretion of PRL is inhibited by the hypothalamus in this species. The objectives of the present study were to determine the effects of hypothalamic deafferentation in the regulation of GH and PRL secretion in prepuberal gilts.

Materials and Methods. *Animals.* Seventeen purebred Yorkshire gilts [118 ± 3.5 (mean and SE) days of age, 40 ± 1.6 kg body wt] were

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used in this study. Each gilt was bilaterally ovariectomized, and an indwelling catheter was inserted into one anterior vena cava (16) 2 days before hypothalamic deafferentation. Gilts were assigned randomly to four treatment groups: AHD, anterior hypothalamic deafferentation (N = 4); CHD, complete hypothalamic deafferentation (N = 5); PHD, posterior hypothalamic deafferentation (N = 4); and SOC, sham-operated control group (N = 4). Hypothalamic deafferentation and sham operation were performed as described by Molina *et al.* (17). On the day of surgery (Day 0), blood samples were collected at 20-min intervals for a 2-hr and 20-min period while the animals were under anesthesia and in early recovery. Twenty-four and forty-eight hours after cranial surgery (Days 1 and 2, respectively) blood samples were collected at 20-min intervals for a 2-hr period. Eight days after hypothalamic deafferentation or sham operation, gilts were killed, and each brain was perfused with buffered neutral Formalin (pH 7.2) via the common carotid arteries. The brain was removed, and a block of tissue containing the hypothalamus and the preoptic region was excised and placed in buffered neutral Formalin for 24 hr. The block of tissue was dehydrated in a sequence of alcohols, embedded in paraplast, and sectioned at 6 μm. Tissue sections were stained with solvent blue 38 (S-3382; Sigma Chemical Co., St. Louis, Mo.)

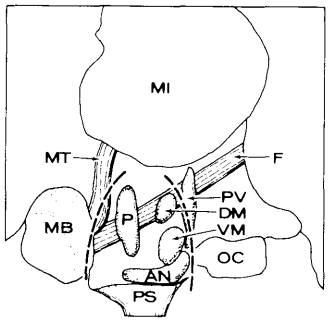

FIG. 1. A camera lucida drawing of the saggital view of the porcine thalamus and hypothalamus with a depiction of the areas isolated by the knife. Interrupted lines define the arc for position of anterior and posterior knife cuts. The mammillary bodies (MB), mammillothalamic tract (MT), fornix (F), dorsomedial nucleus (DM), ventromedial nucleus (VM), arcuate nucleus (AN), posterior nucleus (P), optic chiasm (OC), massa intermedia (MI), and pituitary stalk (PS) are indicated. 2.7×

TABLE I. MEAN PERIPHERAL SERUM CONCENTRATIONS OF GROWTH HORMONE DURING AND AFTER SURGERY IN PREPUBERAL YORKSHIRE GILTS

Surgical group[a]	No. gilts	Overall mean concentration[b] (ng/ml) Day 0	Day 1	Day 2
AHD	4	6.3 ± 0.7	3.3 ± 0.3[c]	3.2 ± 0.3[c]
CHD	5	4.5 ± 0.3	4.2 ± 0.2[d]	4.3 ± 0.4[d]
PHD	4	6.1 ± 0.8	3.9 ± 0.3[c,d]	3.4 ± 0.2[c]
SOC	4	4.1 ± 0.4	5.3 ± 0.8[e]	5.7 ± 1.0[e]

[a] AHD, anterior hypothalamic deafferentation; CHD, complete hypothalamic deafferentation; PHD, posterior hypothalamic deafferentation; SOC, sham-operated control.

[b] Values are means ± SE.

[c–e] Values within columns with different superscripts are different ($P < 0.01$).

and cresyl violet acetate (C-1893; Sigma Chemical Co.), whereas others were stained with hematoxylin and eosin (18). The topographical location of the hypothalamic nuclei and the hypothalamic cuts were reconstructed as previously described by Molina *et al.* (17).

Radioimmunoassay. Serum concentrations of porcine GH (pGH) and prolactin (pPRL) were measured by using double-antibody homologus radioimmunoassays (RIA; 10, 19). The pGH RIA used USDA-pGH-B1 (potency = 3.5 IU/mg) for radioiodination and reference preparations. USDA-pPRL-B1 (potency = 111.0 IU/mg) was the reference preparation and USDA-PRL-I1 (potency = 103.4 IU/mg) was the radioiodination preparation utilized in pPRL RIA. The intraassay coefficients of variation were 12.1 ± 3.4% (mean ± SEM; N = 2/sample, three samples) and 11.2 ± 7.2% (N = 2/sample, three samples) for the pGH and pPRL assays, respectively. The mean interassay coefficients of variation were 11.9 ± 2.1% (N = 4–6/sample, three samples, two assays) and 13.2 ± 4.6% (N = 4–8/sample, three samples, two assays) for pGH and pPRL assays, respectively.

Statistical analysis. The mean of all observations within each sampling period for each animal is the overall mean concentration or level of secretion. Means 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. The histology of coronal sections

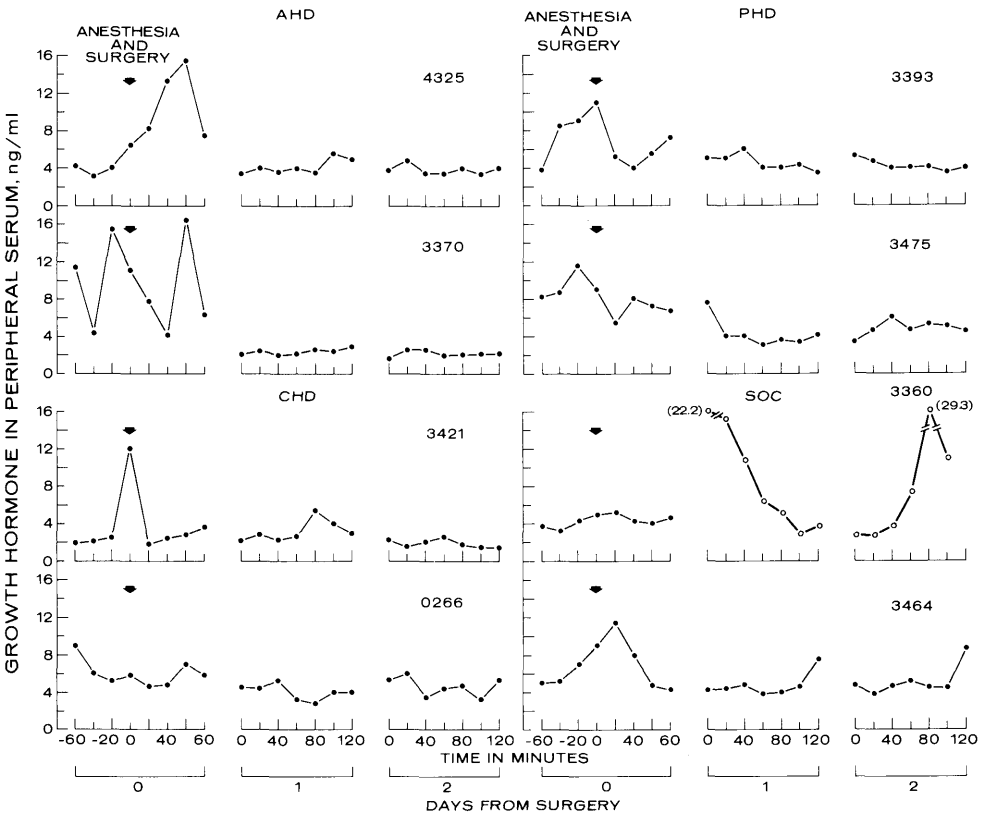

FIG. 2. Peripheral serum concentrations of GH in two representative ovariectomized prepuberal gilts from each treatment group during anesthesia and early recovery (Day 0) and 24 and 48 hr after hypothalamic deafferentation (Days 1 and 2, respectively). Four digit number denotes individual animal.

of the hypothalamus from the gilts in this study has been described previously (17). Histological reconstruction of the hypothalamic cuts indicates that in AHD, the rostral knife cut was caudal to the anterior hypothalamic area (AHA) and rostral to the median eminence. In areas near the midline, the rostral knife cut probably affected the rostral aspect of the arcuate nuclei. In the CHD group, the location of the lesion was between the suprachiasmatic nuclei and the medial ventral hypothalamus. PHD lesions were located within or anteriorly to the mammillary bodies. A camera lucida drawing of the saggital view of the porcine thalamus and hypothalamus with a depiction of the hypothalamic areas isolated by the knife is shown in Fig. 1. No histological evidence of tissue necrosis or blood accumulation was evident in the coronal sections of the pituitary gland and hypothalamus of these gilts.

Sequential bleedings every 20 min, 24 and 48 hr after hypothalamic deafferentation (Days 1 and 2, respectively), reveal that mean GH concentrations in peripheral serum were reduced ($P < 0.01$) in AHD, CHD, and PHD when compared with SOCs (Table I). Episodic GH release observed in the four SOC animals on Days 1 and 2 was obliterated in all gilts after AHD, CHD, and PHD (Figs. 2 and 3). No changes in the pattern of GH secretion were observed during anesthesia and surgery. In contrast prolactin concentrations in peripheral serum were elevated during surgery and anesthesia and during early recovery (Fig. 4). However, PRL concentrations returned to basal levels and remained unchanged during Days 1 and 2 and were similar ($P > 0.05$) in all treated and control groups (Table II).

Discussion. The levels of GH were reduced and the pattern of secretion was significantly altered after hypothalamic deafferentation in the prepuberal pig. Peripheral concentrations

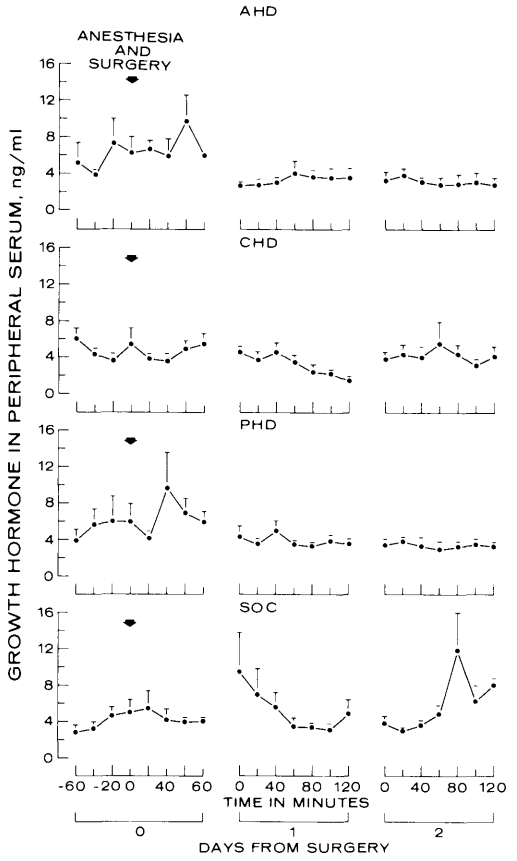

FIG. 3. GH concentrations in peripheral serum in ovariectomized prepuberal gilts during anesthesia and early recovery (Day 0) and 24 and 48 hr after hypothalamic deafferentation (Days 1 and 2, respectively). Groups consisted of 4 AHD, 5 CHD, 4 PHD, and 4 SOC gilts. Values are expressed as means ± SEM.

of GH fluctuated in a manner indicative of episodic secretion in blood obtained from SOC gilts in this study; this pattern of secretion has been observed in other species, such as in rats, sheep, and cattle (20–23). After AHD, CHD, and PHD of gilts the episodic pattern of GH secretion was obliterated. The decrease in mean GH serum levels after deafferentation indicates that anterior and posterior neural links of the medial basal hypothalamus are essential for episodic GH release. Similarly elimination of the pulsatile release of GH had been reported in the pig after hypophysial stalk transection; however, significant elevation in mean basal concentration of the hormone was observed (10). Intravenous administration of 25 μg luteinizing hormone-releasing hormone (LHRH) to these gilts 72 hr after hypothalamic deafferentation elicited an abrupt peak release of luteinizing hormone (LH) in peripheral blood above baseline in all deafferentated groups as well as in the SOCs within 15 min after hormone injection (17). These results clearly indicate that the pituitary gland is responsive to LHRH stimulation after hypothalamic deafferentation.

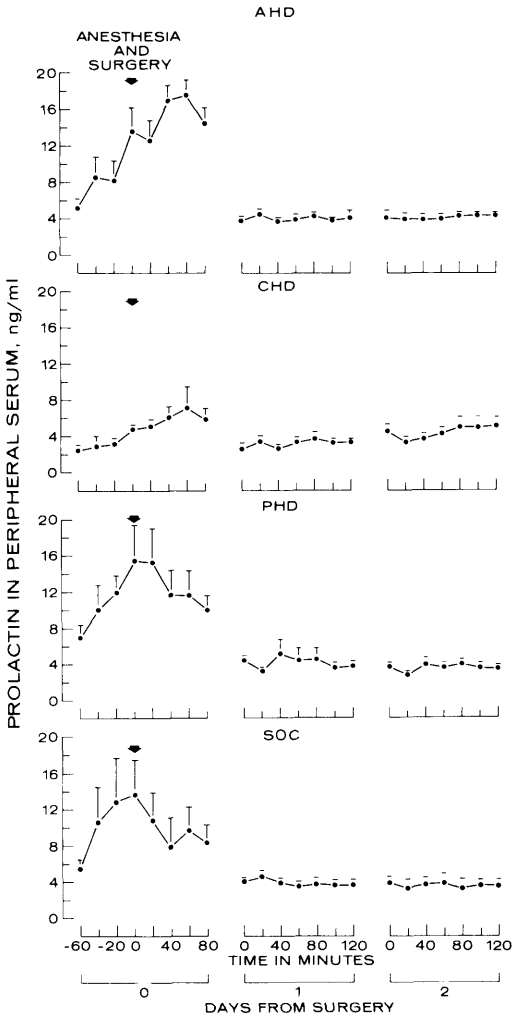

FIG. 4. PRL concentrations in peripheral serum of ovariectomized prepuberal gilts during anesthesia and early recovery (Day 0) and 24 and 48 hr after hypothalamic deafferentation (Days 1 and 2, respectively). Groups consisted of 4 AHD, 5 CHD, 4 PHD, and 4 SOC gilts. Values are expressed as means ± SEM.

TABLE II. MEAN PERIPHERAL SERUM CONCENTRATIONS OF PROLACTIN DURING AND AFTER SURGERY IN PREPUBERAL YORKSHIRE GILTS

Surgical group[a]	No. gilts	Overall mean concentration[b] (ng/ml) Day 0	Day 1	Day 2
AHD	4	12.1 ± 1.0	3.9 ± 0.2[c]	4.0 ± 0.2[c]
CHD	5	4.5 ± 0.5	3.1 ± 0.2[c]	4.3 ± 0.4[c]
PHD	4	11.6 ± 1.0	4.0 ± 0.3[c]	3.6 ± 0.2[c]
SOC	4	10.1 ± 1.1	3.7 ± 0.2[c]	3.6 ± 0.3[c]

[a] AHD, anterior hypothalamic deafferentation; CHD, complete hypothalamic deafferentation; PHD, posterior hypothalamic deafferentation; SOC, sham-operated controls.

[b] Values are means ± SE.

[c] Means within Days 1 and 2 were not significantly different ($P > 0.05$).

PRL concentrations in peripheral serum remained elevated in HST gilts when compared with those sham-operated controls (15). In contrast, the results of this study show that after hypothalamic deafferentation in prepuberal ovariectomized gilts, PRL concentrations return to basal levels, and no significant differences were observed among treated groups and SOCs. Similarly, PRL concentrations in peripheral plasma remained elevated during 14 days after HST in beef calves as compared with those of SOCs (24). After AHD, PHD, and CHD of beef calves, however, PRL blood plasma concentrations remain similar to those of SOC calves (25). Furthermore, AHD in the Suffolk ewe reduced but did not block seasonal changes in PRL secretion (13). The lack of a rise in serum PRL levels after complete hypothalamic deafferentation in the female rat suggests that the hypothalamus does not need neural input to maintain a tonic inhibition on PRL release (14). However, the modest increases in serum PRL concentrations after anterior hypothalamic deafferentation may be caused, at least in part, by increased estrogen release (14). The results from the present study and those reported by Anderson *et al.* (15) may be interpreted to indicate that the hypothalamus by itself appears able to maintain tonic inhibition of PRL release in the pig. No synchrony of identified spikes of serum PRL, GH, and LH was observed after AHD, CHD, and PDH in the present study. PRL and GH serum concentrations were unrelated ($r = 0.12$). Furthermore, no significant correlations were evident between LH and GH or LH and PRL ($r = 0.10$ and 0.14, respectively) in the deafferentated and sham-operated gilts.

The episodic release of porcine GH is abolished after AHD, CHD, and PHD, and a reduction in the overall GH secretion is observed in this species. Moreover, no effect on PRL secretion after hypothalamic deafferentation was evident in this study. The results from the present study clearly indicate that there is a dependency of the ventral medial hypothalamus upon neural connections traversing the anterior or posterior aspects of the porcine hypothalamus for episodic release of GH. Furthermore, these neural pathways seem to play a minor role in mediating changes in PRL secretion in the pig.

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