Brain Neuropeptides in Regulation of Growth and Reproduction in Pigs

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Summary and Implications

Brain hormones exquisitely regulate the secretion of hormones secreted by the pituitary gland that affect growth, metabolism, and reproduction. Oxytocin was one of the first neuroendocrine hormones isolated and characterized in 1955. Currently, 50 neuropeptide hormones ranging from monoamines to polypeptides with > 5,000 molecular weight have been isolated and characterized. We focus on our recent studies concerning hormones affecting growth in young pigs and those that regulate reproduction. Somatic growth in vertebrates is thought to be dependent on pituitary growth hormone (GH); without pituitary GH production or peripheral action, postnatal growth is severely stunted. For example, a deficiency in GH production or GH receptor (GHR) gene has been demonstrated to stunt growth. Young pigs require GH production and secretion from the pituitary gland for continued growth (nitrogen retention); hypophysectomy arrests growth. GH replacement therapy in hypophysectomized pigs causes significant growth but at lesser rate than in sham operated controls. GH is secreted into the peripheral blood in an pulsatile pattern in young pigs and this episodic GH secretion wanes with maturity. Hypophyseal stalk transection eliminates pulsatile GH secretion but basal GH secretion in these animals allows continued growth at a lesser rate than seen in controls. Episodic GH secretion was obliterated by hypothalamic deafferentation in young pigs. Intravenous injection of the brain peptide, GH-releasing hormone (GHRH), stimulates peak release of GH secretion in hypophyseal stalk-transected pigs similar to that in sham-operated controls. Our recent experiments indicate that intravenous injection of a GH-secretagogue causes immediate peak GH release, and coadministration of GHRH and the GH-secretagogue augments GH release. Intracerebroventricular injection of the GH-secretagogue causes a dose-dependent increase in GH release, whereas central administration of somatostatin (SRIH) suppresses peak GH release induced by the GH-secretagogue. Neuropeptide Y and galanin cause modest GH release compared with the GH-secretagogue.

Luteinizing hormone releasing hormone (LHRH) is a hypothalamic decapeptide produced by a placode of LHRH neurons that is crucial for gonadotropin (LH and follicle stimulating hormone, FSH) release by the pituitary gland in the pig. Neurosurgical interventions as described above obliterate LHRH release thus interfering with episodic LH and FSH secretion required for continued reproductive function in male and female pigs.

Introduction

Somatic growth in vertebrates is thought to be dependent on pituitary growth hormone (GH); without pituitary GH production or peripheral GH action, postnatal growth is severely stunted. For example, a deficiency in GH production or GH receptor (GHR) gene has been demonstrated to stunt growth. A notable exception is the guinea pig, in which pituitary gland removal (hypophysectomy) does not alter the growth rate, and treatment with bovine GH (bGH) does not affect growth or increase insulin-like growth factor (IGF)-I levels. Hormones are generally released episodically, but evidence for the requirement of endogenous pulsatile GH secretion for growth in mammals is unknown. GH secretion in the guinea pig is pulsatile and controlled by endogenous GH-releasing hormone (GHRH), somatostatin (SRIH), an inhibitor of GH release, and possibly a GH-releasing peptide receptor. GHR is expressed in various tissues and binds guinea pig GH. Although IGF-I and IGF-II are present in high concentration in guinea pig serum, hypophysectomy does not decrease nor does bGH or ovine GH (oGH) treatment in such animals increase their production. Complex GH binding patterns have been demonstrated not only in the guinea pig but also in rat sera.

The hypothalamus at the base of the brain regulates episodic GH secretion from the pituitary in part by its endogenous release of GHRH, SRIH, and possibly, a yet unidentified GH- secretagogue for which the receptor has been described in the pig, rat, and human. The neurohypophyseal link between the hypothalamus and the pituitary is essential for transporting these releasing and inhibiting hormones. In the young animal, episodic GH secretion occurs during stages of rapid growth and wanes during maturity and senescence. Although aging animals lack robust GH secretion, the pituitary is fully capable of responding to GHRH or GH-secretagogue challenge with supraphysiological GH release.

We have developed neurosurgical techniques in the pig for pituitary removal (hypophysectomy, HYPOX); disconnection of the neurohypophyseal link between the hypothalamus and pituitary (hypophyseal stalk transection, HST); selective isolation of neurons within the hypothalamus by a Halaz knife (hypothalamic deafferentation); central administration of hormones into the brain to determine acute effects on pituitary gland release of hormones into the peripheral blood (intracerebroventricular injection, ICV); and intravenous, intramuscular, and oral hormone administration. These techniques have been used to determine the role of brain peptide hormones in the regulation of pituitary gland secretion of hormones affecting growth and reproduction in the pig.

Materials and Methods

Animals. For growth studies, immature Yorkshire barrows and gilts (30 - 40 kg body weight) were used to determine

effects of HYPOX, HST, hypothalamic deafferentation, sham-operation control (SOC), and hormone replacement by intravenous or intracerebroventricular injection on GH release and growth. Each animal was fitted with an indwelling jugular cannula for sequential blood sampling and hormone administration. We have described previously procedures for these neurosurgical techniques for the pig.

Experimental protocol group 1. HYPOX was carried out at 84 - 89 days of age in Yorkshire male pigs. Porcine GH (pGH) and rat GH (rGH) was injected intramuscularly daily during a period of 40 days after HYPOX; SOC animals were injected with vehicle.

Experimental protocol group 2. HST and SOC were carried out at 211 days of age and 122 kg body weight in ovariectomized Yorkshire gilts. The animals were fitted with jugular cannulas to monitor GH secretion patterns.

Experimental protocol group 3. Hypothalamic deafferentation and SOC were carried out in ovariectomized Yorkshire gilts at 118 days of age and 40 kg body weight. An indwelling jugular cannula was inserted for sequential blood sampling for GH radioimmunoassay of serum.

Experimental protocol group 4. HST and SOC Yorkshire barrows 45 kg body weight were fitted with indwelling jugular cannulas for hormone administration and sequential blood sampling. GHRH alone and in combination with a GH-secretagogue, L-692,585, were intravenously administered to determine GH secretion.

Experimental protocol group 5. Intracerebroventricular cannulas were implanted in Yorkshire barrows (45 kg body weight) for GHRH, and GH-secretagogue, SRIH, NPY, galanin, and vehicle injection. Sequential blood samples were obtained to determine GH secretion in peripheral plasma.

Experimental protocol group 6. LH secretion after hypothalamic deafferentation was determined in ovariectomized Yorkshire gilts (117 kg body weight) subjected to cranial surgery for anterior (AHD), complete (CHD), and posterior (PHD) hypothalamic deafferentation and compared with sham-operated controls (SOC). The animals were fitted with an indwelling jugular cannula for repeat blood sampling for hormone radioimmunoassay.

Hormone radioimmunoassay. A homologous porcine GH radioimmunoassay was developed utilizing reagents supplied by Dr. A. F. Parlow, Pituitary Hormones and Antisera Center, Harbor UCLA Medical Center, Torrance, CA (porcine GH [pGH] for iodination and standards, USDA-I-1; and anti-pGH, AFP10318545). The intra-assay and interassay variances were 6.4 and 18.7%, respectively.

LH blood concentration was quantified in 200-µl aliquots of serum in duplicate by using highly purified porcine LH (USDA-pLH-I-1) for labeling with ^{125}I by the chloramine-T method and for standards (25 pg to 20 ng). Anti-pLH serum (GDN #566) was diluted with 1:400 normal rabbit serum, and antirabbit IGg was diluted 1:45 for second antibody. The intra-assay and interassay variances were 8 and 16%, respectively.

Statistical analyses. The mean of all observations within each sampling period for each animal is the overall mean concentration or level of secretion. Means of secretory patterns across groups were tested by Student's *t* test. Means within treatment groups were tested by paired Student's *t* test.

Results and Discussion

Hypophysectomy of the immature pig leads to a reduced growth rate, but not a complete cessation of growth (0.18 vs. 0.67 kg/day in HYPOX and SOC animals to 200 days of age). Daily injection of pGH, rGH, and phosphate buffer saline (PBS) in HYPOX pigs resulted in weight gain of 0.55, 0.48, and 0.22 kg/day, respectively, compared with 0.39 kg/day in SOC animals during the first 12 days after surgery. Thereafter, growth response was less pronounced, but body weight increases remained higher throughout 40 days compared with those of PBS-injected, hypophysectomized controls.

Hypophyseal stalk transection significantly dampened ($P<.05$) the episodic secretion of GH compared with SOC gilts (Figure 1). These results indicate that synthesis and secretion of GH continue in the absence of hypothalamic control in hypophyseal stalk-transected gilts. Thus, the hypothalamus is required for regulation of both episodic release and the tonic inhibition of basal secretion growth hormone in the pig.

Hypothalamic deafferentation (Figure 2) by AHD, PHD, and CHD significantly decreased ($P<.01$) mean serum concentration of GH compared with SOC gilts (Table 1). Furthermore, episodic GH release evident in SOC animals was obliterated after hypothalamic deafferentation (Figure 3). These results indicate that GH secretion depends upon its neural connection traversing the anterior and posterior aspects of the hypothalamus in the pig.

To investigate the effect of hypophyseal stalk transection on GH-secretagogue activity of the non-peptidyl GH secretagogue L-692,585 in the conscious pig, male castrates were randomly assigned to either HST or SOC.[1] Treatments administered were L-692,585 (100 µg/kg), human GHRH (hGHRH; 20 µg/kg), or L-692,585 + hGHRH at these dosages on days -7 to -3 before surgery and days 3 to 8 after surgery. The results indicated that the GH secretagogue induced an immediate GH response in the intact animal in contrast to hGHRH where the GH release was variable (Fig. 4). Coadministration of the GH-secretagogue + hGHRH induced an immediate GH response of similar magnitude in intact and HST animals (Figure 4). Thus, L-692,585 has a direct but limited action at the level of the pituitary and that an intact hypophyseal stalk is required for a maximal GH response. The findings further suggest that the GH secretagogue stimulates GH secretion by acting in combination with GHRH and interrupting the inhibitory tone of somatotatin (SRIH) on the somatotroph.

Intracerebroventricular injection of the GH-secretagogue caused an immediate dose-dependent increase (3 to 30 µg/kg dose range) ($P<.01$) in GH release in immature castrate male pigs. The GH response was repeatable in a switchback study interspersed with saline-vehicle treatment. ICV injection of SRIH at 2 or 8 µg/kg body weight decreased GH to 2.6 and

2.2 ng/ml plasma compared with saline (4.3 ng/ml). Peak GH response following 2 or 8 µg/kg SRIH + L-692,585 was 30 and 31 ng/ml, respectively, and significantly lower (P<.05) than L-692,585 alone (53 ng/ml). ICV administration of galanin (4 µg/kg body weight) increased (P<.05) GH secretion (10 ng/ml), whereas NPY (4 µg/kg body weight) was not significant (3 ng/ml). These results suggest that endogenous GH secretion was affected by SRIH and galanin but not by NPY, and L-692,585-stimulated GH response appeared to be modulated by all three neuropeptides.

The anatomical disconnection of the anterior neural links of the medial basal hypothalamus by a knife induces profound alterations in reproductive cyclicity in the pig. Episodic LH release after AHD or CHD was abolished (P<.01), but not after PHD or SOC in ovariectomized prepubertal Yorkshire gilts (Figure 5). The results from this study clearly indicate that neural stimuli originating or traversing the neural rostral to median eminence are required for secretion of LH in the pig. Interruption of the neural processes between the anterior hypothalamus and the median eminence by hypothalamic deafferentation in immature gilts abolished episodic LH release; however, basal concentrations of the hormone in the peripheral blood were maintained at reduced levels. Thus, a placode of approximately 1,500 LHRH neurons in the hypothalamus are crucial for maintaining reproductive function in the pig.

Reference

1. Hickey, G. J., J. Drisko, T. Faidley, C. Chang, L. L. Anderson, S. Nicolich, L. McGuire, E. Rickes, D. Krupa, W. Feeney, B. Friscino, P. Cunningham, E. Fraizer, H. Chen, P. Laroque and R. G. Smith. 1996. CNS mediation is critical to the in vivo activity of the growth hormone secretagogue L-692,585. J. Endocrinol. 148: 371 - 380.

Table 1. Mean peripheral serum concentrations of growth hormone during and after surgery in prepuberal Yorkshire gilts.

		Overall mean concentration[b] (ng/ml)		
Surgical group[a]	No. gilts	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<.01).

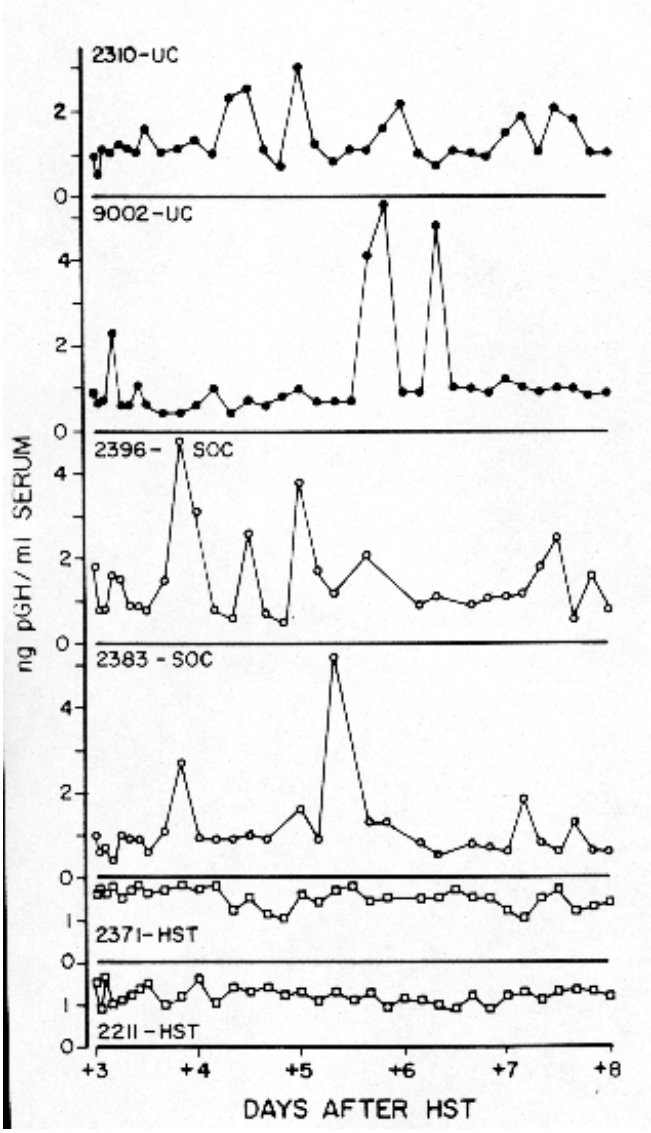

Figure 1. Sequential profiles of peripheral serum concentrations o GH in two gilts from each treatment group (UC. Unoperated control: SOC, sham-operated control: HST. Hypophysial stalk transection) during a 120-hour period from day +3 to day = 8. HST or SOC was performed on day 0. Four-digit numbers designate individual gilts. Symbols indicate unoperated control (•), sham-operated control (0), and hypophysial stalk-transection (☐) animals.

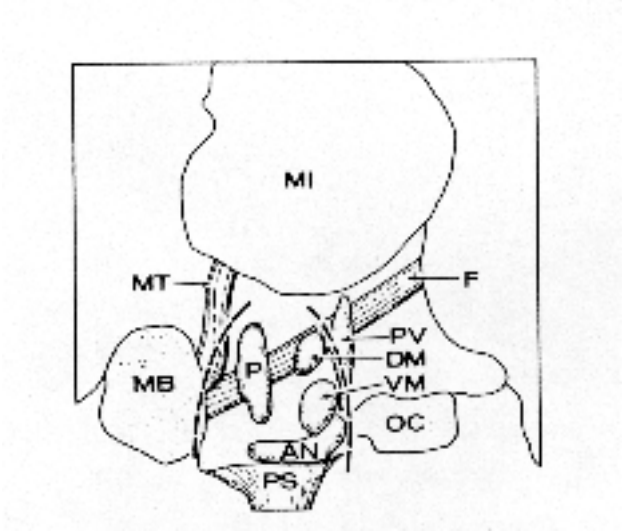

Figure 2. A camera lucida drawing of the saggital view of the procine thalamus and hypothalamus with a depiction of the areas isolated by the knife. Interrupted lines define the arc for position of anterior and posterior knife cuts. The mammillary bodies (<B), mannillothalamic tract (<T), fornix (F), dorsomedial nucleus (DM), ventromedial nucleus (VM), arcuate nucleus (AN), posterior nucleus (P), optic chiasm (OC), massa intermedia (<I), and pituitary stalk (PS) are indicated 2.7X.

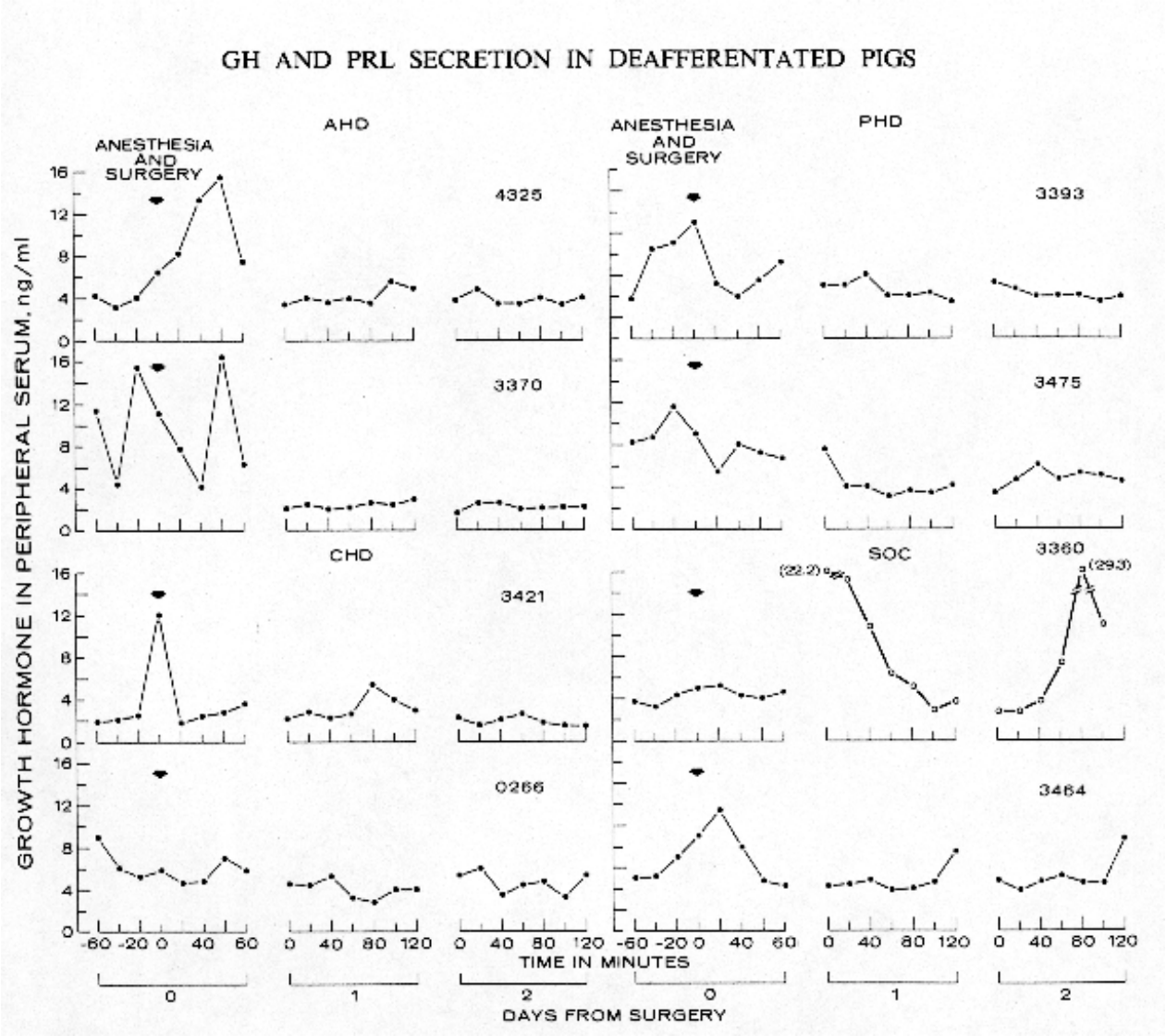

Figure 3. Peripheral serum concentrations of GH in two representative ovariectomized prepuberal gilts from each treatment group during anetsthesia and early recovery (day 0) and 24 and 48 hours after hypothalamic deafferentation (days 1 and 2, respectively). Four digit number denotes individual animal.

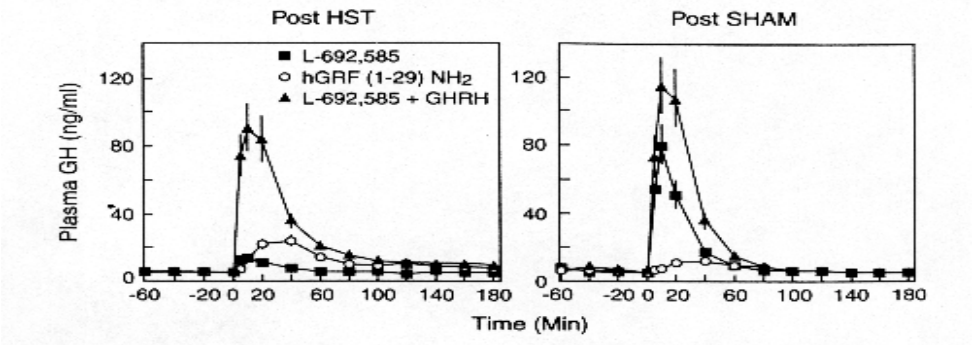

Figure 4. Effects of L-692,585 on GH secretion is markedly attenuated in hypothalamic/pituitary transected (HST) pigs. Activity is restored when GHRH is coadministered with L-692,585 (Hickey et al, 1994b, 1996).

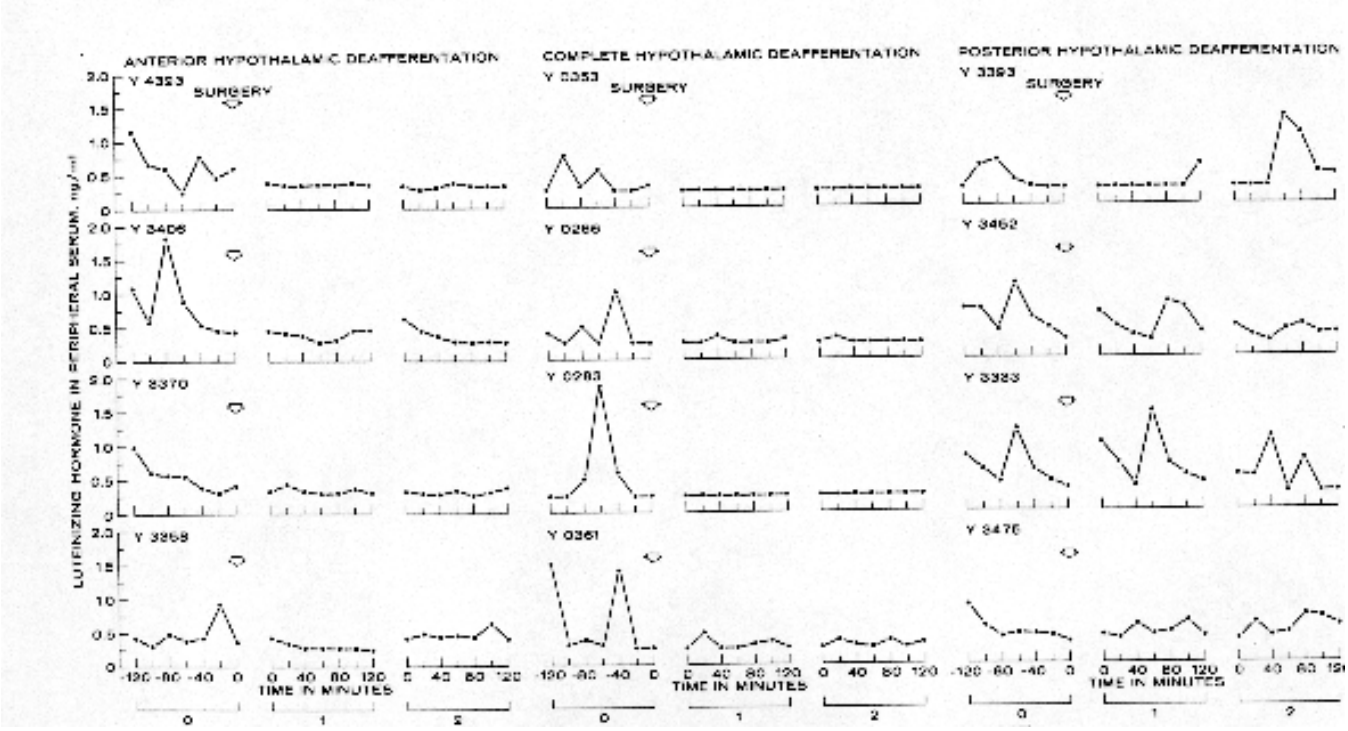

Figure 5.Concentration of LH in peripheral blood serum of individual Yorkshire gilts preceding anesthesia and surgery on day 0 and on days 1 and 2 after AHD, CHD, PHD, and SOC. These prepubertal animals were ovariectomized 2 days preceding surgery (day 0).