

## Central Nervous System Mediation of Growth Hormone Secretagogue in the Pig

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### Summary and Implications

Rapid growth in the young pig requires an episodic pattern of growth hormone (GH) secretion. We developed different neurosurgical techniques to elucidate sites within the hypothalamus, a lower part of the brain, that regulate GH release in this species. A focus of this research is administration of small brain peptide and nonpeptide hormones that acutely affect GH release to define mechanisms of action and their efficacy in augmenting growth.

### Introduction

The hypothalamus in the lower part of the brain regulates both basal and episodic secretion of growth hormone (GH), as well as the secretion of other anterior pituitary hormones such as prolactin, adrenocortropin, follicle stimulating hormone, luteinizing hormone, and thyroid stimulating hormone. For each of these anterior pituitary hormones, small brain peptides (from 189 to 5,108 molecular weight) stimulate or inhibit the secretion of larger proteins (from 4541 to  $\approx$ 32,000 molecular weight).

GH-releasing hormone (GHRH) or factor (GRF) containing 44 amino acids stimulates GH release whereas GH-release-inhibiting factor, somatostatin (SRIF), from the hypothalamus inhibits GH release by the pituitary gland. We have developed neurosurgical techniques of hypophysectomy, hypophyseal stalk transection, hypothalamic deafferentation, and intracerebroventricular cannulation to determine central nervous system regulation of GH secretion in the young, growing pig. With these surgical interventions, selective brain peptide or pituitary hormone replacement is administered intravenously or intracerebroventricularly to determine acute regulation of GH release by radioimmunoassay of GH concentration in sequential blood samples. With the discovery of GH-releasing peptides in 1977 by Bowers and colleagues, consisting of derivatives of the pentapeptide Met-enkephalin, a series of synthetic hexapeptide and nonpeptidyl GH secretagogues are of interest for determining their mechanisms of action in the central nervous system (4). This report summarizes aspects of our studies on central mechanisms regulating GH secretion in the pig.

### Materials and Methods

*Animals.* Yorkshire gilts and castrate males (20–45 kg body weight) were used in these studies. Animals were maintained

in individual pens for the duration of the study, and a ration containing 14% crude protein was available *ad libitum*. Light was provided for 12 hours per day. The animals were fitted with a jugular vein cannula for repeat blood sampling.

*Surgical procedures.* For all surgical procedures the animals were intravenously injected with sodium pentothal or thiamylal sodium and intubated with an endotracheal catheter for maintenance of anesthesia on a closed circuit system of halothane (1–5%) and oxygen (200–500 cc/min). Before surgery the animals were given cortisone acetate (25 mg, intramuscularly) and intravenously infused with 20% mannitol solution during surgery. Postoperatively the animals were infused with 5% glucose solution (100–250 ml) and monitored for body temperature, respiration, and return of normal feed consumption.

a) Hypophysectomy (HYPOX). A supraorbital surgical approach was used to lift the left cerebral hemisphere for exposure to cut the hypophyseal stalk and remove the pituitary gland (1,2). The sella turcica was swabbed with Bouin's fluid to destroy any remaining pituitary cells, and then the recess was filled with thrombin-soaked gel foam. Sham-operated controls (SOC) received the same surgical intervention without removal of the pituitary gland.

b) Hypophyseal stalk transection (HST). A similar surgical approach was used to expose the pituitary gland and sever the hypophyseal stalk, and to insert a nylon barrier to prevent vascular regeneration of the pituitary gland to the stalk at the base of the brain (3,6,8). SOC animals received the same surgical intervention without disturbing the hypophyseal stalk.

c) Hypothalamic deafferentation. Animals were placed in ventral recumbency with the head immobilized in a specially designed stereotaxic apparatus by the procedures we described previously (9,10). The calvarium was exposed with a modified Halasz knife, especially designed for growing pigs. The knife formed an arc of 7-mm height and 3.75-mm radius. For anterior hypothalamic deafferentation (AHD) the knife was inserted between the cerebral hemispheres with the arc in an anterior position, lowered to the floor of the calvarium, elevated 2.5 mm, and rotated 180° twice before returning to midline and removed (Figure 1). For posterior hypothalamic deafferentation (PHD) the knife was inserted with the arc in a posterior position and rotated 180° twice before removal. For complete hypothalamic deafferentation (CHD) the knife was inserted as previously described and rotated 360° twice before removal. Sham-operated controls received the same surgical procedures except the knife was not rotated, and therefore no cut was performed in the hypothalamus.

d) Intracerebroventricular cannulation (icv). We developed stereotaxic coordinates for inserting 18-gauge

stainless steel cannula in the left lateral-cerebral ventricle according to predetermined coordinates (anterior-posterior, +14 mm; lateral, 6 mm to the bregma; horizontal, 18 mm to the dura mater (5,7). Stainless steel screws and cranioplastic cement secured the cannula. Backflow of cerebrospinal fluid indicated the cannula was icv.

*Experimental design and statistical analysis.* For each experiment, appropriate numbers of experimental and SOC were included to determine statistically valid inferences. Experimental units were the individual animals assigned to treatment at random. Temporal endocrine results were analyzed by split-plot analysis of variance. The main plot effect was treatment tested against animal within treatment as the whole-plot error. Student's *t*-test was used for comparison between treatment groups.

### Results

In immature, hypophysectomized control pigs given saline treatment, growth was markedly limited compared with SOC. Daily injection of porcine GH or rat GH in hypophysectomized for 40 days increased daily gain to 0.284 and 0.257 kg/d compared with 0.245 and 0.745 kg/d in HYPOX controls and SOC. Results indicated that low daily levels of GH induced significant increases in body weight and protein anabolism in immature, hypophysectomized pigs but that treatment responses were transitory.

Hypophyseal stalk transection abolished normal episodic release of GH and depressed growth rates compared with SOC immature pigs (Table 1). However, HST pigs continue daily gains significantly greater than seen in saline-treated hypophysectomized animals. The regulation of prolactin (PRL) secretion is tonically inhibited in the intact, growing animal. After HST, PRL blood concentrations significantly increased compared with those in SOC pigs throughout a 9-day postoperative period even though pituitary concentration and content in HST pigs was greatly reduced (Table 1). Thus, PRL is synthesized and immediately released to maintain consistently greater circulating concentrations of PRL in HST compared with SOC pigs.

Hypothalamic deafferentation reduced mean blood serum concentrations of GH after AHD, CHD, and PHD when compared with SOC gilts (Figure 2). Furthermore, episodic GH release evident in SOC animals was obliterated after hypothalamic deafferentation. Prolactin concentrations in peripheral serum of hypothalamic deafferentated gilts remained similar ( $P>0.05$ ) to those in SOC animals. These results indicate that anterior and posterior hypothalamic neural pathways play a minor role in 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.

The HST and stalk-intact pigs provide useful models to determine the effects of analogs of GH-releasing factors (GHRF), somatostatin (SRIF), arginine, and other brain peptides on regulation of GH release by the pituitary gland. HST and SOC gilts were challenged with intravenous injections of human pancreatic GHRF(1-40)OH, thyrotropin releasing hormone, and analogs of rat hypothalamic GHRF. HST animals remained acutely responsive to GHRF by releasing 2-fold greater quantities of GH than seen in SOC controls (Figure 3). This occurred in spite of a 38% reduction in pituitary gland weight and a 32 and 55% decrease in GH concentration and total content, respectively (Table 1). During SRIF infusion, GH remained at similar basal concentrations in HST and control gilts, but increased immediately after stopping SRIF infusion only in the controls. Releasable pituitary GH appears to accumulate during SRIF. GHRF given during SRIF infusion caused a 2-fold greater release of GH than seen in animals receiving only GHRF. Arginine increased GH release in controls, but not in HST gilts, which suggests that it acts through the central nervous system. Thyrotropin releasing hormone acutely elevated circulating PRL in HST gilts, suggesting that it acts directly in the pituitary gland. These results indicate that the isolated pituitary after HST remains acutely responsive to hypothalamic releasing and inhibiting factors for both GH and PRL release 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, castrate male Yorkshire pigs were randomly assigned to either HST or SOC (6). Treatments administered intravenously were L-692,585 (100  $\mu$ g/kg body weight), human GH-releasing factor (1-29)NH<sub>2</sub> (GHRH; 20  $\mu$ g/kg body weight), or L-692,585 + GHRH at the same dosages in days -7 to -3 before brain surgery and days +3 and +8 after surgery, and blood was collected for radioimmunoassay of GH. The magnitude of GH release was greatest in L-692,585 injected-pigs (Figure 4). When L-692,585 and GHRH were intravenously injected together the GH response was more than additive. The pigs then were separated into two experimental groups and subjected to HST or SOC surgery, and again treated with the same dosages of the secretagogues. The magnitude of GH secretion was diminished in stalk-transected pigs treated with L-692,585, but when GHRH was replaced exogenously, L-692,585 provided GH release of a magnitude indistinguishable from that of controls (Figure 4), suggesting that GHRH is released by the hypothalamus in response to L-692,585. When the same HST pigs were given luteinizing hormone-releasing hormone and corticotropin-releasing hormone, normal LH and ACTH responses were measured, indicating that surgical intervention had not compromised the functional integrity of the pituitary gland.

To investigate the acute dose-dependent effect of the GH secretagogue on GH release, L-692,585 was administered icv in castrate Yorkshire male pigs (5). Peak GH plasma levels (ng/ml) following L-692,585 icv treatment at 3 (23\_9; \_ standard error), 10 (25\_10), or 30 \_g/kg (38\_6) occurred at 10 minutes and returned to baseline (<4 ng/ml) by 60 minutes, and were significantly ( $P<0.01$ ) greater than following saline treatment (1.5\_1). These results indicate that L-692,585 when administered icv caused a GH response of equal magnitude to that following intravenous administration.

### Discussion

Different cranial surgical interventions revealed aspects of central nervous system regulation of growth hormone secretion in the young, growing pig. Complete surgical removal of the pituitary gland (hypophysectomy) arrested growth, whereas daily GH replacement in such animals significantly increased growth but at a rate less than seen in SOC animals. In the intact young, growing pig, GH secretion is episodic, with 3 to 6 peaks of 6 to 12 ng/ml of plasma during a 6-hour period. Endogenous secretion of hypothalamic GHRH causes episodic patterns of GH release because after hypophyseal stalk transection they are abolished. Regardless, growth continues in such HST pigs but at a slower rate than seen in SOC. When the GHRH is intravenously injected into HST pigs, they respond with abrupt peak GH release indicating continued GH synthesis by the isolated pituitary gland in spite of reduced gland size (i.e., 38% less than SOC animals). The GH response to GHRH injection in HST pigs is less than sustained in SOC animals. The intravenous injection of the nonpeptidyl GH secretagogue, L-692,585, elicited an abrupt GH release in HST pigs but a peak response less than seen in SOC controls. When the GH secretagogue, L-692,585, was coadministered with GHRH, the GH response was the same magnitude as seen in SOC animals. These results may be interpreted to suggest that the novel GH secretagogue may act through GHRH to augment GH release, interfere with SRIF secretion, or act independently at the level of the hypothalamus and pituitary gland. In the rat, intravenous or icv injection of L-692,585 activates *c-fos* protein expression selectively in the arcuate nucleus; activation is associated with neurosecretory neurons. In contrast, GHRH administered by itself is ineffective in eliciting this immunoactivity. The selective localization of *c-fos* immunoactivity in a specific region of the arcuate nucleus suggests that these GH-secretagogues directly or indirectly target GHRH-containing neurons. The dose-dependent effects of icv injected L-692,585 on GH release in the immature pig reinforces the idea of central activation of neurosecretory neurons in the hypothalamus.

### Conclusions

The central nervous system regulation of an episodic pattern of growth hormone secretion requires endogenous brain peptide hormones that stimulate and inhibit GH release in the young, growing pig. We have developed neurosurgical techniques to reveal sites of action within the basal region of the brain (hypothalamus) that regulate GH release by the pituitary gland in this species. In addition, small peptide and nonpeptide hormones have been administered to reveal in the hypothalamus, neuronal secretory cells that regulate GH secretion. An understanding of hormonal mechanisms within the brain that control GH secretion will lead to effective ways to augment growth in the pig.

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Figure 1. A camera lucida drawing of the sagittal 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. 2.7X

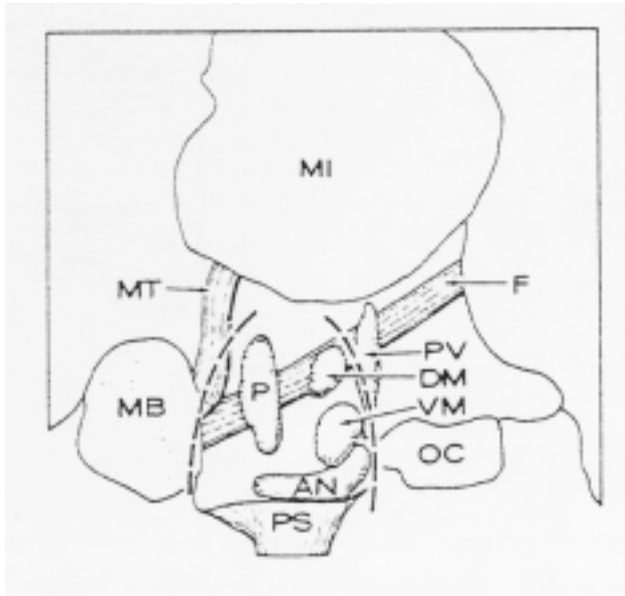


Figure 2. GH concentrations in peripheral serum in ovariectomized prepuberal gilts during anesthesia and early recovery (Day 0) and 24 and 48 hours after hypothalamic deafferentation (Days 1 and 2, respectively). Groups consisted of 4 AHD, 5 CHD, 4 PHD, and 4 SOC gilts. Values are expressed as means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

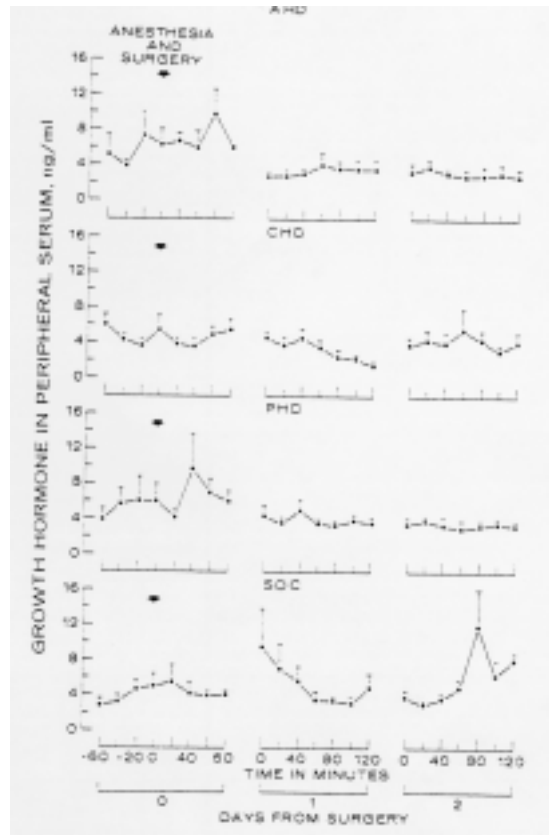


Figure 3. Effect of intravenous injection of hpGHRF(1-40)OH (A and B) or diluent (C) on GH concentrations in peripheral plasma of prepubertal hypophysial stalk-transected and unoperated control gilts. Number of gilts in each group is indicated in parentheses. Values are mean  $\pm$  SEM.

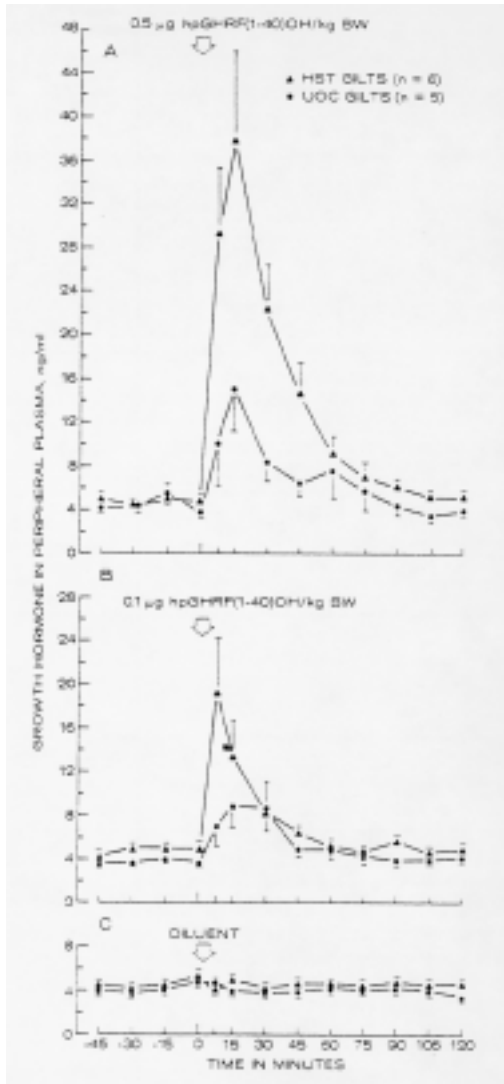
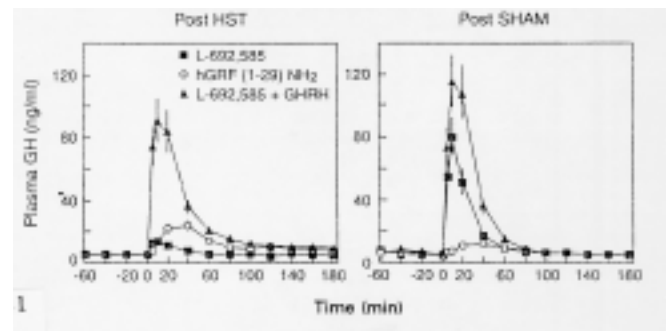


Figure 4. Effects of L-692,585 on GH secretion is markedly attenuated in hypothalamic/pituitary transected (HST) pigs. Activity is restored when GHRH is coadministered with L-692,429 (6).



**Table 1. Effect of HST on adrenal, thyroid, and pituitary glands and PRL and GH in gilts.**

Item	SOC gilts		HST gilts		Ratio
	<i>n</i>	weight	<i>n</i>	weight	HST:SOC
Body (kg)	5	111 _ 4.1 <sup>a</sup>	6	97 _ 5.1	0.874
Adrenal (g)	5	4.9 _ 0.27	6	3.4 _ 0.30 <sup>b</sup>	0.694
Thyroid (g)	5	11.5 _ 1.75	6	11.5 _ 2.51	1.000
Pituitary (mg)	5	342 _ 10	6	213 _ 21 <sup>b</sup>	0.623
Pituitary PRL					
Concentration (_g/mg)		2.1 _ 0.31		0.2 _ 0.05 <sup>c</sup>	0.092
Content (_g)		711 _ 96		44 _ 14 <sup>c</sup>	0.062
Pituitary GH					
Concentration (_g/mg)		14.2 _ 0.99		9.6 _ 3.72 <sup>b</sup>	0.674
Content (_g)		4870 _ 390		2175 _ 937 <sup>b</sup>	0.447

<sup>a</sup>Values are mean \_ SEM.

<sup>b</sup> $P < 0.05$  vs SOC.

<sup>c</sup> $P < 0.001$  vs SOC.