# Endocrine Regulation for Growth in Pigs

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**Summary and Implications**

Endocrine regulation in the brain is important for body growth and metabolism both in human and domestic animals. Growth hormone (GH) has been recognized as a primary regulator that plays an important role in determining body composition to maintain beneficial ratio between skeletal muscle and fat. The main targets of GH actions are liver, muscle, and bone where this action is partially mediated through an increase in insulin-like growth factor-_ (IGF-_). A role for GH in the brain is more critically approached because its pulsatile secretion is regulated by dual system of hypothalamic control: a positive/negative feedback loop. Growth hormone-releasing hormone (GHRH) is released from arcuate neurons in the hypothalamus and transported through the portal blood vessels into the pituitary gland, where it stimulates GH release from somatotrophs (GH-secreting cells in the pituitary gland). Negative feedback is mediated by the release of somatostatin (SS) from hypothalamic neurons that act to inhibit GH release.

A third endocrine pathway controlling GH secretion has been led by discovery of the GH secretagogues (GHSs); growth hormone-releasing peptides (GHRPs) and their pharmacological nonpeptidyl analogs that have a direct effect on in vitro pituitary GH release in both animal and human somatotrophs. GHSs synergize with GHRH and functionally antagonize SS acting on the hypothalamus for GH secretion. Alterations in GH pulsatility are seen in a variety of physiological and pathological circumstances, including puberty, aging, estrous cycle, obesity, starvation, and growth delay. Disconnection of hypothalamic-pituitary stalk results in loss of normal GH pulsatility and suggests that pulsatility is regulated by changes in GHRH, SS, and a natural ligand for GHS receptor. GHSs administrated alone or in combination with GHRH are the most potent and reproducible GH releasers, and useful tools for the diagnosis of GH deficiency when tested in a variety of pathological condition in patients and across many species. Therefore, GHSs present a factor for extending the understanding of GH secretion, as well as a unique therapeutic opportunity.

Neuropeptide Y (NPY) has been well known mediators not only in GH-secreting mechanism by GHSs but also in food intake -relating network in hypothalamic neurons. Previous studies suggest that NPY has negative feedback effect of GH release and stimulatory action in the regulation of appetite having reciprocal relation with hypothalamic hormone such as GHRH, SS, GHS and leptin.

In addition to stimulating body growth, alterations in nutritional status such as obesity or food deprivation significantly influence GH secretion. Leptin secreted by adipocytes that regulates food intake and energy expenditure has recently been shown to play a stimulatory role on GH secretion. Leptin induced GH secretion is suppressed by NPY, so it is possible that NPY mediates leptin action in the hypothalamus.

Another candidate for GH regulation in the hypothalamic area is ghrelin, a GH-releasing acylated peptide as an endogenous bioactive ligand for the GHS receptor. Ghrelin produced by stomach, intestine, placenta, pituitary, and possibly in the hypothalamus is a recently discovered hormone that plays a critical role in the maintenance of energy homeostasis as well as the stimulation of GH release.

GH secretion results from a complex series of interactions that occur both in peripheral tissue and in the central nervous system (CNS). The regulation of growth by elements of the GH axis is a complex process that we have only begun to understand. Further evaluation of physiology and genes involved in the production, secretion, and actions of GH will enhance our understanding of the role and central mechanisms of endocrine regulation affecting growth.

**Review**

***Growth hormone (GH).*** Growth hormone has growth-promoting effects and metabolic actions that are important in many species and acts directly or indirectly on virtually every tissue in the body. These biological events of GH are initiated by hormone binding to its cell surface receptor and trigger gene expression for long term changes that are responsible for the stimulation of growth. Insulin-like growth factor I (IGF-I), a highly conserved to amino acid is one of the key targets for GH. GH has direct effects in many tissues coordinating with locally generated/circulating IGF-I to enhance growth in animals. It is well known that GH treatment induces IGF-I and stimulates IGF-I expression in the liver and other tissues.

GH receptor (GHR) is expressed in many tissues, including liver, heart, fat, testis, skeletal muscle, intestine, kidney, pancreas, and brain. Especially brain is considered as an important target tissue for GH supposed by increasing direct evidence. High-resolution autoradiography and immunocytochemistry and in situ hybridization has been used to confirm GHR expression in the CNS-hypothalamus and hippocampus in rodents and human. Within the hypothalamus, GHRs are involved in a short feedback regulating GH secretion: high GH expression induces periventricular nucleus (PVN) somatostatin (SS) neurons to increase SS expression and arcuate nucleus (ARC) to inhibit GHRH expression, consistent with GH feedback. Decrease of GHR expression in hypophysecromy or in dwarf rat, which has GH deficiency,

shows that central GHR expression is also sensitive to regulation by GH.

In most species, GH is secreted in a pulsatility pattern and is regulated by tightly controlled feedback pathways. Two hypothalamic hormones highly involved in the feedback loop are GHRH and SS. This episodic pulsatility reflects a balanced alteration in two neuroendocrine systems regulating GH release by a positive/negative feedback loop.

***GHRH and SS.*** GHRH is a peptide hormone synthesized and released from the hypothalamic arcuate nucleus (ARC) that stimulates GH secretion from pituitary somatotroph cells (GH secreting cells). The pituitary cell membrane has specific, high-affinity binding sites for GHRH. Binding GHRH to its receptor stimulates adenylate cyclase, resulting increased adenosine 3'5'-cyclic monophosphate (cAMP) production, and indicating that the $G_s$ protein is an intermediate in GHRH action. Because cAMP is an important second messenger for GHRH signaling, GHRH and cAMP stimulate pituitary GH secretion, facilitate GH gene expression, and increase the proliferation of cultured pituitary somatotroph cells.

SS or somatotrophin-releasing inhibiting factor (SRIF) is a 14 amino acid-containing peptide hormone primarily expressed in the hypothalamus. A major physiological function of SRIF is to inhibit GH release and maintain the pulsatile secretion of GH. SRIF inhibits GH release by activating the receptor subtype $sstr_2$ to inhibit $Ca^{2+}$ conductance and $Ca^{2+}$ influx in somatotrophs.

The GH feedback loop by GHRH and SRIF on pulsatile GH release is maintained with time. GHRH expression is increased in the case of GH deficiency, whereas GH treatment reverses changes. Conversely, SRIF expression in the hypothalamus is reduced after hypophysectomy, whereas excess GH stimulates hypothalamic SRIF synthesis and release. The main physiological role of GH feedback on GHRH is to regulate the GH reserve for a much longer time. Although local generation of IGF-I in response to GH secretion in the CNS is considered to be important, changes in GHRH and SRIF are readily observed with GH, but not with IGF-I alone, which implicates sites of direct feedback for GH and not secondary to peripheral IGF-I generation.

***GHS.*** Pulsatile GH secretion from the pituitary somatotrophs was thought to be regulated by episodic changes only in two hypothalamic hormone, GHRH and SRIF. However, the discovery of synthetic GHRP by Bowers and coworkers and several nonpeptidyl GHSs, including L-692,429, L-692,585, and MK-0677 that also act to enhance GH release has brought an emerging perspective in the endogenous regulation of GH secretion. GHRP-6 stimulates pulsatile GH release through the activation of a receptor distinct from the SRIF and GHRH receptor, which belongs of the family of seven transmembrane receptor coupled to GTP binding protein to up-down-regulate cAMP level controlling GH secretion. Peptidyl and nonpeptidyl GHS receptors cloned in the anterior pituitary and hypothalamus are activated by a mediator, a phosphoinositol-protein kinase C intracellular pathway that induces intracellular $Ca^{2+}$ release and depolarization, leading to exocytosis of GH-containing secretory vesicles in the pituitary gland.

GHSs may have dual action to stimulate endogenous GHRH release and suppress endogenous somatostatin release, which implies GHSs require the presence of a functional hypothalamus. In situ hybridization studies in monkey and rat brains show that GHS-R is expressed in arcuate neurons, suggesting that GHS-R ligand stimulates these neurons directly as a GHRH releaser that induces GH secretion from the somatotrophs. GHS and GHRH have a synergistic effect and even very low GHS doses potentiate the GHRH-induced GH rise in human and animals. Hypothalamopituitary disconnection (functional stalk section) fails to respond to GHSs for optimal GH release. Experiment in hypothalamic stalk-sectioned pigs showed that GHS-induced GH release is blocked and synergistic action of GHRH and GHS is absent. Electophysiological studies in vivo show that neurosecretory neurons in the hypothalamic arcuate nucleus are excited by GHSs and are inhibited during electrical stimulation of periventricular nucleus. Also, after intravenous injection of somatostatin, secretagogue-responsive cells are inhibited. Thus, it would appear that a subpopulation of the arcuate cells activated by GHSs is inhibited by central somatostatin action. These important studies link the action of GHS-R ligands with two endogenous regulators of GH release, GHRH and somatostatin, which are important to generate the rhythm of GH pulsatility.

Interestingly, studies in animals have suggested that GHSs may have widespread effect in the brain and interfere with secretion of neuropeptide Y and dopamine, which leads to up-regulation of adrenocorticotropin (ACTH), cortisol, and prolactin secretion. For example, the nonpeptide GHRP analog L-692,429 powerfully stimulated prolactin secretion from pituitary somatomammotrope cells (both GH and PRL secreting cells). This study implies that GHS might include mastopathy, galactorrheas, and/or a loss of libido. A most important reason prompting GHS research is its benefit of oral administration to release GH. Therefore, it would be useful in clinical practice for diagnostic and therapeutic purpose as well as potential in regulating GH secretion in farm animals.

***Neuropeptide Y (NPY).*** NPY has been implicated as a regulator in the control of appetite, body weight gain, and obesity, and is also involved in central mechanism of GH release. NPY expression is GH sensitive and may be the primary target for GH feedback in the ARC. Previous studies showed that ARC NPY mRNA is reduced in GH-deficient dwarf rats and this deficiency is corrected by GH administration. So, ARC NPY is stimulatory effector on somatostatin and inhibitory effector on GHRH. Therefore, GH action of these cells might be involved in food intake activity in the GH/IGF-I axis affecting food utilization. Recently, NPY

has been considered as a mediator of the effects of GHSs, and leptin action.

***Leptin.*** Leptin is newly discovered hormone product of obese gene (*ob*), secreted by adipocytes that regulate food intake and energy expenditure. Leptin has recently been shown to play a stimulatory role on GH secretion by mediating NPY in the hypothalamic area. Alternatively, leptin and NPY could act through parallel pathway to change GH release with NPY, overcoming the stimulatory effect exerted by leptin on plasma GH level. One of the leptin receptor, *ob*-Rb is responsible for the leptin signaling in the brain and expressed in the hypothalamic area: median eminence (ME), arcuate nucleus (ARC), ventromedial nucleus (VMN), and dorsomedial nucleus (DMN). The main physiological role of leptin is to increase energy expenditure and decrease in food intake after signal increasing adiposity to the brain. Also, a study of leptin action on the reproductive system demonstrates that leptin stimulates the reproductive endocrine system and suggests that leptin may serve as a permissive signal to the reproductive system of normal animals. Although the outline of the hypothalamic system for regulation of feeding is now more clear, it is still needed to be investigated to define the mechanisms of neural regulation of feeding.

***Ghrelin.*** Ghrelin is the newest player in the endogenous regulation of GH secretion. Ghrelin, a novel 28 amino acid peptide has recently been purified from rat stomach and subsequently cloned in rat and human. An important physiological role of ghrelin is the endogenous ligand for the GHS-R to induce GH release from pituitary somatotrophs. Ghrelin has been detected in the hypothalamic ARC and in the stomach by using immunocytochemical detection method. This hypothalamic ghrelin has important physiological roles in GH secretion and energy balance. In accordance with synthetic GHSs action, a recent study demonstrated that intracerebroventricular (i.c.v.) and peripheral (i.p.) administration of ghrelin induce increase in plasma GH concentration and this ghrelin-induced GH secretion is also directly opposed by somatostatin action involving mediation through GHRH. An animal study confirmed ghrelin is a regulator of energy balance. The data showed that exogenous ghrelin induces adiposity in rodents by stimulating increase in food intake and a reduction in fat utilization based on the hypothalamic signaling. Adipogenic and orexogenic effects of ghrelin are dissociated from its GH secretion effects and rather are associated with a specific central mechanism of neurons mediated by leptin whose regulation and biological effect are opposed to those of ghrelin. The further understanding of central network and function of ghrelin will enlighten the general frame for hypothalamic machinery in metabolism regulation.