Nutritional regulators of the hypothalamic–pituitary axis in pigs

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Nutritional signals are detected by the central nervous system (CNS) and translated by the neuroendocrine system into signals that alter secretion of LH and growth hormone (GH). Furthermore, these signals directly affect the activity of the pituitary gland independently of CNS input. Insulin-like growth factor I (IGF-I), insulin, leptin and specific metabolites, such as glucose and free fatty acids (FFA), are potential signals of the metabolic status to the brain–pituitary axis. Intravenous injection of a lipid emulsion or glucose suppressed the GH and LH response to GH releasing hormone (GHRH) and GnRH, respectively. Insulin and IGF-I regulation of LH and GH secretion occur at the pituitary gland. Feed deprivation for 24 h suppressed leptin secretion without affecting LH or GH secretion, whereas central administration of leptin resulted in a decrease in feed intake and an increase in GH secretion. Oestrogen-induced leptin gene expression in adipose tissue increased with age and adiposity in pigs. Leptin stimulated GnRH release from hypothalamic tissue *in vitro*. These results identify putative signals that link metabolic status and neuroendocrine control of growth and reproduction by altering endocrine function during periods of fasting, feed restriction and lactation.

Neuroendocrine control of LH and GH secretion

It is generally accepted that there are two modes of LH secretion in pigs (Kraeling and Barb, 1990): pulsatile secretion and surge secretion. These patterns of LH secretion reflect the pattern of GnRH released from neurosecretory neurones within the hypothalamus into the hypothalamic–hypophysial portal system (Goodman, 1988). An LH pulse and an LH surge generator are located within the CNS of pigs (Kraeling and Barb, 1990). The importance of pulsatile GnRH and LH secretion was demonstrated by Lutz *et al.* (1984) and Pressing *et al.* (1992) who induced precocious oestrus and ovulation in intact prepubertal gilts by giving i.v. injections of GnRH at 1 h intervals. In addition, administration of GnRH at 1 h intervals to anoestrous postpartum sows induced oestrus and ovulation (Cox and Britt, 1988). Wetsel *et al.* (1992) reported intrinsic pulsatile secretory activity of immortalized GnRH neurones *in vitro*. Thus, GnRH neurones secrete their product in an autonomous episodic frequency, but interoceptive and exteroceptive factors detected by the CNS are translated by the

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neuroendocrine system into signals that alter the pattern of GnRH and subsequent LH secretion. For example, interoceptive signals, such as gonadal and adrenal steroids, metabolites, and other neuronal signals, act to modulate pulse frequency and amplitude of GnRH pulses.

Many of the interoceptive signals that regulate GnRH and LH secretion also modulate GH secretion. Of particular importance is the role of nutrition and metabolic status in maintaining reproductive function, but also in modulating the GH-releasing hormone (GHRH)–somatostatin–GH axis and subsequent growth. It is well established that the onset of puberty is linked to the attainment of a critical body weight or metabolic mass, indicating an association between mechanisms regulating energy balance and the reproductive and growth axis.

Metabolism and the endocrine system

The importance of nutrition and metabolic state in maintaining reproductive function is well established. The onset of puberty may be linked to attainment of a critical body weight or a minimum percentage of body fat (Frisch, 1984). Alternatively, metabolic mass and food intake, or its correlated metabolic rate, may be the triggering mechanism (Frisch, 1984). Cameron *et al.* (1985) reported that transition from a fed to a fasting state occurred more rapidly in the juvenile than in the mature monkey. Cameron *et al.* (1985) suggested that the dynamic fluctuations in plasma hormones and substrates that occur during postprandial and postabsorptive periods provide signals to the brain that link metabolic status to activation of the reproductive system. Nutritional perturbations delay the onset of puberty, interfere with normal oestrous cycles and alter LH secretion in pigs (for a review, see Prunier and Quesnel, 2000a).

The GH response to nutritional status is variable in pigs. Buonomo *et al.* (1988) reported that serum GH concentrations increased after 48 h of fasting in barrows. In contrast, Booth (1990) reported that feed restriction for 8–14 days failed to affect serum GH concentrations in prepubertal gilts. Acute feed deprivation failed to influence GH secretion during a 28 h fast, although serum GH secretion increased after feeding was resumed. Similarly, Armstrong and Britt (1987) reported that GH secretion increased during the postprandial period in feed restricted gilts. However, antagonism of glucose utilization increased basal and mean serum GH concentrations, but not GH pulse frequency and amplitude (Barb *et al.*, 2001). Thus, GH response to energy availability is equivocal.

Collectively, these data demonstrate that nutritional status affects endocrine function. Contemporary models of energy regulation emphasize physiological signals that control energy intake, partitioning and expenditure, and their sites of action. It is hypothesized that mechanisms regulating energy balance are sensitive to metabolic signals generated by changes in oxidation of metabolic fuels and could account for positive correlations between body fat, fertility and endocrine function (Wade *et al.*, 1996). Identification of specific metabolic signals that influence the reproductive and growth axis remains elusive, primarily because of the large number of substances from the periphery that may act centrally to modify neuronal activity. Similar to the study by Cameron *et al.* (1985) on primates, Barb *et al.* (1997) reported that transition from a fed to fasting state occurred more rapidly in prepubertal than in mature gilts. Different temporal relationships between circulating blood concentrations of glucose, free fatty acids (FFA), ketones, insulin and IGF-I in mature and prepubertal pigs were probably related to a greater glucose production rate, higher metabolic rate, smaller energy reserves and greater growth requirements in prepubertal gilts. In a subsequent study, acute fasting suppressed leptin secretion in prepubertal gilts (Barb *et al.*, 2001). Thus, glucose, FFA, IGF-I, insulin and leptin may provide peripheral signals to the brain that link metabolic status

to activation of the reproductive system and modulation of the growth axis. The intent of this review is to present evidence for nutritional control of the hypothalamic–pituitary axis and putative sites of action of specific metabolites and metabolic hormones in modulating LH and GH secretion. The effects of nutrition and feed restriction on reproductive function in pigs will not be discussed. For further information on nutrition and reproductive function in pigs see Cosgrove and Foxcroft (1996) and Prunier and Quesnel (2000a,b).

Nutritional mediators of LH and GH secretion

Roles of glucose and insulin

Numerous reports have led to the proposal that blood glucose concentration is an accurate reflection and index of the collective effects of energy on reproduction and growth. Moreover, changes in gluconeogenesis may be the mechanism by which altered energy metabolism affects the neuroendocrine axis in pigs. In support of this idea, Barb *et al.* (1997) suggested that the ability of pigs to maintain euglycaemia during acute fasting was primarily due to rapid mobilization of alternative energy stores, such as FFA. This mobilization of energy stores may account for the failure of acute feed deprivation to affect LH secretion. Booth (1990) demonstrated that administration of glucose to feed-restricted gilts induced a rapid increase in episodic LH secretion similar to that observed in response to resumed feeding. In contrast, the LH response to GnRH was lower in satiated prepubertal gilts that received an i.v. injection of glucose compared with that of saline-treated gilts (Barb *et al.*, 1991). These conflicting results may, in part, be related to nutritional state, which has a profound effect on circulating concentrations of metabolites and metabolic hormones. These factors could alter the hypothalamic–pituitary response to glucose challenge.

Blocking of glycolysis with 2-deoxy-D-glucose resulted in a marked reduction in LH pulse frequency and an increase in serum GH concentrations in prepubertal gilts (Barb *et al.*, 2001). Furthermore, an i.v. bolus of glucose suppressed the GH response to GHRH in gilts (Barb *et al.*, 1991) and exposure of pig pituitary cells in culture to 300 or 600 mg glucose dl^{-1} suppressed the LH and GH responses to GnRH and GHRH, respectively (Barb *et al.*, 1995; Tables 1 and 2). The above studies support the hypothesis that glucose is a primary regulator of LH and GH secretion, and that the pituitary gland is a potential site of action.

The physiological relevance of the decrease in serum insulin concentrations during fasting is not clear (Barb *et al.*, 1997). In primiparous lactating sows (Rojkittikhun *et al.*, 1993a) and prepubertal gilts (Barb *et al.*, 2001), a 24 h fast did not affect LH secretion, although serum insulin concentrations were suppressed compared with those of animals that were fed. Increased dietary energy and insulin treatment in gilts at the follicular phase resulted in an increase in serum LH and FSH concentrations, but this effect did not occur in the absence of increased dietary energy (Cox *et al.*, 1987). Rojkittikhun *et al.* (1993b) reported that insulin treatment increased plasma LH concentrations but did not affect LH pulse frequency during the weaning to oestrus interval in primiparous sows. One model used to study the effect of insulin on the hypothalamic–pituitary axis is the diabetes-induced animal. In diabetic ovariectomized gilts, withdrawal of insulin therapy for 4 days prevented the oestradiol-induced preovulatory-like LH surge, but did not affect pulsatile LH secretion (Angell *et al.*, 1996). This finding indicates that diabetes mellitus alters the sensitivity of the hypothalamic–pituitary axis to oestradiol and the responsiveness of the pituitary gland to GnRH. Pituitary cell culture experiments confirmed that the sensitivity of the pituitary gland to GnRH decreased after removal of insulin therapy for 7 days in diabetic pigs (Angell *et al.*, 1996; Table 1).

Table 1. Effects of glucose, free fatty acids or diabetes on LH and GH secretion in the pig

Animal	Hormone	Metabolite[a]	Route	Type response	Result	Reference
Prepubertal gilt	LH	Glucose (1 g kg⁻¹)	i.v.	GnRH challenge	Decreased	Barb *et al.*, 1991
		Liposyn (3 ml kg⁻¹)	i.v.	GnRH challenge	Decreased	Barb *et al.*, 1991
	LH	Liposyn (3 ml kg⁻¹) per h for 9 h	i.v.	Pulse amplitude	Increased	Barb *et al.*, 1991
OVX prepubertal gilt, R	LH	Glucose	i.v.	Pulsatile	Increased	Booth, 1990
OVX diabetic gilt, no insulin	LH	Glucose		Oestradiol-induced surge	Decreased	Angell *et al.*, 1996
	LH	Glucose		Pulsatile secretion	No effect	Angell *et al.*, 1996
Diabetic gilt, no insulin	LH	Glucose		Pulsatile secretion	Increased	Cox *et al.*, 1994
OVX prepubertal gilt	GH	Glucose (1 g kg⁻¹)	i.v.	GHRH challenge	Decreased	Barb *et al.*, 1991
	GH	Liposyn (3 ml kg⁻¹)	i.v.	GHRH challenge	Decreased	Barb *et al.*, 1991
	GH	Liposyn (3 ml kg⁻¹) per h for 9 h	i.v.	Pulsatile	Increased	Barb *et al.*, 1991
OVX diabetic gilt	GH	Blood glucose (465 mg dl⁻¹)		Pulsatile secretion	Increased	Barb *et al.*, 1992

OVX: ovariectomized; R: feed-restricted.

[a]10% Liposym II; Abbott Laboratories, North Chicago, IL.

Table 2. Effects of glucose, oleic or linoleic acids on basal, GnRH and GHRH-induced LH and GH secretion from anterior pituitary cells in culture from prepubertal gilts[a]

	Hormone	Metabolite	Dose	Result
Basal secretion				
	LH	Glucose	300, 600 mg dl^{-1}	No effect
	GH	Glucose	100, 300, 600 mg dl^{-1}	No effect
	LH	Oleic acid	10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} mol l^{-1}	Increased
	LH	Linoleic acid	10^{-6}, 10^{-5}, 10^{-4} mol l^{-1}	Increased
	GH	Oleic acid	10^{-11}, 10^{-9} mol l^{-1}	Decreased
			10^{-7}, 10^{-5} mol l^{-1}	Increased
	GH	Linoleic acid	10^{-9} mol l^{-1}	Increased
Response to GnRH				
	LH	Glucose	600 mg dl^{-1}	Decreased
	LH	Oleic acid	10^{-6}, 10^{-5}, 10^{-4} mol l^{-1}	Decreased
	LH	Linoleic acid	10^{-5} mol l^{-1}	Decreased
Response to GHRH				
	GH	Glucose	100, 300, 600 mg dl^{-1}	Decreased
	GH	Oleic acid	10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} mol l^{-1}	Decreased
	GH	Linoleic acid	10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} mol l^{-1}	Decreased

[a]From Barb *et al.* (1995).

Mean serum GH concentrations and GH pulse frequency were greater in gilts with poorly controlled diabetes than in control pigs. Serum insulin concentrations were lower (0.3 ± 0.02 versus 0.9 ± 0.05 ng ml^{-1}; $P < 0.0001$) and plasma glucose concentrations were higher (465 ± 17 versus 82 ± 17 mg dl^{-1}; $P < 0.05$) in diabetic pigs than in control pigs (Barb *et al.*, 1992). The increase in GH secretion may be due, in part, to higher ($P < 0.0002$) glucose concentrations in the cerebrospinal fluid of diabetic pigs (162 ± 14 mg dl^{-1}) compared with control pigs (51 ± 3 mg dl^{-1}), which alters GHRH and somatostatin secretion from the hypothalamus, which in turn alters pituitary somatotrope activity (Barb *et al.*, 1992).

Two studies were conducted to investigate the idea that insulin acts at either the CNS or pituitary gland to alter LH and GH secretion. Central administration of insulin at doses of 1–100 µg failed to change LH and GH secretion in ovariectomized prepubertal gilts (Barb *et al.*, 1996; Fig. 1). However, insulin suppressed basal GH secretion and the GH response to GHRH for anterior pituitary cells in culture from 180-day-old gilts (C. R. Barb, J. B. Barrett and R. R. Kraeling, unpublished; Fig. 2). Thus, the influence of insulin on the LH and GH axis appears to be a manifestation of plasma glucose concentration. However, subtle effects of insulin on the sensitivity of the pituitary gland to hypothalamic secretagogues cannot be discounted (Tables 3 and 4).

Role of FFA

In pigs, feed deprivation results in a rapid onset of FFA mobilization from peripheral fat deposits, but maintenance of euglycaemia indicates an increase in hydrolysis of triglycerides and FFA oxidation resulting in glucose sparing (Barb *et al.*, 1997). Armstrong and Britt (1987) reported that chronic feed restriction in gilts resulted in cessation of oestrous cycles and lower concentrations of plasma insulin, increased concentrations of FFA and reduced LH pulse
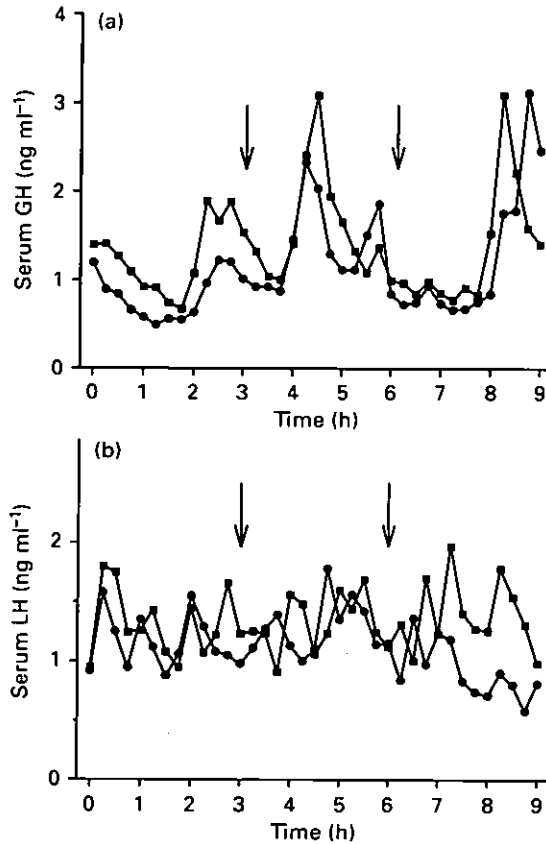
Fig. 1. Concentrations of serum (a) GH and (b) LH in ovariectomized prepubertal gilts receiving intracerebroventricular injection of saline (■; $n = 4$) or 1 µg insulin (●; $n = 4$) at 3 h and 6 h (arrows). Pooled standard errors for LH = 0.2 ng ml⁻¹ and GH = 0.5 ng ml⁻¹.

frequency compared with those of control gilts. Booth (1990) reported similar results in prepubertal gilts that were feed-restricted for 8 days, whereas in prepubertal gilts subjected to short-term fasting, serum concentrations of FFA increased, but there was no change in LH or GH secretion compared with control gilts (Barb *et al.*, 2001). In primiparous lactating sows, concentrations of serum FFA increased on day 12 and day 20 of lactation and this was associated with increased fat mobilization and protein catabolism (Armstrong *et al.*, 1986). Sows that remained anoestrous after weaning had increased plasma glucose concentrations and lower serum concentrations of FFA on day 12 and day 20 than those of oestrous sows. The authors speculated that aberrations in energy metabolism during lactation might have predisposed sows to anoestrus after weaning.

Therefore, do alterations in serum concentrations of FFA influence hypothalamic–pituitary function? In prepubertal gilts, i.v. infusion of a lipid emulsion enhanced the LH response to
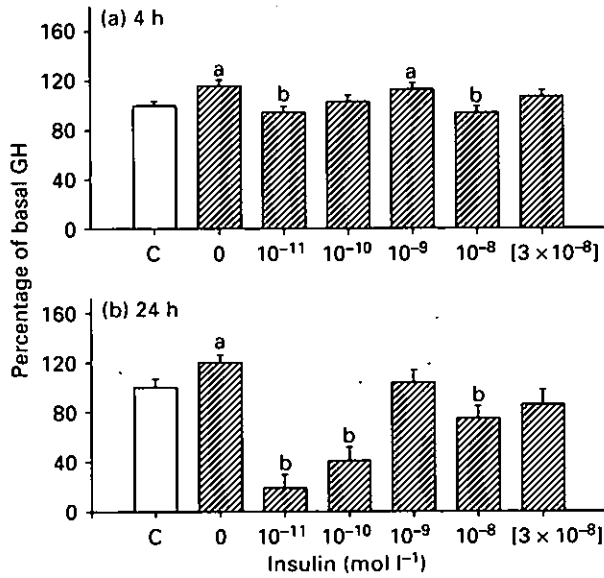
Fig. 2. Effect of insulin ($0–3 \times 10^{-8}$ mol l^{-1}) on growth hormone-releasing hormone (GHRH)-induced (10^{-6} mol l^{-1}) GH secretion in pig pituitary cells during (a) 4 h or (b) 24 h culture periods. Values are mean ± standard errors ($n = 8–12$ wells per treatment). C: control = basal secretion in the absence of treatment. [a]Significantly different from the control ($P < 0.05$). [b]Significantly different from GHRH alone ($P < 0.05$).

GnRH, but suppressed the GH response to GHRH, whereas infusion of the lipid emulsion at 1 h intervals increased serum LH pulse amplitude and GH pulse frequency (Barb et al., 1991; Table 1). The hourly fluctuations in serum concentrations of FFA may have synchronized the endogenous rhythm of GHRH and somatostatin secretion from the hypothalamus and, subsequently, altered somatotrope activity. Cultured pig pituitary cells were used to determine whether the effects of FFA *in vivo* occur at the pituitary gland without benefit of CNS input. Oleic and linoleic acids increased basal LH release. In contrast, oleic acid suppressed GnRH-induced release of LH (Table 2). This response was equivocal for linoleic acid (Barb et al., 1995). It is difficult to explain the differences between the *in vivo* and *in vitro* results. One possibility is that other hypothalamic factors *in vivo* may have altered pituitary gland responsiveness to GnRH (Barb et al., 1991). Alternatively, the lipid emulsion infused in the *in vivo* study (Barb et al., 1991) consisted of linoleic, oleic, palmitic, linolenic and stearic acids, whereas the effects of oleic or linoleic acid alone were evaluated in the *in vitro* study (Barb et al., 1995). Oleic and linoleic acids act directly on the anterior pituitary cells to alter basal and GHRH-induced GH release (Barb et al., 1995; Table 2). Moreover, this event seems to be mediated at the plasma membrane, because oleic and linoleic acids do not block the forskolin-induced release of GH (Barb et al., 1995). Thus, the above findings may explain, in part, the altered neuroendocrine activity observed during periods of fasting, feed restriction and lactation.

Table 3. Effects of leptin, insulin-like growth factor I (IGF-I) or insulin treatment on *in vitro* basal, GnRH and GHRH-induced LH and GH secretion from anterior pituitary cells and GnRH secretion from hypothalamic tissue

Animal[a]	Hormone	Metabolic hormone	Culture	Dose	Result	References
Basal secretion						
Prepubertal gilt	LH	Leptin	Pituitary	10^{-14}, 10^{-13}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-6} mol l^{-1}	Increased	Barb et al., 1999
	GH	Leptin		10^{-6}, 10^{-7} mol l^{-1}	Increased	Barb et al., 1998
Gilt	LH	IGF-I	Pituitary			Whitley et al., 1995
Follicular phase				10^{-11}, 10^{-10}, 10^{-9} mol l^{-1}	Increased	
Luteal phase				10^{-11} mol l^{-1}	Increased	
OVX				10^{-11}, 10^{-10} mol l^{-1}	Increased	
Prepubertal gilt	GH	IGF-I	Pituitary			C. R. Barb, J. B. Barrett and R. R. Kraeling, unpublished
4 h				10^{-9}, 10^{-8}, 3×10^{-8} mol l^{-1}	Increased	
24 h				10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 3×10^{-8} mol l^{-1}	Decreased	
Prepubertal gilt	GH	Insulin	Pituitary			C. R. Barb, J. B. Barrett and R. R. Kraeling, unpublished
4 h				10^{-11} mol l^{-1}	Decreased	
24 h				10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 3×10^{-8} mol l^{-1}	Decreased	Barb et al., 1999
OVX	GnRH	Leptin	Hypothalamic–preoptic area	10^{-12}, 10^{-10}, 10^{-6} mol l^{-1}	Increased	
Response to GnRH						
Prepubertal gilt	LH	Leptin	Pituitary	10^{-7} mol l^{-1}	Decreased	Barb et al., 1999
Gilt	LH	IGF-I	Pituitary			Whitley et al., 1995
Follicular				10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 3×10^{-8} mol l^{-1}	No effect	
Luteal				10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 3×10^{-8} mol l^{-1}	No effect	
OVX				10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 3×10^{-8} mol l^{-1}	No effect	
Response to GHRH						
Prepubertal gilt	GH	Leptin	Pituitary	10^{-11}, 10^{-9} mol l^{-1}	Decreased	Barb et al., 1998
Prepubertal gilt	GH	IGF-I	Pituitary			C. R. Barb, J. B. Barrett and R. R. Kraeling, unpublished
4 h				10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 3×10^{-8} mol l^{-1}	No effect	
24 h				10^{-11}, 10^{-10}, 10^{-8}, 3×10^{-8} mol l^{-1}	Decreased	
Prepubertal gilt	GH	Insulin	Pituitary			C. R. Barb, J. B. Barett and R. R. Kraeling, unpublished
4 h				10^{-11}, 10^{-8} mol l^{-1}	Decreased	
24h				10^{-11}, 10^{-10}, 10^{-8} mol l^{-1}	Decreased	

[a]OVX: ovariectomized.

Role of IGF-I

IGF-I appears particularly suitable for a role in linking somatic growth and development to activation of the reproductive axis. IGF-I exerts neurotrophic and mitogenic effects in the brain via interaction with specific receptors (Sara and Hall, 1990). Although type I IGF receptors are distributed widely throughout the brain, they are more concentrated in the median eminence (Werther *et al.*, 1990) indicating that, in this region, IGF-I is involved in functions other than cellular differentiation. Although IGF-I concentrations in the postnatal hypothalamus appear to be independent of peripheral concentrations (Rotwein *et al.*, 1988), circulating concentrations of IGF-I increased during pubertal development in pigs (Lee *et al.*, 1991). It is now clear that the peripubertal increase in serum IGF-I concentration is from hypothalamic, hepatic (Handelsman *et al.*, 1987) and adipose tissue (Wolverton *et al.*, 1992). Moreover, the pubertal increase in circulating IGF-I concentrations (Lee *et al.*, 1991) occurs concomitantly with an age-related decrease in pituitary response to GHRH (Dubreuil *et al.*, 1987) and an increase in LH secretion (Lutz *et al.*, 1984). These observations indicate that IGF-I may modulate hypothalamic release of GnRH and GHRH–somatostatin and pituitary responsiveness to hypothalamic hormones.

It is proposed that IGF-I acts, in a feedback loop, directly on the pituitary gland and brain to regulate LH and GH secretion. Although most evidence indicates that the effect of IGF-I on the release of pituitary hormones occurs in the CNS, subtle effects on the anterior pituitary cannot be discounted. Hiney *et al.* (1991) reported that IGF-I elicited GnRH release from the median eminence *in vitro*. The release of GnRH is greater after IGF-I than after IGF-II or insulin administration, indicating that IGF-I is the primary signal (Hiney *et al.*, 1991). In addition, release of GnRH from the median eminence, which is devoid of GnRH cell bodies, indicates that activation of gene expression by IGF-I is not involved. In rats, intracerebroventricular administration of IGF-I suppressed pulsatile GH secretion and stimulated brain somatostatin release (Abe *et al.*, 1983). IGF-I inhibited both acute release of GH and GH mRNA content in rat pituitary cells *in vitro* (Yamashita and Melmed, 1986). Moreover, intracerebroventricular administration of IGF-I suppressed GH secretion in pig fetuses (Spencer *et al.*, 1991; Table 4). In a recent experiment, intracerebroventricular administration of 10 µg IGF-I failed to alter LH and GH secretion in ovariectomized prepubertal gilts. Mean serum LH and GH concentrations before treatment were 0.8 ± 0.2 and 1.7 ± 0.6 ng ml^{-1} and after treatment were 1.0 ± 0.2 and 1.7 ± 0.6 ng ml^{-1}, respectively (Barb *et al.*, 1996). Doses up to 75 µg IGF-I failed to alter LH and GH secretion (Barb *et al.*, 1996). IGF-I suppressed basal GH secretion (Table 3) and the GH response to GHRH from pig pituitary cells in culture (C. R. Barb, J. B. Barrett and R. R. Kraeling, unpublished; Fig. 3). Furthermore, Whitley *et al.* (1995) reported that IGF-I-induced LH secretion was greater in pituitary cells from gilts at the follicular phase compared with cells from gilts at the luteal phase and ovariectomized gilts (Table 3). Thus, under certain physiological conditions, such as steroid milieu or nutritional status, endogenous IGF-I may contribute to the regulation of LH and GH secretion from the anterior pituitary in pigs.

Role of leptin

Leptin, secreted by adipose tissue in response to changes in energy availability, serves as a circulating signal of nutritional status and has a profound influence on regulation of the neuroendocrine axis and appetite in rodents (Casanueva and Dieguez, 1999; Ahima and Flier,

Table 4: Effects of leptin, insulin-like growth factor I (IGF-I) or insulin treatment on LH and GH secretion *in vivo*

Animal	Hormone	Hormone and dose	Route	Results	References
Prepubertal gilt	LH	Leptin: 10, 50 or 100 g	ICV	No effect	Barb *et al.*, 1999
	GH	Leptin: 10, 50 or 100 g	ICV	Increased	Barb *et al.*, 1998
OVX prepubertal	LH	IGF-I: 0.1, 1, 10, 25 or 75 g	ICV	No effect	Barb *et al.*, 1996
	GH	IGF-I: 0.1, 1, 10, 25 or 75 g	ICV	No effect	Barb *et al.*, 1996
Fetal pig	GH	IGF-I: 1.5 g	ICV	Decreased	Spencer *et al.*, 1991
OVX prepubertal	LH	Insulin: 1, 10 or 100 g	ICV	No effect	Barb *et al.*, 1996
	GH	Insulin: 1, 10 or 100 g	ICV	No effect	Barb *et al.*, 1996
Barrow	GH	Insulin: 0.3 iu kg^{-1} body weight	i.v.	Increased	Bonneau 1993
OVX gilt	LH	Insulin: 6 ng	ICV	Increased	Cox *et al.*, 1990
OVX gilt	GH	Insulin: 3, 6 or 12 ng	ICV	No effect	Barb *et al.*, 1990
Postpartum sow	LH	Insulin: 0.5 iu kg^{-1} body weight	i.v.	No effect	Rojkittikhun *et al.*, 1993b
Gilt follicular phase	LH	Insulin: 0.1 iu kg^{-1} body weight 4 × day +9, 960 kcal Me day^{-1}	i.v.	Increased	Cox *et al.*, 1987
		Insulin: 0.1 iu kg^{-1} body weight 4 × day +5, 771 kcal Me day^{-1}	i.v.	No effect	Cox *et al.*, 1987

OVX: ovariectomized; Me: metabolizable energy; ICV: intracerebroventricular.

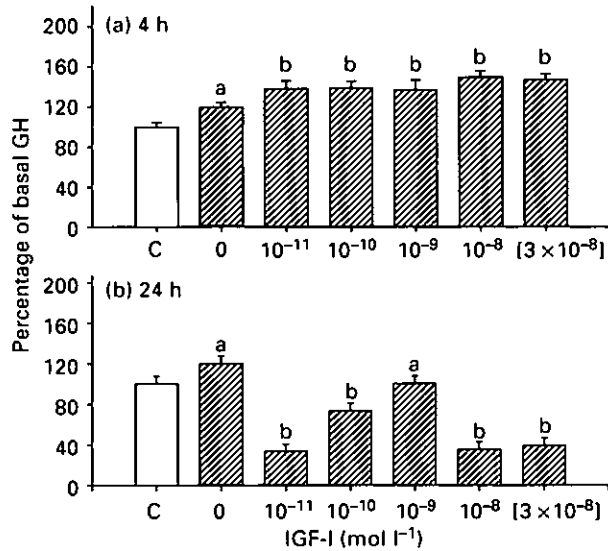**Fig. 3.** Effect of insulin-like growth factor I (IGF-I) $(0–3 \times 10^{-8}$ mol $l^{-1})$ on growth hormone-releasing hormone (GHRH)-induced $(10^{-6}$ mol $l^{-1})$ GH secretion in pig pituitary cells during (a) 4 h or (b) 24 h culture periods. Values are mean ± standard errors $(n = 8–12$ wells per treatment). C: control = basal secretion in the absence of treatment. [a]Significantly different from the control $(P < 0.05)$. [b]Significantly different from GHRH alone $(P < 0.05)$.

2000). We reported that serum leptin concentrations increased with age (Qian *et al.*, 1999) and decreased in response to feed deprivation (Barb *et al.*, 2001). In the ovariectomized prepubertal gilt, oestrogen-induced leptin mRNA expression in adipose tissue occurred at the time of expected puberty in intact gilts (Qian *et al.*, 1999) and was associated with greater LH secretion (Barb *et al.*, 2000). Furthermore, expression of the long form leptin receptor (OB-Rl) mRNA in the hypothalamus had increased by 3.5 months of age and remained high at 6 months of age in the prepubertal gilt demonstrating an age-dependent increase in OB-Rl expression (Lin *et al.*, 2001). Thus, leptin may be an important link between metabolic status and the neuroendocrine system.

Gonadotrophin secretion. The multifaceted effects of leptin appear to be mediated by the hypothalamus. In pigs, the presence of biologically active leptin receptors in the hypothalamus and pituitary gland indicate that leptin acts through the hypothalamic–pituitary axis (Lin *et al.*, 2000). In support of this proposal, leptin increased LH secretion from pig pituitary cells *in vitro* and GnRH release from hypothalamic tissue *in vitro* (Barb *et al.*, 1999; Table 3). Co-localization of leptin receptor mRNA with neuropeptide Y (NPY) gene expression provides strong evidence that hypothalamic NPY is a potential target for leptin (Cunningham *et al.*, 1999). In pigs, central administration of NPY suppressed LH secretion (Barb, 1999). However, fertility was restored only partially in the *ob/ob* mouse with a homozygous null mutation for NPY (Erickson *et al.*, 1996). In addition, leptin failed to affect *in vitro* NPY release from pig hypothalamic–preoptic area tissue fragments (C. R. Barb, unpublished). Therefore,

the action of leptin at the CNS may be mediated via other hypothalamic factors in addition to NPY.


Growth hormone axis. Growth hormone, unlike other pituitary hormones, exerts biological effects on most tissues in the body and plays an important role in the regulation of metabolism and energy balance (Etherton and Bauman, 1998). Altered GH secretion is associated with changes in body composition, metabolism and fasting (Muller *et al.*, 1999). A common factor associated with all of the above situations is a change in adiposity or change in energy metabolism.

We assessed the role of leptin in modulating GH secretion by intracerebroventricular administration of leptin to pigs that had been fed. GH secretion increased markedly in pigs that were fed normally, and maximum concentrations of GH occurred at 15–30 min after intracerebroventricular injection (Barb *et al.*, 1998), which is similar to the GH response to exogenously administered GHRF (Barb *et al.*, 1991; Table 4). More-over, central admin-istration of leptin reduced feed intake in a dose-dependent manner and this effect was still apparent at 48 h after leptin treatment (Barb *et al.*, 1998). In addition to a hypothalamic site of action, we demonstrated that leptin stimulated basal GH secretion and inhibited GHRH-induced GH secretion from pituitary cells in culture (Barb *et al.*, 1998; Table 3). Taken together, these results support the hypothesis that adipose tissue secretes a protein signal that acts on the CNS to regulate GH secretion and feed intake.

Leptin, a metabolic signal, regulates LH and GH secretion. In pigs, pulsatile leptin secretion decreased significantly after 24 h of a 28 h fast with no subsequent change in LH and GH secretion or in subcutaneous back fat thickness. However, plasma glucose and serum insulin and IGF-I concentrations were lower in fasted animals compared with control animals (Barb *et al.*, 2001). Prepubertal gilts were treated with 2-deoxy-D-glucose, a competitive inhibitor of glycolysis, to determine whether the effects of metabolic fuel restriction on LH and GH secretion were due to reduced serum leptin concentrations. Treatment with 2-deoxy-D-glucose increased mean serum GH concentrations, but failed to affect the frequency and amplitude of GH pulses. However, 2-deoxy-D-glucose suppressed LH pulse frequency, but failed to alter mean serum LH concentrations and LH pulse amplitude. Serum leptin concentrations were unchanged by 2-deoxy-D-glucose treatment (Barb *et al.*, 2001). These results indicate that acute effects of energy deprivation on LH and GH secretion are independent of changes in serum leptin concentrations and that there are two distinct sites at which leptin and glucose modulate the neuroendocrine axis.

Conclusion

Evidence has been presented that supports the concept that metabolites and metabolic hormones affect both hypothalamic hormone secretion and, hence, anterior pituitary function and anterior pituitary hormone secretion, directly. In general, glucose, FFA, insulin and IGF-I act primarily at the anterior pituitary gland to modulate pituitary responsiveness to GHRH and GnRH, whereas there are two distinct sites of action for leptin in regulating LH and GH secretion. Although leptin is an important metabolic signal, other metabolic cues, such as glucose, FFA, insulin and IGF-I, may play a role in modulating the neuroendocrine axis during pubertal development, lactation and during periods of acute and chronic undernutrition (Fig. 4).
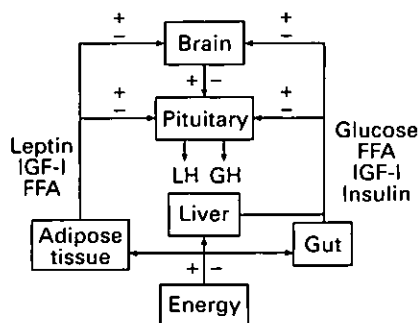
Fig. 4. Putative sites of action for metabolic fuels and metabolic hormones in neuroendocrine control of LH and GH secretion in pigs. Changes in circulating concentrations of glucose, free fatty acids (FFA), insulin, insulin-like growth factor I (IGF-I) and leptin in response to energy availability can be both inhibitory and stimulatory to LH and GH secretion.

References

Abe H, Molitch ME, Van Wyk JJ and Underwood LE (1983) Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetized rats *Endocrinology* 113 1319–1324

Ahima RS and Flier JS (2000) Leptin *Annual Review of Physiology* 62 413–437

Angell CA, Tubbs RC, Moore AB, Barb CR and Cox NM (1996) Depressed luteinizing hormone response to estradiol *in vivo* and gonadotropin-releasing hormone *in vitro* in experimentally diabetic swine *Domestic Animal Endocrinology* 13 453–463

Armstrong JD and Britt JH (1987) Nutritionally-induced anestrus in gilts: metabolic and endocrine changes associated with cessation and resumption of estrous cycles *Journal of Animal Science* 65 508–523

Armstrong JD, Britt JH and Kraeling RR (1986) Effect of restriction of energy during lactation on body condition, energy metabolism, endocrine changes and reproductive performance in primaparous sows *Journal of Animal Science* 63 1915–1925

Barb CR (1999) The brain–pituitary–adipocyte axis: role of leptin in modulating neuroendocrine function *Journal of Animal Science* 77 1249–1257

Barb CR, Cox NM, Matamoros IA, Kraeling RR and Rampacek GB (1990) Growth hormone (GH) secretion after intracerebroventricular (ICV) administrations of insulin (INS) in the gilt *Journal of Animal Science* 68 (Supplement 1) 310 (Abstract)

Barb CR, Kraeling RR, Barrett JB, Rampacek GB, Campbell RM and Mowles TF (1991) Serum glucose and free

fatty acids modulate growth hormone and luteinizing hormone secretion in the pig *Proceedings of the Society for Experimental Biology and Medicine* 198 636–642

Barb CR, Cox NM, Carlton CA, Chang WJ and Randle RF (1992) Growth hormone secretion, serum, and cerebral spinal fluid insulin and insulin-like growth factor-1 concentrations in pigs with streptozotocin-induced diabetes mellitus *Proceedings of the Society for Experimental Biology and Medicine* 201 223–228

Barb CR, Kraeling RR and Rampacek GB (1995) Glucose and free fatty acid modulation of growth hormone and luteinizing hormone secretion by cultured porcine pituitary cells *Journal of Animal Science* 73 1416–1423

Barb CR, Kraeling RR and Rampacek GB (1996) Luteinizing hormone and growth hormone (GH) secretion after intracerebroventricular (ICV) administration of IGF-I or insulin (INS) in the prepuberal gilt *Journal of Animal Science* 74 (Supplement 1) 240 (Abstract)

Barb CR, Kraeling RR, Rampacek GB and Dove CR (1997) Metabolic changes during the transition from the fed to the acute feed-deprived state in prepuberal and mature gilts *Journal of Animal Science* 75 781–789

Barb CR, Yan X, Azian MJ, Kraeling RR, Rampacek GB and Ramsay TG (1998) Recombinant porcine leptin reduced feed intake and stimulates growth hormone secretion in swine *Domestic Animal Endocrinology* 15 77–86

Barb CR, Barrett JB, Kraeling RR and Rampacek GB (1999) Role of leptin in modulating neuroendocrine function: a metabolic link between the brain–pituitary and adipose tissue *Reproduction in Domestic Animals* 34 111–125

Barb CR, Kraeling RR, Rampacek GB and Estienne MJ (2000) Current concepts of the onset of puberty in the gilt *Reproduction in Domestic Animals* (Supplement 6) 82–89

Barb CR, Barrett JB, Kraeling RR and Rampacek GB (2001) Serum leptin concentrations, luteinizing hormone and growth hormone secretion during feed and metabolic fuel restriction in the prepubertal gilt *Domestic Animal Endocrinology* 20 47–63

Bonneau M (1993) Growth hormone response to GRF and insulin-induced hypoglycemia in Yorkshire and Meishan pigs *American Journal of Physiology: Endocrinology and Metabolism* 264 E54–E59

Booth PJ (1990) Metabolic influences on hypothalamic–pituitary ovarian function in the pig *Journal of Reproduction and Fertility Supplement* 40 89–100

Buonomo FC, Grohs DL, Baile CA and Campion DR (1988) Determination of circulating levels of insulin-like growth factor II (IGF-II) in swine *Domestic Animal Endocrinology* 5 323–329

Cameron JL, Koerker DJ and Steiner RA (1985) Metabolic changes during maturation of male monkeys: possible sign for onset of puberty *American Journal of Physiology* 249 E385–E391

Casanueva FF and Dieguez C (1999) Neuroendocrine regulation and actions of leptin *Frontiers in Neuroendocrinology* 20 317–363

Cosgrove JR and Foxcroft GR (1996) Nutrition and reproduction in the pig: ovarian aetiology *Animal Reproduction Science* 42 131–141

Cox NM and Britt JH (1988) Pulsatile administration of gonadotropin releasing hormone to lactating sows: endocrine changes associated with induction of fertile estrus *Biology of Reproduction* 27 1126–1137

Cox NM, Stuart MJ, Althen TG, Bennett WA and Miller HW (1987) Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during follicular growth *Journal of Animal Science* 64 507–516

Cox NM, Barb CR, Kesner PJ, Kraeling RR, Matamoros IA and Rampacek GB (1990) Effects of intracerebroventricular administration of insulin on luteinizing hormone in gilts *Journal of Reproduction and Fertility Supplement* 40 63 (Abstract)

Cox NM, Meurer KA, Carlton CA, Tubbs RC and Mannis DP (1994) Effect of diabetes mellitus during the luteal phase of the oestrous cycle on preovulatory follicular function, ovulation and gondatrophins in gilts *Journal of Reproduction and Fertility* 101 77–86

Cunningham MJ, Clifton DK and Steiner RA (1999) Leptin's action on the reproductive axis: perspectives and mechanisms *Biology of Reproduction* 60 216–222

Dubreuil P, Pelletier G, Petitclerc D, Lapierre H, Couture Y, Brazeau P, Gaudreau P and Morisset J (1987) Influence of age and sex on basal secretion of growth hormone (GH) and on GH-induced release by porcine GH-releasing factor pGRF(1-29NH2) in growing pigs *Domestic Animal Endocrinology* 4 299–307

Erickson JC, Hollopeter G and Palmiter RD (1996) Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y *Science* 274 1704–1707

Etherton TD and Bauman DE (1998) Biology of somatotropin in growth and lactation of domestic animals *Physiological Reviews* 78 745–761

Frisch RE (1984) Body fat, puberty and fertility *Biological Reviews* 59 161–188

Handelsman DJ, Spaliviero JA, Scott CD and Baxter RC (1987) Hormonal regulation of the peripubertal surge of insulin-like growth factor-I in the rat *Endocrinology* 120 491–496

Goodman RL (1988) Neuroendocrine control of the ovine estrus cycle. In *The Physiology of Reproduction*, Vol 1, pp 1929–1970 Eds E Knobil, JD Neill, LL Ewing, GS Greenwald, CL Markert and DW Pfaff. Raven Press, New York

Hiney JK, Ojeda SR and Dees WL (1991) Insulin-like growth factor-I: a possible metabolic signal involved in the regulation of female puberty *Neuroendocrinology* 54 420–423

Kraeling RR and Barb CR (1990) Hypothalamic control of gonadotrophin and prolactin secretion in pigs *Journal of Reproduction and Fertility Supplement* 40 3–7

Lee CY, Bazer FW, Etherton TD and Simmen FA (1991) Ontogeny of insulin-like growth factors (IGF-I and IGF-II) and IGF-binding proteins in porcine serum during fetal and postnatal development *Endocrinology* 128 2336–2344

Lin J, Barb CR, Matteri RL, Kraeling RR, Chen X, Meinersmann RJ and Rampacek GB (2000) Long form leptin receptor mRNA expression in the brain, pituitary and other tissues in the pig *Domestic Animal Endocrinology* 19 53–61

Lin J, Barb CR, Kraeling RR and Rampacek GB (2001) Developmental changes in the long form leptin receptor and related neuropeptide gene expression in the pig brain *Biology of Reproduction* 64 1614–1618

Lutz JB, Rampacek GB, Kraeling RR and Pinkert CA (1984) Serum luteinizing hormone and estrogen profiles before puberty in the gilt *Journal of Animal Science* 58 686–691

Muller EE, Locatelli V and Cocchi D (1999) Neuroendocrine control of growth hormone secretion *Physiological Reviews* 79 511–607

Pressing A, Dial GD, Esbenshade KL and Stroud CM (1992) Hourly administration of GnRH to prepubertal gilts: endocrine and ovulatory responses from 70 to 190 days of age *Journal of Animal Science* 70 232–242

Prunier A and Quesnel H (2000a) Nutritional influences on the hormonal control of reproduction in female pigs *Livestock Production Science* 63 1–16

Prunier A and Quesnel H (2000b) Influence of the nutritional status on ovarian development in female pigs *Animal Reproduction Science* 60–61 185–197

Qian H, Barb CR, Compton MM, Hausman GJ, Azain MJ, Kraeling RR and Baile CA (1999) Leptin mRNA expression and serum leptin concentrations as influenced by age, weight, and estradiol in pigs *Domestic Animal Endocrinology* 16 135–143

Rojkittikhun T, Uvnäs-Moberg K and Einarsson S (1993a) Plasma oxytocin, prolactin, insulin and LH after 24 h of fasting and after refeeding in lactating sows *Acta Physiologica Scandinavica* 148 413–419

Rojkittikhun T, Einarsson S, Zilinskas H, Edqvist LE, Uvnäs-Moberg K and Lundeheim N (1993b) Effects of insulin administration at weaning on hormonal patterns and reproductive performance in primiparous sows *Zentralblatt fur Veterinarmedizin Reihe A* **40** 161–168

Rotwein P, Burgess JD, Milbrandt JD and Krause JE (1988) Differential expression of insulin-like growth factor genes in rat central nervous system *Proceedings National Academy of Sciences USA* **85** 265–269

Sara VR and Hall K (1990) Insulin-like growth factors and their binding proteins *Physiological Reviews* **70** 591–614

Spencer GSG, Macdonald AA, Buttle HL, Moore LG and Carlyle SS (1991) Intracerebral administration of insulin-like growth factor I decreases circulating growth hormone levels in the fetal pig *Acta Endocrinologica (Copenhagen)* **124** 563–568

Wade GN, Schneider JE and Li HY (1996) Control of fertility by metabolic cues *American Journal of Physiology* **270** E1–E19

Werther GA, Abate M, Hogg A *et al.* (1990) Localization of insulin-like growth factor-I mRNA in rat brain by *in situ* hybridization – relationship to IGF-I receptors *Molecular Endocrinology* **4** 773–778

Wetsel WC, Valenca MM, Merchenthaler I, Liposits Z, Lopez FJ, Weiner RI, Mellon PL and Negro-Vilar A (1992) Intrinsic pulsatile secretory activity of immortalized luteinizing hormone-releasing hormone-secreting neurons *Proceedings National Academy of Sciences USA* **89** 4149–4153

Whitley NC, Barb CR, Utley RV, Popwell JM, Kraeling RR and Rampacek GB (1995) Influence of stage of the estrous cycle on insulin-like growth factor-I modulation of luteinizing hormone secretion in the gilt *Biology of Reproduction* **53** 1359–1364

Wolverton CK, Azain MJ, Duffy JY, White ME and Ramsay TG (1992) Influence of somatotropin on lipid metabolism and IGF gene expression in porcine adipose tissue *American Journal of Physiology* **263** E637–E645

Yamashita S and Melmed S (1986) Insulin-like growth factor I action on rat anterior pituitary cells: suppression of growth hormone secretion and messenger ribonucleic acid levels *Endocrinology* **118** 176–182