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Role of neuropeptides and amino acids in controlling secretion of hormones from the anterior pituitary gland in pigs

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All reproductive processes involve one or more of the protein hormones secreted from the anterior pituitary gland: LH, FSH, prolactin, growth hormone, ACTH and thyroid-stimulating hormone (TSH). Primary hormones of reproduction, such as LH and FSH, directly regulate a reproductive activity. For example, LH and FSH stimulate follicular growth and the associated secretion of oestradiol in sows. In contrast, secondary hormones of reproduction such as TSH are permissive and regulate other physiological systems that indirectly, but profoundly, influence reproduction. Reproduction in pigs can be enhanced by developing strategies to alter and control secretion of hormones from the anterior pituitary gland. However, the successful manipulation of adenohypophysial hormone secretion will require a sound understanding of the mechanisms controlling the function of the hypothalamic–pituitary axis. Hypothalamic hormones including GnRH, dopamine, growth hormone-releasing hormone (GHRH), somatostatin, corticotrophin-releasing hormone (CRH) and thyrotrophin-releasing hormone (TRH) are synthesized in perikarya that possess axons that terminate at the median eminence. These hormones are released into the hypothalamo–hypophysial portal vasculature, travel to the anterior pituitary gland and stimulate or inhibit secretion of adenohypophysial hormones. Secretion of hypothalamic hormones is ultimately controlled by a variety of neurotransmitters and neuropeptides, the most studied in swine being the endogenous opioid peptides (EOP) and more recently, the excitatory amino acids (ExAA). In general, EOP inhibit GnRH and hence LH secretion, and this effect involves the central catecholaminergic system. A definitive role for EOP in the modulation of FSH release remains to be determined. EOP stimulate secretion of GHRH and thus growth hormone release, and depending on the animal model studied, EOP exert either stimulatory or inhibitory influences on prolactin secretion. ExAA, working via *N*-methyl-D-aspartate (NMDA) receptors at the central nervous system, stimulate secretion of LH, FSH, growth hormone and prolactin in appropriate animal models. However, in certain situations, an inhibitory effect of ExAA on LH secretion has been demonstrated. The modulation of growth hormone and prolactin secretion by ExAA involves EOP. Research investigating the function of ExAA and EOP in the physiological control of swine reproduction warrants further scrutiny.

Neuroendocrine Control of Adenohypophysial Hormone Secretion

Hypothalamic hormones, including GnRH, dopamine, GHRH, somatostatin, CRH and TRH, are synthesized in perikarya that possess axons that terminate at the median eminence. These hormones are released into the hypothalamo–hypophysial portal blood vessels, travel to the anterior pituitary gland, and either stimulate or inhibit secretion of adenohypophysial hormones. Anterior pituitary

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gland hormones stimulate target tissues to release hormones and metabolites that in turn modify adenohypophysial hormone secretion via negative and positive feedback mechanisms. Because of space limitations, these intricate feedback loops will not be discussed in this review.

Gonadotrophins

Administration of GnRH stimulates LH secretion, follicular growth and ovulation in prepubertal gilts (Lutz *et al.*, 1985) and hypophysial stalk-transected gilts injected with pregnant mares' serum gonadotrophin (Kraeling *et al.*, 1990). In pigs, as in other mammals, there are two distinct modes of LH secretion that presumably reflect two patterns of GnRH secretion from the central nervous system: (1) episodes of LH release (i.e. pulsatile secretion), the frequency and amplitude of which vary with the reproductive status of the animal, circulating steroidal milieu or with both factors, and (2) preovulatory surge secretion. With regard to gonadotrophins, this review focuses primarily on the neuroendocrine control of pulsatile secretion of LH. The neuroendocrine control of the preovulatory surge of gonadotrophins in pigs has been thoroughly covered in the review by Kraeling *et al.* (1992).

Kineman *et al.* (1988) described GnRH-immunostained cell bodies in the medial preoptic area, diagonal band of broca, lateral hypothalamic area, paraventricular nucleus, periventricular zone, suprachiasmatic nucleus and medial basal hypothalamus of ovariectomized and ovary-intact gilts. These perikarya had axons that projected to the median eminence.

Using push–pull cannulae, Leshin *et al.* (1992) demonstrated that pulsatile release of LH is associated with pulsatile secretion of GnRH, as detected in the vasculature of the anterior pituitary gland of ovariectomized gilts. Disrupting the GnRH signal to the anterior pituitary gland by transection of the hypophysial stalk (Kraeling *et al.*, 1990) or by active or passive immunization against GnRH (Esbenshade *et al.*, 1990) abolished pulsatile release of LH in gilts. Secretion of FSH was also suppressed after immunization against GnRH (Esbenshade *et al.*, 1990).

Prolactin

Prolactin secretion is tonically inhibited by signals from the central nervous system in pigs. Kraeling *et al.* (1994) reported high circulating concentrations of prolactin in ovariectomized gilts that were subjected to transection of the hypophysial stalk.

Several studies suggest that dopamine inhibits prolactin secretion in swine. For example, bromocryptine, a dopamine agonist, suppressed prolactin secretion in lactating sows, ovariectomized gilts and ovariectomized gilts that were subjected to transection of the hypophysial stalk (Kraeling *et al.*, 1982, 1994).

In pigs, TRH is a putative prolactin-releasing hormone (Dubreuil *et al.*, 1990; Kraeling *et al.*, 1994). The prolactin response to a TRH challenge was attenuated by bromocryptine treatment in ovariectomized, hypophysial stalk-transected gilts, suggesting that the suppression of dopaminergic pathways is a necessary antecedent to the mechanism by which TRH stimulates prolactin secretion (Kraeling *et al.*, 1994).

Growth hormone

GHRH stimulates, and somatostatin inhibits, growth hormone secretion in swine (Dubreuil *et al.*, 1990). Immunocytochemical studies have revealed GHRH neurones in the arcuate and ventromedial nuclei, and somatostatin neurones in the periventricular region of gilts (Leshin *et al.*, 1994). GHRH and somatostatin nerve fibres project ventrally into the median eminence.

Active immunization against GHRH abolished episodic secretion of growth hormone in the lactating sow (Armstrong *et al.*, 1990). In contrast, active immunization against somatostatin increased growth hormone secretion in prepubertal boars and gilts (Dubreuil *et al.*, 1989). Disrupting the GHRH signal to the anterior pituitary gland by transection of the hypophysial stalk resulted in a

loss of pulsatile growth hormone secretion in ovariectomized gilts. Nevertheless, basal growth hormone concentrations were increased, perhaps due to the decreased amount of somatostatin reaching the adenohypophysis (Klindt *et al.*, 1983).

Other anterior pituitary gland hormones

Relatively little is known about the neuroendocrine control of ACTH and TSH in swine. However, CRH stimulated secretion of cortisol in ovariectomized gilts and in gilts that were ovariectomized and subjected to transection of the hypophysial stalk, presumably by enhancing ACTH secretion (Estienne *et al.*, 1988). Serum concentrations of cortisol before CRH administration were similar for hypophysial stalk-transected and control gilts, indicating that basal secretion of cortisol was maintained in the absence of central inputs (Estienne *et al.*, 1988). Finally, treatment of gilts or lactating sows with TRH enhanced thyroxine secretion, probably as a consequence of increased secretion of TSH (Dubreuil *et al.*, 1990; Barb *et al.*, 1991a).

Catecholamines

The catecholamines noradrenaline, adrenaline and dopamine are synthesized by neurones within the central nervous system. The conversion of tyrosine to dihydroxyphenylalanine (DOPA) is catalysed by tyrosine hydroxylase and is the rate-limiting step in catecholamine biosynthesis. DOPA is converted to dopamine via the action of aromatic decarboxylase. Dopamine is converted to noradrenaline and then adrenaline via the action of dopamine β-hydroxylase and phenylethanolamine-*n*-methyltransferase, respectively. Leshin *et al.* (1996) located tyrosine hydroxylase and dopamine-β-hydroxylase immunopositive neurones in the hypothalamus of the gilt. It has been hypothesized that in pigs, nerve cells that release catecholamines act as interneurones and participate in the control of secretion of hypothalamic hormones and hence anterior pituitary gland function by EOP and ExAA. Thus, a brief description of the effects of catecholamines on secretion of adenohypophysial hormones is warranted.

Central administration of noradrenaline to adult, gonadectomized miniature pigs had dichotomous effects on LH release (Parvizi and Ellendorff, 1982). Whether noradrenaline stimulated or inhibited secretion of LH depended on the dose administered and specific site of hypothalamic injection.

Reports concerning the effects of dopamine on LH secretion in swine are equivocal. Kraeling *et al.* (1982) reported that the dopamine agonist bromocryptine decreased serum concentrations of LH in lactating sows. However, Bevers *et al.* (1983) reported that LH secretion increased in bromocryptine-treated lactating sows. Treatment with bromocryptine had no effect on LH secretion in ovariectomized gilts (Kraeling *et al.*, 1982).

AIMAX (synonym = methallibure) is a derivative of dithiocarbamoylhydrazine and has actions similar to diethyldithiocarbamate, a potent inhibitor of dopamine β-hydroxylase. Treatment of rats with AIMAX decreased LH secretion, and suppressed noradrenaline synthesis and increased dopamine content in the hypothalamus (Chang *et al.*, 1995). When fed to ovariectomized gilts with (Chang *et al.*, 1993a) or without (Kesner *et al.*, 1987) progesterone replacement therapy, AIMAX suppressed pulsatile gonadotrophin secretion. Moreover, the oestradiol-induced LH surge was abolished and the FSH surge attenuated, in AIMAX-fed, ovariectomized gilts (Kesner *et al.*, 1987). Effects of AIMAX were manifested at the hypothalamus since pituitary responsiveness to exogenously administered GnRH was uncompromised by treatment (Kesner *et al.*, 1987). These findings suggest that catecholamines stimulate both pulsatile and surge secretions of gonadotrophins in swine.

Ingestion of AIMAX had no effect on serum concentrations of growth hormone and prolactin in ovariectomized or ovariectomized, steroid-treated gilts (Kesner *et al.*, 1987; Chang *et al.*, 1993a), and failed to alter pulsatile secretion of growth hormone in boars (D. S. Broughton, M. J. Estienne, J. M. Harter-Dennis, C. R. Barb and T. G. Hartsock, unpublished). AIMAX also had no effect on circulating

concentrations of the thyroid hormones and cortisol, suggesting that secretion of TSH and ACTH was unaffected in ovariectomized gilts (Kesner *et al.*, 1987).

Endogenous Opioid Peptides

Endorphins, enkephalins and dynorphins are collectively called EOP and are natural ligands for receptors that also bind opiates. Proopiomelanocortin (POMC) is the precursor for β-endorphin. Using immunocytochemical techniques, Kineman *et al.* (1989) located POMC perikarya within the arcuate area in gilts. These cell bodies had fibres that projected to the medial basal hypothalamus, periventricular zone, preoptic area and median eminence.

Effect of EOP and EOP agonists on secretion of anterior pituitary gland hormones

The role of EOP in controlling LH secretion in pigs has been scrutinized in several thorough reviews (Barb *et al.*, 1991b; Kraeling *et al.*, 1992). In general, treatment of pigs with EOP or their agonists decreased secretion of LH. For example, morphine, an agonist of EOP, suppressed LH secretion when administered to ovariectomized gilts via the lateral cerebral ventricle (Estienne *et al.*, 1990). However, intravenous injections of morphine failed to alter LH secretion in immature boars (Trudeau *et al.*, 1988).

Responses to EOP agonists with regard to FSH secretion have been less consistent. For example, intracerebroventricular injection of morphine suppressed FSH release in ovariectomized gilts in some experiments, but not others (Estienne *et al.*, 1990; Barb *et al.*, 1992a). Morphine inhibited FSH secretion when intravenously administered to immature boars (Trudeau *et al.*, 1988).

Results of several studies suggest that EOP stimulate secretion of prolactin and growth hormone. Intracerebroventricular administration of morphine increased circulating concentrations of prolactin and growth hormone in ovariectomized gilts (Estienne *et al.*, 1990; Barb *et al.*, 1992a).

Effect of EOP antagonists on secretion of anterior pituitary gland hormones

The classic method for studying the physiological role of an endogenous neuromodulator is to use a selective antagonist to block its effects. Several studies have been conducted in which male and female pigs in various reproductive states were treated with the EOP receptor antagonist naloxone.

Stimulation of LH secretion by treatment with naloxone supports the concept that EOP have a physiological role in inhibiting gonadotrophin secretion. Intravenous treatment with naloxone increased LH secretion in luteal phase, but not follicular phase, gilts (Barb *et al.*, 1986a). Injections of naloxone also increased serum concentrations of LH in lactating sows (Barb *et al.*, 1986b; Mattioli *et al.*, 1986; De Rensis *et al.*, 1993).

Treatment with naloxone stimulated LH release in ovariectomized gilts only if the animals were treated with exogenous progesterone (Barb *et al.*, 1986a, 1988). In contrast, naloxone treatment failed to alter LH release in ovary-intact and ovariectomized, progesterone-treated prepubertal gilts (Barb *et al.*, 1988). However, naloxone increased LH secretion when gilts were ovariectomized before puberty and were treated with progesterone and the EOP antagonist at an age when contemporary gilts had begun to display oestrous cycles (Barb *et al.*, 1988). This study suggests that, in gilts, the development of EOP modulation of LH secretion is a brain maturational process that is independent of gonadal influences. In contrast, naloxone had no effect on LH release in immature boars or barrows, but increased LH secretion in barrows treated with testosterone so as to establish adult concentrations of androgen (Patchev *et al.*, 1987; Trudeau *et al.*, 1989).

Similar to experiments in which equivocal responses to EOP agonists were found, studies examining the effects of naloxone on FSH secretion have yielded conflicting results. For example, naloxone increased FSH secretion in immature boars (Trudeau *et al.*, 1989) and lactating sows (Barb *et al.*, 1987), but had no effect on FSH release in ovariectomized gilts, or ovariectomized, prepubertal gilts with or without progesterone therapy (Barb *et al.*, 1992a). De Rensis *et al.* (1993) reported that

naloxone had no effect on FSH secretion in postpartum sows. Interestingly, Barb *et al.* (1992a) also reported that naloxone treatment suppressed FSH secretion in prepubertal gilts and in mature, ovariectomized gilts treated with progesterone.

Changes in LH release without concomitant alterations in FSH secretion and vice versa support the notion that there are separate releasing hormones for the two gonadotrophins in pigs. Alternatively, divergent patterns of LH and FSH secretion following treatment with EOP agonists and antagonists could be related to circulating concentrations of steroids and gonadal peptides or the existing pattern of GnRH release. Jayes *et al.* (1997) suggested that high GnRH pulse frequency was more effective in acutely releasing LH than FSH, and inhibits FSH synthesis and secretion. In contrast, low GnRH pulse frequency supports FSH synthesis and release but is not effective in increasing LH concentrations.

Naloxone treatment suppressed secretion of growth hormone (Armstrong *et al.*, 1990; Barb *et al.*, 1991a) and prolactin (Mattioli *et al.*, 1986; Barb *et al.*, 1987) in lactating sows. De Rensis *et al.* (1993) reported that naloxone treatment suppressed prolactin secretion when administered at day 10 of lactation but not when given during the first 78 h postpartum.

Naloxone increased prolactin secretion in luteal phase gilts (Barb *et al.*, 1986a) and in mature, ovariectomized, progesterone-treated gilts (Barb *et al.*, 1992a), but had no effect on prolactin release in mature, ovariectomized gilts or ovariectomized, prepubertal gilts with or without progesterone treatment (Barb *et al.*, 1992a). Thus, it appears that EOP exert both stimulatory and inhibitory effects on prolactin secretion in swine.

The effects of naloxone on ACTH and TSH secretion in swine have not been reported. However, naloxone treatment stimulated cortisol secretion in cyclic and ovariectomized gilts (Barb *et al.*, 1986a; Estienne *et al.*, 1988) and inhibited cortisol secretion in lactating sows (Barb *et al.*, 1991a).

Site of action for effects of EOP on adenohypophysial hormone secretion

The notion that the EOP restraint on gonadotrophin secretion is manifested primarily at the hypothalamus is supported by studies *in vitro* in which naloxone stimulated GnRH secretion from the hypothalamic–preoptic area collected from gilts (Barb *et al.*, 1994). Moreover, naloxone failed to increase LH secretion in gilts treated with antisera to GnRH (Chang *et al.*, 1993a). Naloxone had no effect on the LH and FSH response to GnRH when the EOP receptor antagonist and secretogogue were administered concomitantly in immature boars (Trudeau *et al.*, 1989).

Treatment with naloxone in sows injected with control serum decreased growth hormone concentrations and the frequency of growth hormone pulses to values reported for saline-treated, GHRH-immunized sows (Armstrong *et al.*, 1990). This finding suggests that high circulating concentrations of growth hormone in the lactating sow are primarily due to an opioid-modulated increase in GHRH secretion.

Naloxone-stimulated secretion of cortisol in ovariectomized gilts was abolished by transection of the hypophysial stalk (Estienne *et al.*, 1988). Since serum cortisol concentrations increased in these animals following CRH and ACTH administration, Estienne *et al.* (1988) concluded that naloxone increased cortisol secretion in swine by acting at the hypothalamus to alter release of CRH and subsequent ACTH secretion.

Despite the abundance of evidence suggesting that modulation of anterior pituitary gland hormone secretion by EOP in swine is primarily a consequence of central actions, there may be subtle effects of EOP directly on the adenohypophysis. β-Endorphin suppressed basal and GnRH-induced LH secretion by pig pituitary cells *in vitro* (Barb *et al.*, 1990). In contrast, chronic exposure to naloxone increased basal secretion of LH and enhanced pituitary responsiveness to GnRH (Barb *et al.*, 1990).

Mechanism of action for effects of EOP on adenohypophysial hormone secretion

Catecholamines stimulate, and EOP inhibit, secretion of GnRH and hence LH in pigs. Several lines of evidence suggest that EOP and catecholamines interact in regulating GnRH secretion (Fig. 1). Anatomical studies have revealed that POMC, tyrosine hydroxylase and dopamine

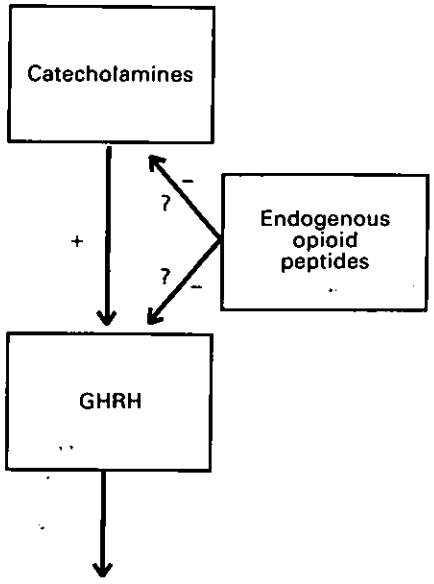

Fig. 1. Possible neuronal pathways by which endogenous opioid peptides inhibit secretion of GnRH, and hence LH, release in swine.

β–hydroxylase immunopositive neurones are located in the vicinity of GnRH neurones in the hypothalamus (Kineman *et al.*, 1988, 1989; Leshin *et al.*, 1996).

Treatment with AIMAX abolished naloxone stimulation of LH secretion in ovariectomized, progesterone-treated gilts (Chang *et al.*, 1993a). The prolactin response to naloxone was not attenuated in ovariectomized, progesterone-treated gilts fed AIMAX, suggesting EOP modulation of prolactin secretion is independent of the noradrenaline system (Chang *et al.*, 1993a).

Excitatory Amino Acids

A group of amino acids that includes glutamate and aspartate has been implicated as the major family of neurotransmitters in the mammalian central nervous system. The ExAA satisfy the main criteria for classification as neurotransmitters, namely: (1) presynaptic localization in specific nerve terminals, (2) specific release by physiological stimuli in concentrations great enough to evoke a postsynaptic response, (3) identity of action (i.e., postsynaptic actions of the naturally occurring transmitter are mimicked by the candidate substance, including response to antagonists), and (4) existence of neuronal mechanisms, such as re-uptake and metabolism, that terminate neurotransmitter action.

Studies in other species have demonstrated that glutamate and aspartate, and receptors for these ExAA, appear in many regions of the brain including the hypothalamus and median eminence. There are several types of receptor that are stimulated by glutamate and these are named according to their selective agonists. Receptor types include *N*-methyl-D-aspartate (NMDA), kainate, and D,L-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) (Petralia and Wenthold, 1996).

Secretion of hormones from the anterior pituitary gland following administration of ExAA

The ExAA agonist, *N*-methyl-D,L-aspartate (NMA) is a potent activator of the NMDA receptor. The ability of intravenously administered NMA to alter secretion of hormones from the anterior pituitary gland has been confirmed in several experiments in pigs (Table 1). In contrast to the

Table 1. Effects of intravenous injections of *N*-methyl-D,L-aspartate (NMA) on circulating concentrations of hormones secreted from the anterior pituitary gland in swine

Hormone and model	Dose of NMA (mg kg^{-1} body weight)	Result	References
LH			
Prepubertal gilts	1.25, 2.5 or 5.0	No effect	Estienne *et al.*, 1995
	10.0	Increased	Estienne *et al.*, 1995
Cyclic gilts – luteal phase	10.0	Increased	Hurlock *et al.*, 1995
– follicular phase	10.0	No effect	Hurlock *et al.*, 1995
Lactating sows	1.5 or 3.0	No effect	Sesti and Britt, 1992
	5.0	Increased	Sesti and Britt, 1992
	10.0	Increased	Sesti and Britt, 1993, 1994
Ovariectomized gilts	10.0	Decreased	Barb *et al.*, 1992b
		Decreased	Chang *et al.*, 1993b
		Decreased	Hurlock *et al.*, 1995
		Decreased	Popwell *et al.*, 1996
Ovariectomized,	10.0	Increased	Sesti and Britt, 1992
oestradiol-treated gilts	10.0	No effect	Barb *et al.*, 1992b
Ovariectomized,	10.0	Decreased	Barb *et al.*, 1992b;
progesterone-treated gilts			Chang *et al.*, 1993b
Boars	1.25, 2.5, 5.0 or 10.0	No effect	Broughton *et al.*, 1996
Barrows	2.5	No effect	Popwell *et al.*, 1996
FSH			
Lactating sows	10.0	Increased	Sesti and Britt, 1993, 1994
Growth Hormone			
Prepubertal gilts	1.25 or 5.0	No effect	Estienne *et al.*, 1995
	2.5 or 10.0	Increased	
Ovariectomized gilts	10.0	Increased	Barb *et al.*, 1992b; Chang *et al.*, 1993b
Ovariectomized,	10.0	Increased	Barb *et al.*, 1992b
oestradiol-treated gilts			
Ovariectomized,	10.0	Increased	Barb *et al.*, 1992b;
progesterone-treated gilts			Chang *et al.*, 1993b
Boars	1.25, 2.5, 5.0 or 10.0	Increased	Broughton *et al.*, 1996
	2.5	Increased	Estienne *et al.*, 1996c
Barrows	1.25	No effect	Estienne *et al.*, 1996b
	2.5 or 5.0	Increased	Estienne *et al.*, 1996b
Prolactin			
Ovariectomized gilts	10	Increased	Barb *et al.*, 1992b; Chang *et al.*, 1993b
Ovariectomized,	10	Increased	Barb *et al.*, 1992b
oestradiol-treated gilts			
Ovariectomized,	10	No effect	Barb *et al.*, 1992b
progesterone-treated gilts	10	Increased	Chang *et al.*, 1993b

extensive studies on NMDA receptor regulation of adenohypophysial hormone secretion, the role of other ExAA receptors in controlling secretion of anterior pituitary gland hormones has received scant attention.

Effects of ExAA on gonadotrophin secretion. Typically, secretion of LH and FSH is increased by NMA in animal models that are characterized as having suppressed gonadotrophin secretion. For

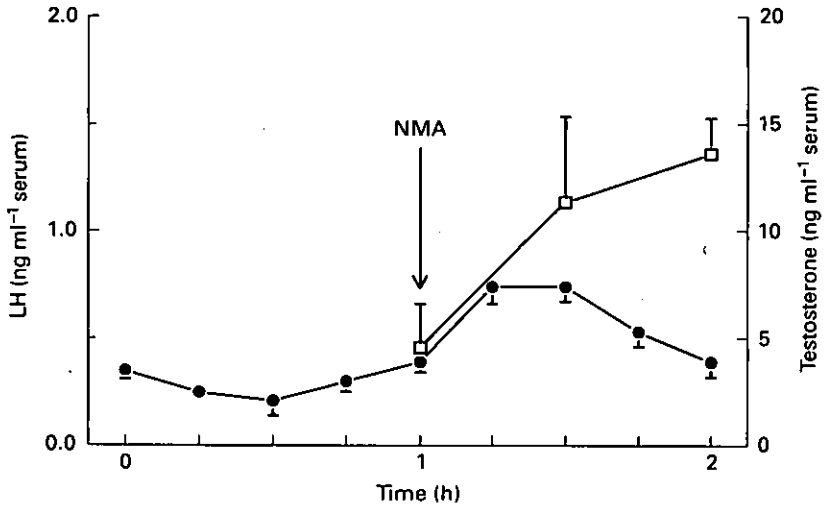

Fig. 2. Serum concentrations of LH (●) and testosterone (□) in mature boars receiving an intravenous injection of *N*-methyl-D,L-aspartate (NMA; 10 mg kg^{-1} body weight). Blood samples were collected at 15 min intervals for 2 h and NMA was administered at 1 h (arrow). All of the samples were analysed for concentrations of LH, and testosterone concentrations were determined in samples collected at 1.0, 1.5 and 2.0 h. Values are means±standard errors ($n = 5$). NMA administration resulted in an increase in LH ($P < 0.01$) and testosterone ($P < 0.05$) secretion.

example, NMA stimulated LH release in ovariectomized, oestradiol-treated gilts (Sesti and Britt, 1992), and enhanced secretion of both LH and FSH in lactating sows (Sesti and Britt, 1992, 1993, 1994). NMA stimulated LH release throughout lactation in both multiparous and primiparous sows, while FSH secretion increased when the secretogogue was administered between day 7 and day 21 postpartum in multiparous sows and between day 14 and day 21 postpartum in primiparous sows (Sesti and Britt, 1993, 1994).

Work in our laboratory demonstrated that administration of NMA increased LH secretion in prepubertal gilts (Estienne *et al.*, 1995) and in gilts treated during the luteal, but not follicular, phase of the oestrous cycle (Hurlock *et al.*, 1995). Failure of follicular phase gilts to respond to NMA in that study was perhaps due to oestradiol-induced alterations in pituitary sensitivity to GnRH. Indeed, the response to a GnRH challenge was compromised in follicular phase compared with luteal phase or ovariectomized gilts (Hurlock *et al.*, 1995).

Broughton *et al.* (1996) reported that NMA at doses of 1.25–10 mg kg^{-1} body weight failed to alter LH release in immature boars that were approximately 6 months of age. However, we recently demonstrated that NMA, at a dose of 10 mg kg^{-1} body weight, increased secretion of LH and testosterone in mature boars that were approximately 13 months of age (Fig. 2). These findings are consistent with the concept that, in boars, there is an age-related change in sensitivity of the gonadotrophin response to NMA.

In contrast to the studies showing stimulation of LH secretion by NMA, Barb *et al.* (1992b) and Chang *et al.* (1993b) reported that NMA had no effect on LH release in ovariectomized, oestradiol-treated gilts and decreased LH secretion in ovariectomized, progesterone-treated gilts. Treatment with NMA also decreased circulating concentrations of LH in ovariectomized gilts (Barb *et al.*, 1992b; Chang *et al.*, 1993b; Hurlock *et al.*, 1995; Popwell *et al.*, 1996). These studies suggest that, in addition to well-documented stimulatory effects, under appropriate conditions, ExAA may also suppress gonadotrophin secretion.

Effect of ExAA on growth hormone secretion. The effects of ExAA on growth hormone secretion, growth performance and carcass quality in domestic animals were summarized by Estienne *et al.* (1996a). Barb *et al.* (1996) reported that intravenous administration of aspartate (50–150 mg kg^{-1} body

weight) or glutamate (100–150 mg kg^{-1} body weight) increased serum concentrations of growth hormone in prepubertal gilts. Moreover, infusion of NMA evoked growth hormone release in ovariectomized gilts receiving no treatment or steroid replacement therapy (Barb *et al.*, 1992b; Chang *et al.*, 1993b), prepubertal gilts (Estienne *et al.*, 1995), barrows (Estienne *et al.*, 1996b) and boars (Broughton *et al.*, 1996; Estienne *et al.*, 1996c).

The D,L racemic mixture of NMA has been used in neuroendocrine experiments but no attempt was made to determine which specific isomer was responsible for increasing blood concentrations of adenohypophysial hormones. However, using barrows, we demonstrated that the pure D isomer of NMA, at a dose of 1.25 mg kg^{-1} body weight, increased serum concentrations of growth hormone in a manner comparable to that which occurs after treatment with the D,L racemic mixture of NMA at a dose of 2.5 mg kg^{-1} body weight (Estienne *et al.*, 1996b). In contrast, injection of the pure L isomer of NMA (1.25 mg kg^{-1} body weight) did not increase secretion of growth hormone. These results do not preclude the possibility that higher doses of the pure L isomer of NMA may evoke hypersecretion of growth hormone.

Effect of ExAA on secretion of other anterior pituitary gland hormones. Barb *et al.* (1992b) reported that the injection of NMA increased serum concentrations of prolactin in ovariectomized and ovariectomized, oestradiol-treated gilts, but not in ovariectomized, progesterone-treated gilts. However, in a subsequent study, NMA increased prolactin secretion in both ovariectomized and ovariectomized, progesterone-treated gilts (Chang *et al.*, 1993b).

Circulating concentrations of ACTH and TSH following treatment with NMA have not been reported for swine. However, Barb *et al.* (1992b), Chang *et al.* (1993b) and Popwell *et al.* (1996) demonstrated that NMA increased serum concentrations of cortisol in ovariectomized and ovariectomized, steroid-treated gilts, and barrows, perhaps as a consequence of pituitary release of ACTH.

ExAA-induced secretion of hormones from the adenohypophysis: mechanism of action

Secretion of adenohypophysial hormones induced by exogenous ExAA is primarily a consequence of action at the central nervous system rather than a direct pituitary effect. Administration of NMA had no effect on serum concentrations of LH in ovariectomized gilts that were passively immunized against GnRH (Sesti and Britt, 1992). Active immunization against GHRH abolished the ability of NMA to increase circulating concentrations of growth hormone in ovariectomized gilts (Barb *et al.*, 1996). Similarly, NMA-induced increases in growth hormone secretion were abolished by treating barrows with antiserum to GHRH (Estienne *et al.*, 1996b).

Although most of the evidence suggests a central site for the effects of ExAA on release of pituitary hormones, subtle effects of the compounds directly on the anterior pituitary gland cannot be discounted. Studies *in vitro* demonstrated a positive effect of aspartate and glutamate on growth hormone secretion by pituitary cells collected from prepubertal gilts (Barb *et al.*, 1996). Barb *et al.* (1993) also reported that NMA stimulated growth hormone release from pituitary cells collected from ovariectomized gilts and gilts in the luteal phase of the oestrous cycle, but not from pituitary cells obtained from gilts in the follicular phase of the oestrous cycle. Treatment with NMA increased LH secretion from pituitary cells collected from ovariectomized, luteal phase and follicular phase gilts (Barb *et al.*, 1993).

Neural loci at which intravenously administered ExAA may act to stimulate release of hypothalamic hormones include the areas of the brain that lack a distinct blood–brain barrier. Price *et al.* (1981) reported preferential uptake of subcutaneously administered aspartate and glutamate by circumventricular organs of the rat brain. Therefore, exogenously administered ExAA may affect hypothalamic hormone secretion in pigs by influencing function of cell bodies in specific nuclei and(or) nerve terminals in the median eminence. However, these results do not preclude the existence of endogenous ExAA neurotransmission within the blood–brain barrier.

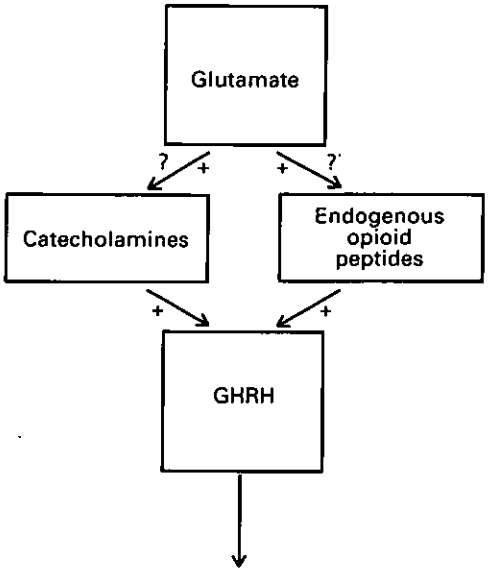

Fig. 3. Possible neuronal pathways by which excitatory amino acids such as glutamate stimulate release of growth hormone-releasing hormone (GHRH), and hence, growth hormone secretion in pigs.

Results from our laboratory are consistent with the concept that stimulation of adenohypophysial hormone secretion (at least growth hormone) by NMA is modulated by NMDA receptors. Treatment of barrows with ketamine hydrochloride, a noncompetitive NMDA receptor antagonist, attenuated the growth hormone response to NMA (Estienne *et al.*, 1996b).

It is unclear whether ExAA affect hypothalamic hormone secretion by a direct effect on neurones that secrete these hormones or indirectly through effects on interneurones (Fig. 3). As previously mentioned, drugs with opiate-like activity alter adenohypophysial hormone secretion in swine and it has been hypothesized that NMA-induced effects on secretion of LH, growth hormone and prolactin are mediated through EOP. In support of this hypothesis, administration of naloxone attenuated the growth hormone and prolactin responses to NMA in ovariectomized and ovariectomized, progesterone-treated gilts (Chang *et al.*; 1993b). In that study, however, responsiveness to NMA with regard to LH secretion was unaffected by naloxone.

We recently conducted an experiment to test the hypothesis that NMA-induced growth hormone secretion is modulated by catecholamine neurotransmission (D. S. Broughton, M. J. Estienne, J. M. Harter-Dennis, C. R. Barb and T. G. Hartsock, unpublished). Boars were fed a daily ration containing 0 or 125 mg AIMAX daily for 9 days ($n = 5$ per treatment). On day 8, blood samples were collected every 15 min for 8 h. Four and six hours after sampling was initiated, boars allowed each ration received intravenous injections of NMA (2.5 mg kg^{-1} body weight) or saline. The next day the experiment was repeated, but boars that previously received NMA were administered saline and vice versa. Before NMA or saline injections, mean serum growth hormone concentrations and the frequency and amplitude of growth hormone pulses were similar for AIMAX and control groups. Injections of NMA significantly increased mean serum growth hormone concentrations and growth hormone pulse amplitude in both groups in a similar fashion (Fig. 4). Treatment with NMA did not alter frequency of growth hormone pulses. These results suggest that in boars, catecholamines: (1) do not play a significant role in modulating pulsatile secretion of growth hormone, and (2) do not mediate the effects of NMA on growth hormone secretion.

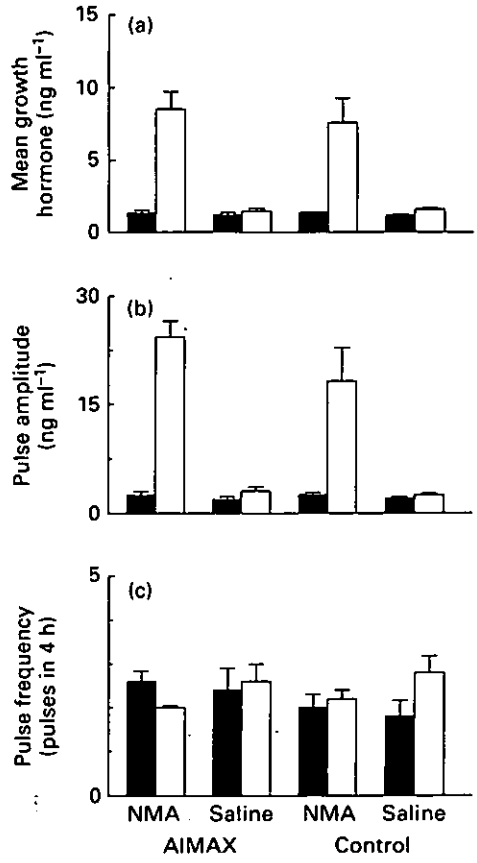

Fig. 4. Mean growth hormone concentrations (a) and the (b) amplitude and (c) frequency of growth hormone pulses in immature boars fed the central catecholamine synthesis inhibitor AIMAX (125 mg daily for 9 days) or a control diet, and injected intravenously with *N*-methyl-D,L-aspartate (NMA; 2.5 mg kg^{-1} body weight) or saline. Blood samples were collected every 15 min for 8 h and NMA was administered at 4 h and 6 h. (■): the 4 h period before NMA or saline injections; (□): the 4 h period after initiation of NMA or saline injections. Values are means±standard errors ($n = 5$). Administration of NMA resulted in an increase ($P < 0.01$) in mean growth hormone concentrations and growth hormone pulse amplitude in both AIMAX-fed and control boars. The frequency of growth hormone pulses was unaffected ($P > 0.1$) by NMA injections.

ExAA as physiological modulators of adenohypophysial hormone secretion

Although exogenous administration of ExAA alters secretion of adenohypophysial hormones, a physiological role for aspartate and (or) glutamate in control of the hypothalamic–pituitary axis in pigs remains to be determined. If ExAA are involved in physiological control of a particular hormone, then treatment with an ExAA antagonist should have the opposite effect to the ExAA agonist.

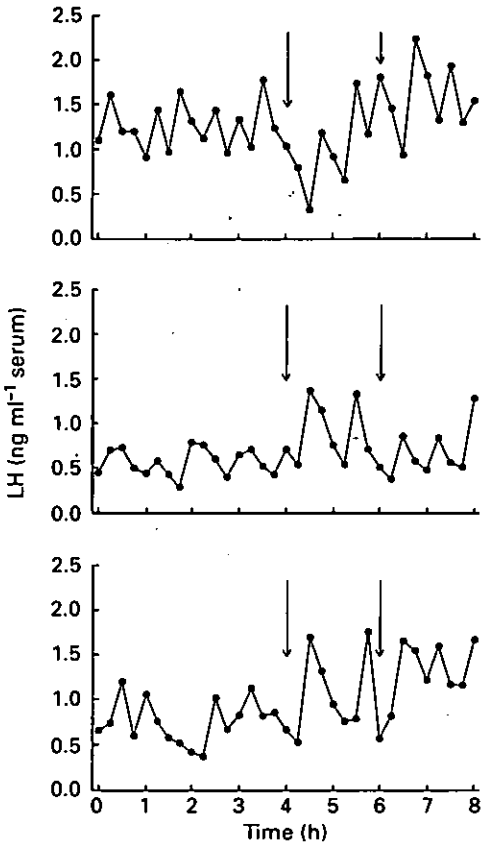

Fig. 5. Serum LH profiles in individual, ovariectomized gilts receiving intravenous injections of the competitive *N*-methyl-D-aspartate receptor antagonist D,L-2-amino-5-phosphonovaleric acid (AP5; 13 mg kg^{-1} body weight). Blood samples were collected at 15 min intervals for 8 h. AP5 injections were administered at 4 h and 6 h and are indicated by the arrows. Frequency of LH pulses was not altered ($P > 0.1$) by AP5 injections. There was, however, a tendency ($P < 0.09$) for LH pulses of greater amplitude after AP-5 treatment.

Experiments during which gonadotrophin secretion was assessed in pigs treated with ExAA antagonists yielded conflicting data. Equivocal results could arise because ExAA neurotransmission may have dichotomous effects on LH secretion. As mentioned earlier, exogenously administered ExAA can both stimulate and inhibit LH secretion. In addition, exogenously administered ExAA and ExAA antagonists may act at different areas of the brain owing to the ability or inability to cross the blood–brain barrier.

Popwell *et al.* (1996) reported that intramuscular administration of ketamine hydrochloride (20 mg kg^{-1} body weight) decreased concentrations of LH in serum of ovariectomized gilts, a finding consistent with the notion that ExAA are involved in modulating gonadotrophin secretion. In contrast, the frequency of LH pulses was unaltered in a recently conducted experiment during which ovariectomized gilts received intravenous injections of D,L-2-amino-5-phosphonovaleric acid (AP5), a competitive NMDA receptor antagonist. There was, however, a tendency for greater

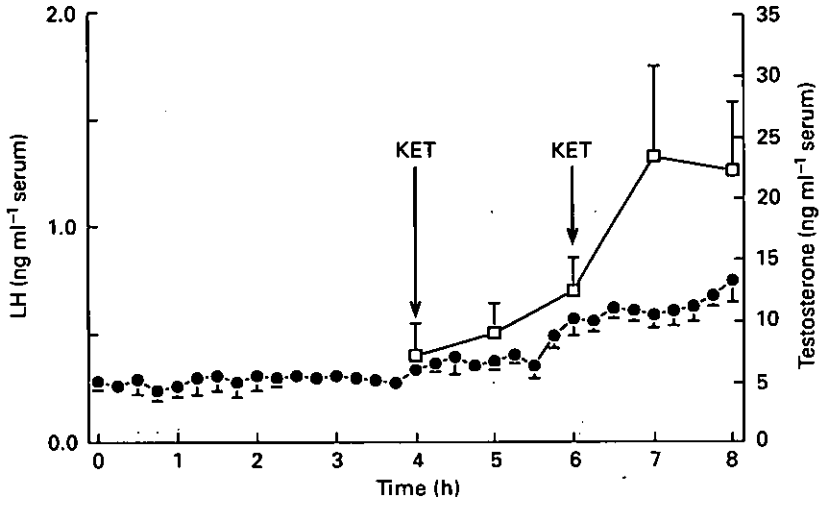

Fig. 6. Serum concentrations of LH (●) and testosterone (□) in immature boars receiving intramuscular injections of the noncompetitive *N*-methyl-D-aspartate receptor antagonist ketamine hydrochloride (KET; 19.9 mg kg^{-1} body weight). Blood samples were collected at 15 min intervals for 8 h and KET was administered at 4 h and 6 h. All samples were analysed for concentrations of LH, and testosterone concentrations were determined in samples collected each hour beginning at 4 h. Values are means±standard errors ($n = 4$). Administration of KET increased LH ($P < 0.01$) and testosterone ($P < 0.07$) secretion.

amplitude LH pulses after AP5 treatment (Fig. 5; M. J. Estienne, J. M. Harter-Dennis, and C. R. Barb, unpublished). The dose of AP5 used (13 mg kg^{-1} body weight) was equimolar to the dose of NMA used previously to stimulate secretion of LH in gilts (Estienne *et al.*, 1995; Hurlock *et al.*, 1995).

We conducted an experiment in which fasted boars, weighing approximately 125 kg each, were treated intramuscularly with ketamine hydrochloride (19.9 mg kg^{-1} body weight) or saline (Estienne *et al.*, 1996c). Fasting increased serum concentrations of growth hormone. However, circulating growth hormone concentrations were similar for boars treated with ketamine hydrochloride and saline, suggesting that endogenous ExAA acting via the NMDA receptor are not involved in the physiological control of growth hormone secretion. Interestingly, ketamine hydrochloride increased circulating concentrations of both LH and testosterone (Fig. 6), a finding consonant with the theory that ExAA inhibit LH release in pigs.

Conclusion

Evidence has been presented that supports the concept that EOP and ExAA affect secretion of hypothalamic hormones and hence function of the anterior pituitary gland in pigs. In general, EOP inhibit LH secretion and this effect involves the central catecholaminergic system. A definitive role for EOP in the modulation of FSH release remains to be determined. EOP stimulate secretion of growth hormone and, depending on the animal model studied, EOP exert either a stimulatory or an inhibitory influence on prolactin secretion. ExAA, acting via NMDA receptors, stimulate secretion of LH, FSH, growth hormone and prolactin in appropriate animal models. However, in certain situations an inhibitory effect of ExAA on LH secretion has been demonstrated. The modulation of growth hormone and prolactin secretion by ExAA involves EOP. Research investigating the role of non-NMDA ExAA receptors in the control of adenohypophysial hormone secretion and the function of ExAA in the physiological control of swine reproduction warrant scrutiny.

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