

Neuroendocrine regulation of reproduction in male domestic animal species: Role of excitatory amino acids

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Abstract

The ability to control and enhance reproduction in male domestic animal species requires a sound understanding of the mechanisms functioning within the hypothalamic-pituitary-testicular axis. Recent evidence suggests that the excitatory amino acids (ExAA) are important neurotransmitters that participate in the neuroendocrine control of anterior pituitary hormone secretion. In general, treatment with agonists of different ExAA receptor types, such as n-methyl-D,L-aspartate (NMA), stimulate release of most anterior pituitary hormones in boars, bulls, and rams, and these effects are manifested mainly at the level of the central nervous system. In some experimental models, inhibitory effects of ExAA on LH secretion have also been reported. Whether ExAA act directly on neurons that secrete hypothalamic-releasing hormones or indirectly by action on other neuronal systems is not clear. Data from boars, however, suggest that the catecholamines do not modulate the effects of NMA on LH or GH secretion. Although some data indicate that the ExAA are involved in the processes that culminate in sexual maturation, more research is needed before definitive conclusions can be drawn.

Key Words: Excitatory Amino Acid Derivatives, Neurohormones, Pituitary, Testes, Males

Introduction

Glutamate belongs to a family of neurotransmitters known as the excitatory amino acids (ExAA). Studies in numerous species have demonstrated glutamate immunoreactivity in locations throughout the central nervous system, including neurons in the hypothalamus and nerve terminals in the median eminence. There are several types of receptors 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). It is not the purpose of this review to provide a detailed description of the anatomical localization and function of glutamate neurons and receptors. For excellent reviews focusing on these topics, readers are encouraged to see van den Pol et al. (1996) and Petralia and Wenthold (1996).

The ExAA are now believed to be the principal neurotransmitters in the brain, and research investigating their part in neuroendocrine control of anterior pituitary hormone secretion has intensified. Moreover, the role of ExAA in reproduction is receiving increasing scrutiny. The objective of this review is to describe our current understanding of the neuroendocrine regulation of reproduction in male domestic animal species and the specific role of ExAA.

Effects of ExAA on Secretion of Gonadotropins and Testosterone

The hypothalamic hormone GnRH is synthesized in perikarya with axons that terminate at the median eminence. This

hormone is released into the hypothalamo-hypophysial portal vasculature, travels to the anterior pituitary gland, and stimulates secretion of the gonadotropins LH and FSH. The gonadotropins provide direct control of testicular function, including testosterone secretion and spermatogenesis.

The majority of studies examining the effects of ExAA on LH secretion have employed the D, L racemic mixture of n-methyl-aspartate (NMA) to stimulate NMDA receptors. In general, treatment with NMA stimulates LH secretion in intact male farm animals, but not in castrated ones, as summarized in Table 1. Consistent with a stimulation of LH release, circulating concentrations of testosterone, but not of estradiol, are also increased by i.v. injection of NMA (10 mg/kg BW) in mature boars (Estienne et al., 2000).

Few studies have been conducted during which LH secretion was assessed in male farm animals treated with agonists of non-NMDA, ExAA receptors. Kumar et al. (1993), however, reported that i.v. injections of AMPA, at a dose of .2 mg/kg BW, stimulated LH secretion in Soay rams exposed to a photoperiod characteristic of long days. The effects of kainate receptor agonists on LH secretion in male domestic animal species have not been evaluated.

The effects of ExAA on FSH secretion in male domestic animal species have not been previously reported. Shown in Figure 1 are serum concentrations of FSH in mature, cross-bred boars (401 d of age and approximately 179 kg BW) before and after i.v. injection of NMA at a dose of 10 mg/kg BW or 0.9% saline vehicle (M. J. Estienne, C. R. Barb, and R. R. Kraeling, unpublished data). There was no effect of NMA ($P > 0.1$) on circulating FSH concentrations. In these same boars, however, NMA caused robust increases in circu-

lating LH concentrations (Estienne et al., 2000), suggesting that there are differences in the neuroendocrine control of LH and FSH secretion in boars.

Stimulation of Gonadotropin Secretion by ExAA: Site of Action

Because glutamate and other ExAA have been identified as neurotransmitters throughout the central and peripheral nervous systems, it has been assumed that the effects of these amino acids on gonadotropin secretion are a consequence of action at the level of the central nervous system to effect GnRH release. Data to support this concept in male domestic animal species, however, are limited. Moreover, glutamate and other ExAA may have direct actions on the anterior pituitary gland. Indeed, treatment with NMA stimulated LH secretion from pituitary cells collected from luteal-phase, follicular-phase, and ovariectomized gilts (Barb et al., 1993).

Using an immunological approach, Sesti and Britt (1992), sought to determine the site of action of ExAA on LH secretion in swine. Administration of NMA had no effect on circulating concentrations of LH in ovariectomized gilts that were passively immunized against GnRH. These data are difficult to interpret, however, because other researchers have reported that NMA actually suppressed LH secretion in the ovariectomized gilt model (Barb et al., 1992; Chang et al., 1993a; Popwell et al., 1996; Estienne et al., 1998).

We recently conducted an *in vitro* experiment to determine the effects of NMA on GnRH secretion from the central nervous system in boars (M. J. Estienne, K. V. McElwain, J. B. Barrett, and C. R. Barb, unpublished data). Crossbred boars ($n = 11$), approximately 187 d of age and 115 kg BW, were killed, and one-half of the hypothalamic-preoptic area (**HYP-POA**) and the whole median eminence (**ME**) were separated, and each was placed into separate microchambers of a perfusion system as previously described (Barb et al., 1994). Effluent was collected continuously in 5-min fractions for 3 h (Fraction 1 = h 0). At h 1, explants were exposed to NMA at concentrations of 10^{-4} M ($n = 4$) or 10^{-8} M ($n = 4$). Control explants ($n = 3$) were not exposed to NMA.

Prior to NMA, GnRH secretion from the ME was 97.4 ± 9.6 pg/fraction across treatments. Secretion of GnRH increased by 29% ($P < 0.03$) after 10^{-4} M NMA, but it was not affected ($P > 0.1$) after exposure to 10^{-8} M NMA and controls (Figure 2). The GnRH response to 10^{-4} M NMA for an individual ME explant is shown in Figure 3. Secretion of GnRH from the HYP-POA was unaffected ($P > 0.1$) by exposure to NMA, and overall concentrations were 58.6 ± 13.5 pg/fraction (data not shown).

Across treatments, exposure to 60 mM KCl at h 2 stimulated ($P < 0.01$) GnRH secretion from the ME by 160% (data from representative explant shown in Figure 3) and HYP-POA by 83% (data not shown), illustrating viability of the tissues. These data are consistent with the notion that NMA stimulates LH secretion in boars, at least in part, by causing the release of GnRH from the ME.

Stimulation of GnRH Secretion: Mechanism of Action

The ExAA may affect GnRH secretion by a direct effect on GnRH neurons. Alternatively, ExAA may alter GnRH release by acting upon other neurotransmitter systems that modulate GnRH secretion.

Catecholamines, including norepinephrine, epinephrine, and dopamine, are synthesized by neurons located within the central nervous system. Tyrosine is converted to dihydroxyphenylalanine (**1-DOPA**) via the action of tyrosine hydroxylase, and this is the rate-limiting step in catecholamine biosynthesis. Aromatic decarboxylase converts 1-DOPA to dopamine. Dopamine is converted to norepinephrine by dopamine β -hydroxylase, and norepinephrine is transformed to epinephrine via the action of phenylethanolamine-n-methyl transferase.

A role for catecholamines in control of LH secretion in swine is supported by several anatomical and pharmacological studies. Leshin et al. (1996) located tyrosine hydroxylase and dopamine- β -hydroxylase immunopositive neurons that were in the vicinity of GnRH neurons within hypothalamic nuclei (Kineman et al., 1988). Intracerebroventricular injection of norepinephrine stimulated LH secretion in Gottingen boars. However, direct application of norepinephrine into periventricular structures inhibited LH secretion (Parvizi and Ellendorff, 1978).

Methallibure (synonym = **AIMAX**) is a derivative of di-thiocarbamoylhydrazine and has actions similar to diethylthiocarbamate, a potent inhibitor of dopamine β -hydroxylase activity. Treatment of rats with AIMAX suppressed norepinephrine synthesis, increased hypothalamic content of dopamine, and suppressed LH secretion (Chang et al., 1995). Similarly, feeding of AIMAX to gilts inhibited LH secretion (Kesner et al., 1987; Chang et al., 1993b). The estradiol-induced LH surge was abolished, and the FSH surge was attenuated in AIMAX-fed, ovariectomized gilts (Kesner et al., 1987). Pituitary responsiveness to a GnRH challenge was unaffected by feeding of AIMAX to gilts, a finding consistent with a hypothalamic site of action for the compound (Kesner et al., 1987).

We recently conducted an experiment to test the hypothesis that NMA-induced LH secretion is modulated by catecholamine neurotransmission in boars (M. J. Estienne and C. R. Barb, unpublished data). Crossbred boars, 245 d of age and approximately 125 kg BW, received 2.27 kg of a feed containing 0 ($n = 4$) or 125 mg of AIMAX ($n = 4$) daily for 8 d. On d 8, blood samples were collected every 15 min for 4 h via indwelling jugular vein catheters (Sample 1 collected at h 0). At h 2 and 3, all boars received an *i.v.* injection of 10 mg of NMA/kg BW.

Results of this experiment are shown in Figure 4. Injections of NMA increased ($P < 0.01$) mean serum concentrations of LH by 84% and testosterone by 78% in both groups in a similar fashion. Additionally, NMA increased LH pulse frequency by 250% ($P < 0.01$), but not pulse amplitude ($P > 0.1$), in AIMAX-fed boars. In control boars, NMA decreased LH pulse frequency by 38% ($P < 0.09$) and increased ($P <$

0.02) the amplitude of LH pulses by 216%. Several conclusions can be derived from examination of these data. First, the frequency of LH pulses ($P < 0.01$) and mean concentrations of testosterone ($P < 0.07$) were less in AIMAX-fed boars than in control boars (Figure 4). These findings suggest that catecholamines participate in neuroendocrine control of GnRH secretion in domestic boars. Second, various characteristics of LH secretion and testosterone release increased after NMA in AIMAX-fed boars despite an assumed suppression of norepinephrine synthesis, suggesting that catecholamines do not modulate the effects of NMA on gonadotropin secretion. Third, a lower frequency of higher-amplitude LH pulses during NMA treatment in control boars suggests a resetting and slowing of the LH "pulse generator," with the frequency being dictated by the exogenous NMA injections. Accordingly, enhanced amplitude of pulses could be due to increased releasable pools of LH in the anterior pituitary gland.

Effects of ExAA Antagonists on Gonadotropin Secretion

If ExAA are involved in the physiological control of gonadotropin secretion, then treatment with an ExAA antagonist should have the opposite effect of the ExAA agonists.

Few studies have been conducted in which male domestic animal species have been treated with ExAA antagonists.

Treatment of prepubertal bull calves with MK-801, an NMDA receptor antagonist, decreased mean LH concentrations and frequency and amplitude of LH pulses (Shahab et al., 1995). These findings are consistent with the concept that maintenance of pulsatile LH secretion involves an NMDA-receptor-mediated drive to GnRH-releasing neurons.

Results in other species are equivocal. In mature rams housed under a long-day photoperiod, treatment with CGP 37849, an NMDA receptor antagonist, or 6,7 dinitroquinoxaline-2, 3-dione (DNQX), a non-NMDA receptor antagonist, failed to alter LH secretion.

In NMA-treated barrows, ketamine hydrochloride, a NMDA receptor antagonist, suppressed mean serum LH concentrations (Popwell et al., 1996). Interestingly, ketamine hydrochloride increased circulating concentrations of both LH and testosterone in feed-deprived boars (Estienne et al., 1997). This finding is consonant with the theory that in addition to stimulatory effects on gonadotropin secretion, ExAA may also inhibit LH release in certain situations. Inhibition of LH secretion by ExAA agonists in various swine models, including ovariectomized gilts (Barb et al., 1992; Chang et al., 1993a; Popwell et al., 1996; Estienne et al., 1998) and ovariectomized, progesterone-treated gilts (Barb et al., 1992; Chang et al., 1993a), was reported previously. Additionally, Kittok (1999) reported that NMA decreased the frequency of LH pulses in wethers.

Role of ExAA in Sexual Development

The ExAA have been implicated in the ontogeny of neuroendocrine mechanisms that culminate in puberty. Administering NMA in a manner so as to produce a peripubertal

pattern of LH release advanced onset of puberty by 7 d in female rats (Urbanski and Ojeda, 1987). Prolonged intermittent treatment with NMA (once every 3 h) in rhesus monkeys caused precocious puberty with full activation of the hypothalamic-pituitary-Leydig cell axis and initiation of spermatogenesis (Plant et al., 1989). Finally, daily treatment with MK-801 delayed onset of puberty in female rats (Urbanski and Ojeda, 1990).

Few studies have examined the role of ExAA in sexual development of male domestic farm animals. Shahab et al. (1995) treated Holstein bull calves with MK-801 at 1, 12, and 24 wk of age. Basal LH secretion increased in calves from 1 to 12 wk of age, with establishment of frequent, high-amplitude pulses. Mean serum LH concentrations and pulse amplitude, but not frequency, were lower at 24 than at 12 wk of age. Administration of MK-801 did not affect LH secretion in 1- and 12-wk-old calves, but at the highest dose tested it decreased mean serum LH concentrations and the frequency and amplitude of LH pulses in 24-wk-old calves. The authors concluded that ExAA operating via NMDA receptors do not mediate initiation of pulsatile secretion of LH during the juvenile phase in bulls. However, maintenance of pulsatile LH secretion in 24-wk-old prepubertal calves does involve NMDA receptors.

One interpretation of the data of Shahab et al. (1995) is that at younger ages (1 and 12 wk of age) NMDA receptors are not anatomically and/or functionally linked to GnRH neurons. If so, then there should be age-related changes in the ability of NMA to evoke LH secretion. Some data support this hypothesis for swine.

Estienne et al. (2000) conducted three experiments examining the effects of NMA on circulating concentrations of various hormones. In Exp. 1, NMA at i.v. doses of 1.25, 2.5, 5, or 10 mg/kg BW failed to alter circulating concentrations of LH and testosterone in 185-d-old boars. In Exp. 2, mature boars (401 d of age) responded to an i.v. challenge of 10 mg of NMA/kg BW with increased LH and testosterone secretion. Finally, in Exp. 3, boars at 152, 221, or 336 d of age were treated i.v. with 10 mg of NMA/kg BW. The percentages of boars responding to NMA with increased LH secretion were as follows: 50% (2/4) at 152 d of age, 75% (3/4) at 221 d of age, and 100% (4/4) at 336 d of age. Similar results were found in prepubertal heifer calves. Treatment with 4.7 mg of NMA/kg BW i.v. caused increased LH secretion in 0 (0/5), 60 (3/5), 60 (3/5), 80 (4/5), 100 (5/5), and 100% (5/5) of the calves that were 4, 8, 12, 24, 36, and 48 wk of age, respectively (Honaramooz et al., 1998).

Recently, we conducted an experiment to determine the effects of two doses of NMA on LH and testosterone secretion in developing boars, our hypothesis being that there are age-related changes in the magnitude of the LH secretory response to NMA (M. J. Estienne and C. R. Barb, unpublished data). Crossbred boars received either 5 or 10 mg of NMA/kg BW i.v. ($n = 4/\text{dose}$) at approximately 150, 180, 210, 240, and 270 d of age. Blood samples were collected for 1 h before and 1 h after NMA.

For boars receiving the low dose of NMA, there was an effect of time (i.e., before vs after NMA; $P < 0.01$) but no

effect of time \times age on characteristics of LH secretion, suggesting that the boars responded similarly to NMA regardless of age (Figure 5). In these same animals, however, there was an effect of time \times age ($P < 0.06$; Figure 6) on serum concentrations of testosterone. Boars that were 210, 240, or 270 d of age ($P < 0.04$), but not 150 or 180 d of age ($P > 0.1$), exhibited increases in testosterone secretion following the NMA challenge. In contrast, boars receiving the high dose of NMA responded ($P < 0.01$) with increases in LH and testosterone secretion at all ages (data not shown).

These data suggest that, in contrast to our previous work (Estienne et al., 2000), boars 150 d or older respond to NMA with increased secretion of LH. Additionally, peak values of LH were slightly higher after treatment with 10 compared to 5 mg of NMA/kg BW. This finding could help explain why testosterone levels increased in the 150- and 180-d-old boars treated with the high, but not the low, dose of NMA. Perhaps testicular responsiveness to gonadotropin stimulation was lower in the younger boars.

Effects of ExAA on Other Hormones

Because adenohipophysial hormones other than LH and FSH can profoundly influence reproduction, a review of the effects of ExAA on these endocrine products is warranted.

Growth Hormone

Many studies have been conducted during which NMA stimulated secretion of growth hormone in male farm animals (Table 2). These effects are most likely a consequence of activation of NMDA receptors. Pretreatment of barrows with ketamine hydrochloride attenuated the GH response to NMA in barrows (Estienne et al., 1996). Moreover, treatment of barrows with the pure D, but not the pure L, isomer of NMA increased circulating GH concentrations (Estienne et al., 1996). Effects of non-NMDA receptor agonists on GH release in boars, rams, and bulls, however, are unknown.

Passive immunization against GHRH blocked NMA stimulation of GH secretion in barrows (Estienne et al., 1996). Additionally, NMA stimulated GHRH secretion from the HYP-POA and ME of boars in vitro (Estienne et al., 1999). These data are consistent with a central site for the effects of NMA on GH secretion in male swine.

It is unclear whether ExAA affect GHRH secretion directly or indirectly through effects on interneurons. Treatment of young boars with AIMAX failed to alter the GH response to NMA, suggesting that catecholamines do not mediate the effects of ExAA on GH secretion (Estienne et al., 1997).

Prolactin, ACTH, and TSH

Kumar et al. (1993) reported that NMA, but not AMPA, stimulated prolactin secretion in mature rams. Interestingly, treatment with CGP 37849, but not DNQX, also stimulated prolactin secretion. Thus, ExAA may both stimulate and inhibit prolactin secretion in rams.

The effects of ExAA on secretion of ACTH in male domestic farm animals have not been thoroughly addressed. Increased cortisol secretion following NMA treatment can probably be used as an index of the effects of the ExAA agonist on CRF, and subsequent ACTH, secretion. In fetal sheep, NMA stimulated ACTH secretion (Brooks and Howe, 1996). Moreover, Reyes et al. (1990) observed that intracerebroventricular infusion of CRF antiserum prevented NMA-induced cortisol secretion in ovariectomized rhesus monkeys.

In a recent experiment (M. J. Estienne and C. R. Barb, unpublished data), crossbred boars, 245 d of age and approximately 125 kg BW, received feed containing 0 ($n = 4$) or 125 mg of AIMAX ($n = 4$) daily for 8 d. On d 8, blood samples were collected every 15 min for 4 h via indwelling jugular vein catheters (Sample 1 collected at h 0). At h 2 and 3, all boars received i.v. injections of 10 mg of NMA/kg BW. Injections of NMA increased ($P < 0.06$) serum concentrations of cortisol in both groups; however, the magnitude of increase was greater ($P < 0.02$) for AIMAX-fed than for control boars (Figure 7). The mechanism by which AIMAX enhances responsiveness of the hypothalamic-pituitary-adrenal axis to NMA is not readily apparent.

Summary

Excitatory amino acids (ExAA) such as glutamate and aspartate are important neurotransmitters that play an important role in neuroendocrine control of anterior pituitary hormone secretion. The gonadotropins LH and FSH control testicular function, including testosterone secretion and spermatogenesis. In male domestic animal species, ExAA agonists, such as n-methyl-D, L-aspartate (NMA), effectively stimulate LH secretion, and subsequent testosterone release, by acting within the brain to stimulate secretion of GnRH. Stimulatory effects of ExAA agonists on FSH secretion, however, have not been demonstrated. Moreover, in some situations, ExAA may inhibit gonadotropin secretion. The effects of NMA on GnRH secretion are manifested either directly on the GnRH neuron or indirectly through non-catecholaminergic neurotransmission. In addition to effects on LH, ExAA are powerful stimulators of secondary hormones of reproduction, such as GH. For example, NMA stimulates GH secretion by acting at the brain to cause release of GHRH. Although data are equivocal regarding a role for ExAA in sexual maturation, ExAA play an important role in control of hormone secretion, and thus reproduction, in male domestic farm animals. Developing techniques to alter ExAA function holds promise as a means for controlling and enhancing reproduction in boars, bulls, and rams.

Implications

Luteinizing hormone (LH) stimulates testosterone secretion, and these hormones play critical roles in reproduction in male domestic animals. Moreover, growth hormone (GH) stimulates growth and muscle accretion. N-methyl-D,L-aspartate increases blood concentrations of LH, testosterone,

and GH and mimics the actions of excitatory amino acids (ExAA), physiologically important neurotransmitters in the brain. Developing techniques to alter ExAA function could be an effective means for controlling and enhancing reproduction and growth in male domestic animal species.

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Table 1. Effects of n-methyl-D, L-aspartate (NMA) on circulating concentrations of LH in male domestic animal species

Animal model	Dose (i.v), mg/kg BW	Result	Reference
Mature boars	10.0	Increased	Estienne et al., 2000
Barrows	2.5	No effect	Popwell et al., 1996
Mature rams	4.0	Increased	Kumar et al., 1993
	5.0	Increased	Kittok, 1999
Wethers	12.0	No effect	Estienne et al., 1989
	5.0	No effect ^a	Kittok, 1999
Bull calves	1.75	Increased	Shahab et al., 1993
Bull calves + estradiol	1.75	Increased	Shahab et al., 1993
Steers	1.75	No effect	Shahab et al., 1993

^aSuppressed LH pulse frequency.

Table 2. Studies during which n-methyl-D, L-Aspartate (NMA) increased secretion of growth hormone (GH) in male domestic animal species

Animal model	Dose (i.v.), mg/kg BW	Reference
Boars	1.25, 2.5, 5.0, or 10	Estienne et al., 2000
Barrows	2.5 or 5.0	Estienne et al., 1996
Wethers	12	Estienne et al., 1989
Bull calves	1.75	Shahab et al., 1993
Bull calves + estradiol	1.75	Shahab et al., 1993
Steers	1.75	Shahab et al., 1993

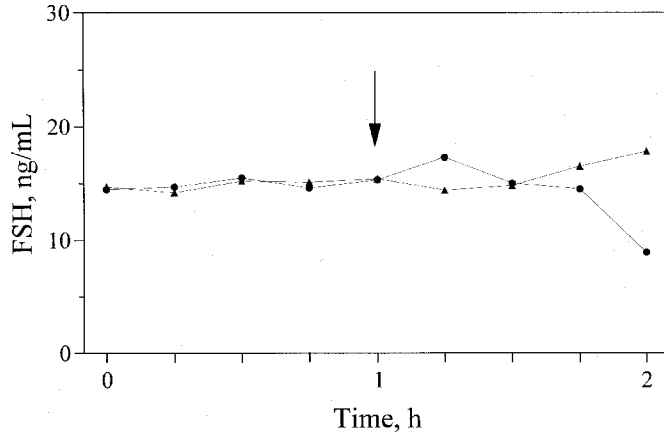


Figure 1. Serum FSH concentrations in mature boars treated i.v. with n-methyl-D, L-aspartate (NMA; 10 mg/kg BW; ●) or .9% saline (▲). Blood samples were collected every 15 min for 2 h, and injections, represented by the vertical arrow, occurred at h 1. Values are means \pm SE ($n = 5$ /treatment), and the pooled SE is 1.1 ng/mL. There was no effect of treatment ($P > 0.1$) on concentrations of FSH in serum.

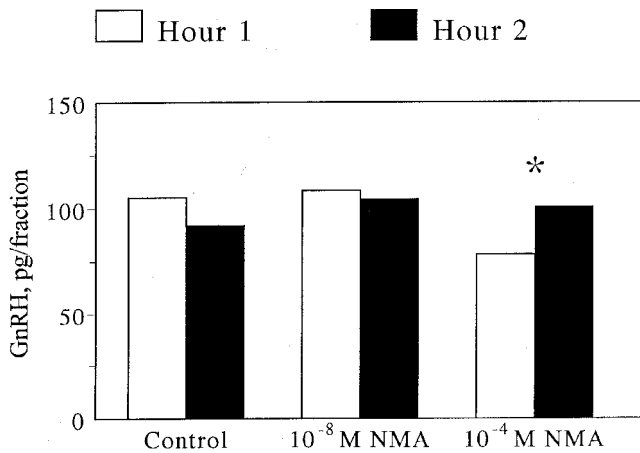


Figure 2. Concentrations of GnRH in effluent collected from boar median eminence explants perfused with n-methyl-D, L-aspartate (NMA) and control explants. Values represent means for fractions collected every 5 min for 1 h before (Hour 1) and 1 h after (Hour 2) treatment with NMA ($n = 4$ per dose). Control explants ($n = 3$) were not exposed to NMA. Pooled SE is 6.5 ng/mL. The 10^{-4} M dose of NMA increased ($*P < 0.03$) concentrations of GnRH.

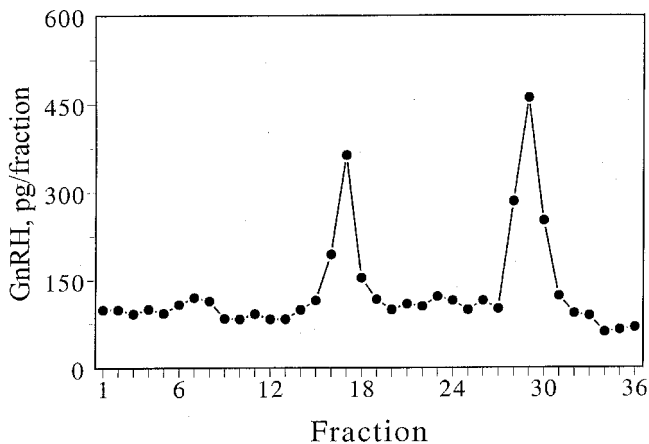


Figure 3. Concentrations of GnRH in effluent collected from a boar median eminence explant perfused with n-methyl-D, L-aspartate (NMA; 10^{-4} M) and KCl (60 mM). Fractions were collected at 5-min intervals and NMA and KCl was administered after collection of fraction 12 and fraction 24, respectively. Note the robust increases in GnRH concentrations following NMA and KCl.

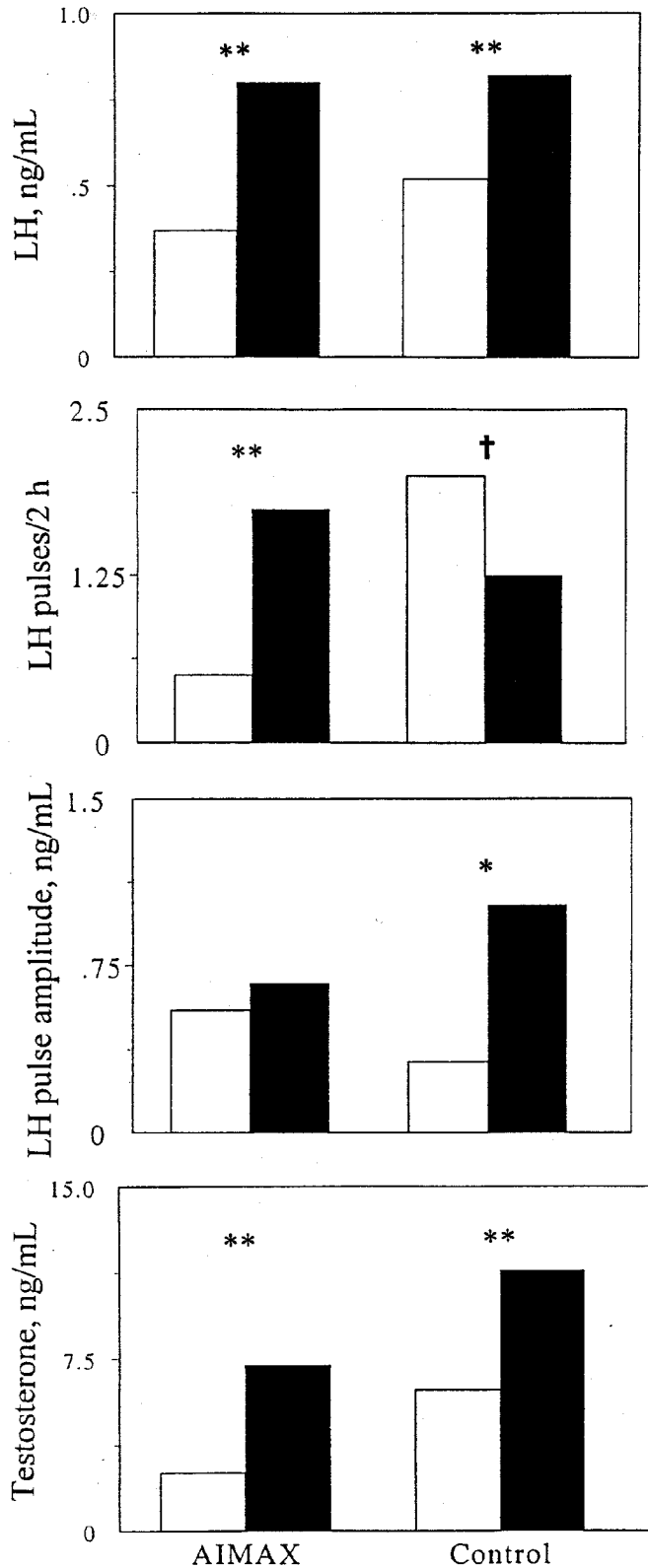


Figure 4. Characteristics of LH secretion and testosterone concentrations in boars fed daily a ration containing AIMAX (125 mg; $n = 4$) or a control ration ($n = 4$) for 8 d. On d 8, blood was sampled every 15 min for 4 h. All boars received n-methyl-D, L-aspartate (NMA; 10 mg/kg BW) at h 2 and 3. The open bars represent the means for samples collected before NMA, and the darkened bars represent the means for samples collected after the initiation of NMA injections. Injections of NMA increased (** $P < 0.01$) mean concentrations of LH and testosterone in both groups. Injections of NMA increased LH pulse frequency (** $P < 0.01$) in AIMAX-fed boars. In control boars, NMA decreased LH pulse frequency († $P < 0.09$) and increased (* $P < 0.02$) the amplitude of LH pulses. The pooled SE is 0.07 ng/mL for LH, .2 for LH pulses/2 h, 0.09 ng/mL for LH pulse amplitude, and 0.9 ng/mL for testosterone.

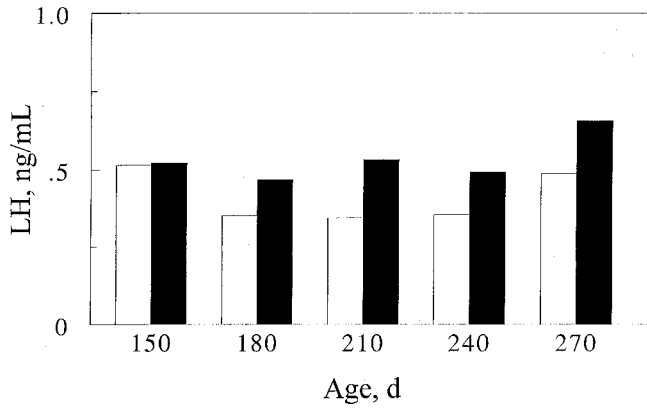


Figure 5. Serum LH concentrations in boars of various ages (four observations per age) treated with n-methyl-D, L-aspartate (NMA; 5 mg/kg BW). Bars represent the means for samples collected at 15-min intervals for 1 h before (□) and 1 h after (■) injection of NMA, and the pooled SE is 0.03 ng/mL. Across ages, NMA increased ($P < 0.01$) serum LH concentrations.

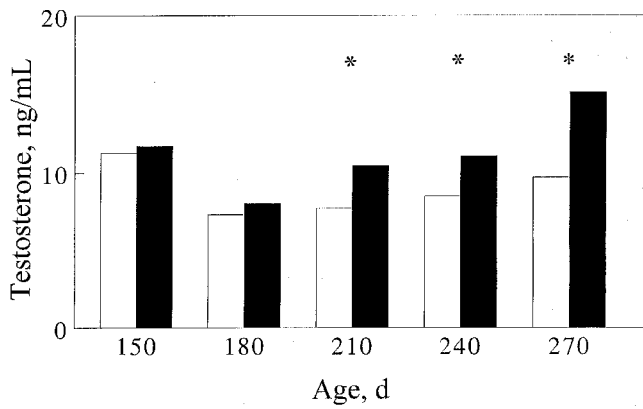


Figure 6. Serum testosterone concentrations in boars of various ages (four observations per age) treated with n-methyl-D, L-aspartate (NMA; 5 mg/kg BW). Bars represent the means for samples collected at 15-min intervals for 1 h before (□) and 1 h after (■) injection of NMA, and the pooled SE is 0.75 ng/mL. Treatment with NMA increased (*; $P < .04$) serum concentrations of testosterone in 210-, 240-, and 270-d-old boars.

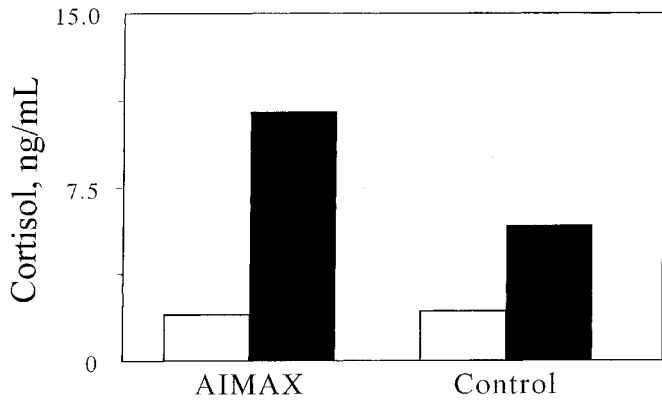


Figure 7. Cortisol concentrations in serum of boars fed daily a ration containing AIMAX (125 mg; $n = 4$) or a control ration ($n = 4$) for 8 d. On d 8, blood was sampled every 15 min for 4 h. All boars received n-methyl-D, L-aspartate (NMA; 10 mg/kg BW) at h 2 and 3. The open bars represent the means for samples collected before NMA, and the solid bars represent the means for samples collected after the initiation of NMA injections. The pooled SE is 1 ng/mL. The magnitude of increase in cortisol concentrations after NMA was greater ($P < 0.02$) for AIMAX-fed boars.