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Serum concentrations of luteinizing hormone, growth hormone, testosterone, estradiol, and leptin in boars treated with n-methyl-D,L-aspartate

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ABSTRACT: Three experiments were conducted to determine the effects of n-methyl-D,L-aspartate (NMA), an agonist of the excitatory amino acid glutamate, on secretion of hormones in boars. In Exp. 1, boars (185.0 ± .3 d of age; mean ± SE) received i.v. injections of either 0, 1.25, 2.5, 5, or 10 mg of NMA/kg BW. There were no effects of NMA (P > .1) on secretion of LH and testosterone. Treatment with NMA, however, increased (P < .01) circulating GH concentrations in a dose-dependent manner. In Exp. 2, boars (401 d of age) received an i.v. challenge of NMA at a dose of 10 mg/kg BW or .9% saline. Treatment with NMA, but not saline (P > .1), increased serum concentrations of LH (P < .01), GH (P < .01), and testosterone (P < .06). In Exp. 3, boars that were 152, 221, or 336 d of age were treated i.v. with NMA (10 mg/kg BW). Across ages, treatment with NMA increased circulating concentrations of LH (P < .07) and testosterone (P < .01). However, NMA increased (P < .01) mean GH concentrations in only the oldest boars. Treatment with NMA had no effect (P > .1) on circulating concentrations of estradiol or leptin; however, estradiol concentrations increased (P < .03) with age. In summary, NMA increased secretion of LH, GH, and testosterone in boars. However, endocrine responses to treatment with NMA may be influenced by age of the animal. Finally, NMA did not influence circulating concentrations of estradiol or leptin.

Key Words: LH, Somatotropin, Testosterone, Estradiol, Leptin, Boars


Introduction

Glutamate is an excitatory amino acid and a major neurotransmitter in the mammalian central nervous system. Treatment with n-methyl-D,L-aspartate (NMA), an agonist of glutamate, has been shown to increase pituitary secretion of LH and GH under appropriate conditions in a variety of domestic animal species (for reviews, see Estienne et al., 1996, 1997).

For example, NMA at an i.v. dose of 10 mg/kg BW, but not at doses of 1.25, 2.5, or 5 mg/kg BW, increased circulating concentrations of LH in prepubertal gilts (Estienne et al., 1995). In that study, GH secretion was increased by NMA at doses of 2.5 and 10 mg/kg BW.

More recently, NMA at a dose of 10 mg/kg BW, increased LH release in gilts during the luteal phase of the estrous cycle but not during the follicular phase of the estrous cycle, or following ovariectomy. In contrast, NMA evoked secretion of GH irrespective of the reproductive status of the treated gilt (Estienne et al., 1998).

Developing strategies to control pituitary function could enhance reproduction and growth in boars. The successful manipulation of adenohypophysial hormone secretion, however, requires a sound understanding of the mechanisms operating within the hypothalamic-pituitary axis. For boars, a paucity of information exists regarding neurotransmitter systems that modulate secretion of hormones from the anterior pituitary gland. Furthermore, the endocrine effects of NMA administration in boars have not been determined. Thus, the objective of these experiments was to determine the effects of NMA on serum concentrations of LH and GH in boars. Also assessed during the course of this investigation were circulating concentrations of testosterone, estradiol, and leptin, both before and after treatment with NMA.
Materials and Methods

General. The experiments were conducted at the University of Maryland Eastern Shore Swine Research and Education Facility in Princess Anne. Boars were individually penned in an environmentally controlled room and exposed to a 12 h:12 h light:dark cycle (lights on 0700) and an ambient temperature of 15.5°C. Animals were allowed ad libitum access to water and were fed fortified corn-soybean meal diets (Southern States Cooperative, Baltimore, MD) that met or exceeded NRC recommendations (1998) for the various nutrients.

One day before an experiment, boars were fitted with indwelling jugular vein catheters (Kraeling et al., 1982) that were used for collecting sequential blood samples and for administering NMA (D,L racemic mixture; Sigma Chemical Co., St. Louis, MO). The NMA was dissolved in .9% saline.

Experiment 1. Blood samples (10 mL) were collected from Yorkshire × Poland China boars (n = 5; 185.0 ± .3 d of age; mean ± SE; and 113.4 ± 2.6 kg BW) every 15 min for 2 h (1000 to 1200) on five consecutive days. One hour after the start of the experiment each day, boars received i.v. injections of 0 (.9% saline vehicle), 1.25, 2.5, 5, or 10 mg of NMA/kg BW. Each boar received a different dose of NMA each day as per a 5; 401 d of age; and 179.1 ± .9, 156.5 ± .5, and 201.0 ± 1.3 kg BW, respectively, were used (n = 4 per age group). On the day of the experiment, blood was sampled every 15 min for 2 h (1400 to 1600). One hour after the initiation of blood sampling, all boars received an i.v. injection of NMA at a dose of 10 mg/kg BW.

Blood Handling Procedures and Radioimmunoassays. Blood samples were allowed to clot overnight at 4°C, and serum was harvested following centrifugation. Serum was stored at −20°C until RIA were performed. All samples were assayed for LH (Kesner et al., 1987) and GH (Barb et al., 1991) using previously reported procedures.

All samples were analyzed for testosterone concentrations using a commercially available RIA kit (Diagnostic Systems Laboratories, Webster, TX) validated for porcine serum in our laboratory. Parallelism was demonstrated by showing that estimates of testosterone concentrations were not influenced by volume of serum assayed (3.125 to 50 μL). Recovery of .44 to 5.48 ng of added testosterone averaged 85.1% (n = 9). Assay sensitivity averaged .1 ng/mL, which corresponded to 90% maximum binding of the label. The intra- and interassay CV determined by replicating a single serum pool containing 4.8 ng of testosterone/mL four times in each of six assays were 8.4 and 11.2%, respectively.

Samples collected during Exp. 3 were also analyzed for concentrations of estradiol (Estienne et al., 1998) and leptin (Qian et al., 1999) using previously reported procedures.

Statistical Analyses. For Exp. 1, mean concentrations of LH, GH, and testosterone were determined for each boar for the 1-h periods before and after treatment. The differences between these values (∆LH, ∆GH, and ∆testosterone) were analyzed as previously described (Estienne et al., 1995, 1996). The main effects of boar, day, and dose of NMA on ∆LH, ∆GH, and ∆testosterone were determined using ANOVA. Treatment effects were analyzed for linear, quadratic, and cubic components to assess the dose-response relationship of LH, GH, and testosterone to NMA.

For Exp. 2 and 3, mean hormone concentrations for the 1-h periods before and after i.v. injections were determined for each boar. Data were then subjected to ANOVA for a repeated measures design. For Exp. 2, the model included treatment (NMA or .9% saline), boar within treatment, period, and treatment × period as possible sources of variation. For Exp. 3, the model included age, boar within age, period, and age × period as possible sources of variation. If significant interactions were detected for Exp. 2 (i.e., treatment × period) or Exp. 3 (i.e., age × period), subsequent one-way ANOVA (Gill and Hafs, 1971) were conducted to determine the effect of period within treatment (Exp. 2) and the effect of period within age (Exp. 3).

Results

Experiment 1. Mean concentrations of LH, GH, and testosterone were determined for the 1-h period before and the 1-h period after injection of NMA for each boar. The difference between these values (∆LH, ∆GH, and ∆testosterone) for boars receiving various doses of NMA are shown in Figure 1.

There was an effect of boar (P < .07), but no effects (P > .1) of day or dose of NMA on ∆LH. Overall LH concentration was .60 ± .05 ng/mL.

There was an effect of dose of NMA (P < .01) but no effects (P > .1) of boar or day on ∆GH. Orthogonal comparisons revealed linear (P < .01), quadratic (P < .01), and cubic (P < .01) relationships between dose of NMA and ∆GH (Figure 1). Among treatments, preinjection concentration of GH was 2.03 ± .17 ng/mL of serum. After administration of 0, 1.25, 2.5, 5, or 10 mg NMA/kg BW, mean GH concentrations were 2.91 ± .68, 4.50 ± .72, 9.41 ± .17, 13.6 ± 1.06, and 8.16 ± .67 ng/mL of serum, respectively.

No effects (P > .1) of boar, day, or dose of NMA on ∆testosterone were seen. Overall testosterone concentration was 7.82 ± .45 ng/mL of serum.
Endocrine responses to n-methyl-D,L-aspartate

Figure 1. The differences in mean concentrations of LH, GH, and testosterone (ΔLH, ΔGH, and ΔTestosterone, respectively) between the 1-h period before and the 1-h period after i.v. injection of various doses of n-methyl-D,L-aspartate (NMA). Values are means ± SE (five observations per dose). There was an effect of dose of NMA on ΔGH (P < .01) but not on (P > .1) ΔLH or Δtestosterone. The relationship between dose of NMA and ΔGH had linear (P < .01), quadratic (P < .01), and cubic (P < .01) components.

Experiment 2. Mean concentrations of LH, GH, and testosterone for the 1-h periods before and after injections of NMA or .9% saline are shown in Table 1. There was an effect of treatment × period for mean LH (P < .004), GH (P < .01), and testosterone (P < .04) concentrations. Treatment with NMA increased concentrations of LH (Figure 2). Across ages, treatment with NMA increased mean LH concentrations by 44% (P < .07).

An effect of age × period (P < .09) on mean concentrations of GH was seen (Figure 3). Treatment with NMA increased (P < .01) mean GH concentrations by 382% in boars that were 336 d of age but did not affect (P > .1) GH levels in boars from the other two age groups.

There was an effect (P < .01) of period, but no effects (P > .1) of age or age × period on mean concentrations of testosterone (Figure 4). Across ages, treatment with NMA increased mean testosterone concentrations by 53% (P < .01).

Experiment 3. There was an effect (P < .07) of period, but no effects (P > .1) of age or age × period on mean concentrations of LH (Figure 2). Across ages, treatment with NMA increased mean LH concentrations by 44% (P < .07).

An effect of age × period (P < .09) on mean concentrations of GH was seen (Figure 3). Treatment with NMA increased (P < .01) mean GH concentrations by 382% in boars that were 336 d of age but did not affect (P > .1) GH levels in boars from the other two age groups.

There was an effect (P < .01) of period, but no effects (P > .1) of age or age × period on mean concentrations of testosterone (Figure 4). Across ages, treatment with NMA increased mean testosterone concentrations by 53% (P < .01).

Table 1. Mean serum concentrations (ng/mL) of LH, GH, and testosterone in boars before and after treatment with n-methyl-D,L-aspartate (NMA) or with .9% saline

<table>
<thead>
<tr>
<th>Item</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMA</td>
<td>.30 ± .05</td>
<td>.60 ± .06</td>
</tr>
<tr>
<td>Saline</td>
<td>.25 ± .04</td>
<td>.25 ± .02</td>
</tr>
<tr>
<td>GH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMA</td>
<td>3.32 ± .73</td>
<td>7.23 ± .84</td>
</tr>
<tr>
<td>Saline</td>
<td>2.51 ± .11</td>
<td>2.84 ± .18</td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMA</td>
<td>4.35 ± 1.01</td>
<td>8.40 ± 1.47</td>
</tr>
<tr>
<td>Saline</td>
<td>4.36 ± 1.7</td>
<td>4.97 ± 2.38</td>
</tr>
</tbody>
</table>

*Values are means ± SE.

bBefore treatment vs after treatment differ (P < .01).

cBefore treatment vs after treatment differ (P < .06).
Figure 3. Mean (± SE) serum GH concentrations before (open bars) and after (solid bars) i.v. injection of 10 mg of n-methyl-D,L-aspartate (NMA)/kg BW in boars of various ages (n = 4 per age). Blood samples were collected at 15-min intervals for 1 h before and 1 h after injections of NMA. There was an effect (P < .09) of age × period. Treatment with NMA increased (P < .01; *) mean GH concentrations in the oldest boars only.

An effect (P < .03) of age, but no effects (P > .1) of period or age × period on mean concentrations of estradiol were identified (Figure 5). There were no effects (P > .1) of age, period, or age × period on mean concentrations of leptin (Figure 6).

Discussion

Glutamate is an excitatory amino acid and satisfies the main criteria for classification as a neurotransmitter (van den Pol et al., 1996). Glutamate and receptors for this neurotransmitter are found in the central nervous system in pigs, as well as in many other species (Petralia and Wenthold, 1996). Several types of receptors are stimulated by glutamate, including the N-methyl-D-aspartate (NMDA), kainate, and D,L-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Petralia and Wenthold, 1996). N-Methyl-D,L-aspartate is a potent agonist of the NMDA receptor and has been used to stimulate LH secretion in prepubertal gilts (Estienne et al., 1995), lactating sows (Sesti and Britt, 1992, 1993, 1994), ovariectomized gilts treated with estradiol (Sesti and Britt, 1992), and gilts in the luteal phase of the estrous cycle (Estienne et al., 1998). Increased secretion of LH following treatment with NMA is due primarily to NMA-induced release of GnRH from the hypothalamus. Tal et al. (1983) reported that NMA failed to alter basal or GnRH-induced gonadotropin release from rat or monkey pituitary glands in vitro. However, NMA increased LH secretion from pituitary cells collected from ovariectomized and intact gilts (Barb et al., 1993).

In contrast to studies in which NMA increased LH secretion, NMA had no effect on LH release in barrows (Popwell et al., 1996), gilts in the follicular phase of the estrous cycle (Estienne et al., 1998), or in ovariectomized gilts treated with estradiol (Barb et al., 1992). Moreover, NMA decreased LH secretion in ovariectomized gilts (Barb et al., 1992; Chang et al., 1993; Popwell et al., 1996; Estienne et al., 1998) and ovariectomized gilts treated with progesterone (Barb et al., 1992; Chang et al., 1993).

In the current study, various doses of NMA failed to alter circulating LH concentrations when administered to boars that were approximately 185 d of age (Exp. 1). In contrast, NMA given to boars that were 401 d of age (Exp. 2) increased LH secretion. Thus, we hypothesized that there was an age-related change in the ability of NMA to elicit LH release in boars.

The results reported for Exp. 3 do not support this hypothesis. That there was an effect of period but no age × period interaction suggests that NMA increased serum concentrations of LH regardless of age of boar. However, in a subsequent analysis of the data, boars were classified as “responding” to NMA if an increment in LH concentrations greater than two times the intrainassay CV occurred within 15 min after injection. Based on this criterion, 50% (2/4) of the 152-d-old boars, 75% (3/4) of the 221-d-old boars, and 100% (4/4) of the 336-d-old boars responded to NMA with increases in LH secretion. Moreover, applying this criterion to results obtained in Exp. 1 (185-d-old boars treated with the 10 mg NMA/kg BW dose) and 2 (401-d-old boars), revealed...
response rates of 80% (4/5) and 100% (5/5), respectively. Perhaps there are subtle, age-related changes in responsiveness to NMA with regard to the ability of the compound to evoke LH release in boars.

Luteinizing hormone is a potent stimulator of testosterone secretion. As expected, the effects of NMA on concentrations of testosterone paralleled the effects of the compound on levels of LH. Indeed, testosterone secretion was increased by NMA in Exp. 2 and 3, but not in Exp. 1. Although there was no effect of NMA on circulating estradiol concentrations (Exp. 3), estradiol levels increased with advancing age in boars, as has been previously reported (Allrich et al., 1982).

Previous experiments demonstrated that injections of NMA increased serum concentrations of GH in ovariectomized gilts with or without steroid replacement therapy (Barb et al., 1992; Chang et al., 1993; Estienne et al., 1998), prepubertal gilts (Estienne et al., 1995), luteal and follicular phase gilts (Estienne et al., 1998), and barrows (Estienne et al., 1996). The current study extends these findings to boars.

Treatment with NMA increased GH secretion in a dose-dependent manner in 185-d-old boars (Exp. 1), with the intermediate doses of the compound (2.5 and 5.0 mg/kg BW) being most effective. Perhaps the inability of NMA to stimulate GH secretion in the younger boars (152 and 221 d of age) in Exp. 3 was due to the high dose of the compound (10 mg/kg BW) administered. In contrast, 10 mg NMA/kg BW readily increased GH secretion in older boars in Exp. 2 (401 d of age) and Exp. 3 (336 d of age). These results are consistent with the notion that the effects of NMA on GH secretion change with age in boars.

Pretreatment of barrows with ketamine hydrochloride, an NMDA receptor antagonist, blocked the ability of NMA to increase GH secretion in barrows (Estienne et al., 1996). Moreover, immunization against GRF, either passively in barrows (Estienne et al., 1996) or actively in gilts (Barb et al., 1996), abolished NMA-induced GH secretion. Thus, NMA most likely stimulates GH secretion in swine by activating an NMDA receptor, which causes the release of GRF from the median eminence.

Leptin is a recently discovered protein hormone that is produced by adipose tissue. Barb et al. (1998) reported that leptin inhibited feed intake and stimulated GH secretion in gilts. More recently, Qian et al. (1999) reported that serum leptin concentrations increased with age (42 to 154 d of age) in ovary-intact gilts but were unaffected by age or estradiol treatment in ovariectomized gilts (90 to 210 d of age). Leptin mRNA expression in adipose tissue was, however, greater in estradiol-treated, ovariectomized gilts than in controls (Qian et al., 1999). In the current investigation, serum leptin concentrations in boars did not change between 152 and 336 d of age, despite dramatic increases in circulating concentrations of estradiol.

In summary, NMA increased secretion of LH, GH, and testosterone in boars, and endocrine responses to the compound may be influenced by age of the treated animal. The NMA did not influence circulating concentrations of estradiol or leptin.

**Implications**

Luteinizing hormone (LH) stimulates testosterone secretion, and these hormones play critical roles in re-
production in boars. Moreover, growth hormone (GH) stimulates growth and muscle accretion. In the present investigation, N-methyl-D,L-aspartate increased blood concentrations of LH, testosterone, and GH in boars. N-Methyl-D,L-aspartate mimics the actions of glutamate, a physiologically important neurotransmitter in the brain. Thus, glutamate may have an important role in the control of hormone secretion and, thus, reproduction and growth in boars.

**Literature Cited**


