Title
Differential gonadotropin responses to N-methyl-D,L-aspartate in intact and castrated male rats

Permalink
https://escholarship.org/uc/item/0zz7g1jz

Journal
Biology of Reproduction, 48(4)

ISSN
0006-3363

Authors
Strobl, FJ
Luderer, U
Besecke, L
et al.

Publication Date
1993

DOI
10.1095/biolreprod48.4.867

License
CC BY 4.0

Peer reviewed
Peripheral administration of N-methyl-D,L-aspartate (NMA), a neuroexcitatory amino acid agonist, probably stimulates LH release through an increase in endogenous LHRH secretion. In the present study, NMA and a potent LHRH antagonist were used to determine the degree to which release of FSH is similarly dependent upon the acute secretion of LHRH. A second aim was to compare responsiveness of LHRH neurons to NMA in castrated and intact male rats. Adult male rats were castrated (n = 10) or sham castrated (n = 11) on the morning of Day 0. After 8 days, rats were fitted with atrial catheters between 0900 and 1200 h; at 2100 h they received s.c. either oil vehicle or 100 μg of an LHRH antagonist. Starting at 0900 h on Day 9, 0.5-ml blood samples were collected every 10 min for 5 h. After 1 h of sampling each animal received i.v. 5 mg of NMA in 0.5 ml 0.9% saline. An hour later each rat received i.v. 500 ng of LHRH in 0.5 ml saline. Plasma LH, FSH, and prolactin (PRL) levels were determined by RIA. In the oil-treated sham castrates, mean plasma LH levels were increased by 110% (p < 0.01) within 10 min and remained elevated for 30 min after the injection of NMA. The profile of this LH secretory response was similar to or slightly more robust than endogenous LH pulses observed previously. The NMA-induced LH release was completely blocked by pretreatment with LHRH antagonist. In both oil- and antagonist-treated sham-castrated rats, NMA administration failed to elicit a concomitant increase in plasma FSH levels. In both castrated groups, neither LH nor FSH release was elevated after administration of NMA.

Peripheral administration of NMA stimulates the release of LH from pituitary tissue [9]. The significance of NMA-activated LHRH neurosecretion is, at present, not completely clear; it may simply reflect a general ability of the drug to excite virtually any neuronal system through ubiquitously distributed NMA receptors. Alternatively, exogenous NMA may activate receptors for endogenous excitatory amino acids that normally play a physiological role in the regulation of LHRH pulses [4], LH surges [10], or puberty [11], as suggested in experiments using specific NMA and non-NMA receptor antagonists [4, 10, 11].

The effects of NMA administration on FSH secretion have received much less attention. In monkeys [7] it has been noted that NMA injections stimulate secretion of both LH and FSH pulses, with both actions being preventable by administration of LHRH antagonist [7]. The ability of NMA to induce pulses of FSH in rodents, however, has not yet been examined. For this reason, and in view of the renewed debate about the dependence of pulsatile FSH secretion on pulsatile LHRH release in the rat [12–18], the present study was carried out to fully characterize profiles of FSH and LH secretion after NMA administration. It was reasoned that if pulsatile LHRH secretion normally functions to stimulate both LH and FSH pulses, then the stimulation of a physiologically proportioned, endogenous LHRH discharge should, in turn, evoke coincident LH and FSH responses. Moreover, both responses should be susceptible to blockade by prior treatment with LHRH antagonist. If, on the other hand, pulsatile LHRH secretion does not normally support pulsatile FSH secretion, then the stimulation of LH by NMA would be unaccompanied either by a significant FSH response or by a FSH response resistant to blockade by LHRH antagonist, i.e., dependent upon the actions of another releasing factor.
A second major aim of these experiments was to compare, for the first time, the effects of NMA in testes-intact and castrated rats. Previous studies have shown that LHRH content [19], in vitro release [20], immunocytochemical signal [21], and electrically stimulated LHRH release [22, 23] are decreased after castration, even as LH levels are greatly elevated [24]. We recently proposed that these paradoxical LHRH decrements may be explained as secondary consequences of the increased LHRH pulse frequency [17] that follows removal of gonadal feedback suppression of the hypothalamic LHRH pulse generator [17, 25, 26]. Implicit in this hypothesis is the notion that an increase in the frequency of LHRH pulse generation results in the proportional diminution of the readily releasable LHRH pool. To test this hypothesis, we sought to determine, using a standard dose of NMA as LHRH secretagogue [27], whether the releasability of LHRH/LH is diminished in castrated versus intact rats.

In a companion study [28], related issues were investigated in female rats through use of NMA challenges at two different stages of the estrous cycle and after ovariectomy.

MATERIALS AND METHODS

Animals

Adult male Charles River (Wilmington, MA) Sprague-Dawley rats (220–380 g) were individually housed in a temperature-controlled room with lights-on from 0500 to 1900 h. Animals had free access to tap water and standard laboratory rat chow.

Experimental Procedures

Rats were anesthetized with methoxyflurane and then castrated (n = 10) or sham castrated (n = 11) on the morning of Day 0 between 0900 and 1100 h. Eight days later, between 0900 and 1200 h, each rat was again anesthetized and fitted with an atrial catheter. On the same day at 2100 h, each rat received s.c. either LHRH antagonist ([Ac-B(2)-D-NAL'-4-Fd-Phe3-D-Trp3-D-Arg9]-LHRH, 100 µg/250 µl sesame oil; Wyeth-Ayerst Research, Philadelphia, PA) or oil vehicle. The dose and the time of administration were based on previously published work from our laboratory [15]. Starting at 0900 h the next morning, 0.5-ml blood samples were withdrawn from the catheter every 10 min for 3 h. Blood samples were dispensed into sample tubes for centrifugation, storage at −20°C, and subsequent assay for LH, FSH, and PRL by RIA. After each blood sample was removed, an equivalent volume of a blood replacement mixture was slowly injected back through the sampling catheter. Preparation of the blood replacement mixture, containing male rat erythrocytes and human plasma protein fraction (Plasmanate; Cutter Laboratories, Berkeley, CA), has been described by Ellis and Desjardins [24]. The blood replacement mixture contains no detectable immunoreactive LHRH, LH, or FSH. Immediately following the first hour of blood sampling, each animal received 5 mg i.v. NMA (N-methyl-D,L-aspartate; Sigma Chemical, St. Louis, MO) in 0.5 ml 0.9% saline. This dose had previously been found to elicit physiologically proportioned LH pulses in male rats [27]. To test LH and FSH responsiveness to LHRH under each experimental condition, each rat also received a large dose (500 ng i.v.) of LHRH (Sigma) 1 h after NMA. This amount of LHRH was previously determined (unpublished trials) to be the minimal LHRH dose that stimulates FSH, as well as LH, secretion in intact male rats.

Additional groups of sham-castrated (n = 8) and 9-day-castrated (n = 8) rats were decapitated; median-eminence tissue was quickly obtained, homogenized in 0.1 N HCl, and centrifuged at 2000 × g. The supernatants were extracted in methanol/water (4:1 v/v) and centrifuged again at 2000 × g. The supernatants from the latter centrifugation were then transferred to sample storage vials and evaporated to dryness. The residues were stored at −20°C for LHRH RIA.

Hormone Assays

Levels of LH, FSH, and PRL in blood samples were determined by RIA through use of materials supplied by NIDDK (Bethesda, MD). The standards used in these assays were rLH-RP-3, rFSH-RP-2, and rPRL-RP-3; the levels of sensitivity were 30 pg/tube for LH, 400 pg/tube for FSH, and 160 pg/tube for PRL. The within-group variable was time of blood sampling, each animal receiving 5 mg i.v. NMA (N-methyl-D,L-aspartate; Sigma Chemical, St. Louis, MO) in 0.5 ml 0.9% saline. This dose had previously been found to elicit physiologically proportioned LH pulses in male rats [27]. To test LH and FSH responsiveness to LHRH under each experimental condition, each rat also received a large dose (500 ng i.v.) of LHRH (Sigma) 1 h after NMA. This amount of LHRH was previously determined (unpublished trials) to be the minimal LHRH dose that stimulates FSH, as well as LH, secretion in intact male rats.

Additional groups of sham-castrated (n = 8) and 9-day-castrated (n = 8) rats were decapitated; median-eminence tissue was quickly obtained, homogenized in 0.1 N HCl, and centrifuged at 2000 × g. The supernatants were extracted in methanol/water (4:1 v/v) and centrifuged again at 2000 × g. The supernatants from the latter centrifugation were then transferred to sample storage vials and evaporated to dryness. The residues were stored at −20°C for LHRH RIA.

Statistical Analysis

All results are expressed as the mean ± SE. Analysis of variance (ANOVA) with repeated measures was used to assess differences in LH, FSH, and PRL secretion between and within groups. Between-groups variables tested were treatment (vehicle vs. LHRH antagonist) and surgery (intact vs. castrated). The within-group variable was time of blood sampling. Post hoc comparisons between mean values at each time point were made using Newman-Keuls test. Results were considered significant if p < 0.05.

RESULTS

Effect of LHRH Antagonist on LH and FSH Secretion

The s.c. administration of 100 µg LHRH antagonist 12 h before blood sampling failed to significantly suppress LH and FSH levels in the sham-castrated animals (Fig. 1A and 2A). In the castrated rats, LHRH antagonist significantly (p
< 0.0001) reduced LH release by 83% (Fig. 1B) to levels observed in the sham-castrated animals, while plasma FSH concentrations were inhibited (p < 0.001) to only 45% of levels measured in oil-treated controls (Fig. 2B).

**Effect of NMA on LH and FSH Secretion**

Mean plasma LH and FSH concentrations during the hour before and the hour after the i.v. injection of 5 mg NMA in oil- and LHRH antagonist-treated sham-castrated and castrated rats are depicted in Figures 1 and 2. In the oil-treated sham castrates, mean plasma LH levels were significantly (p < 0.01) increased by 110% within 10 min and remained elevated for at least 30 min following injection of NMA (Fig. 1A). This NMA-induced increase in LH release was completely blocked in animals pretreated with LHRH antagonist. Although there was an apparent increase in LH release in the oil-treated castrated males at 10 min after NMA injection, this rise was significant only by the less stringent post hoc t-test (Fig. 1B). In both oil- and LHRH antagonist-treated sham-castrated (Fig. 2A) and castrated (Fig. 2B) animals, FSH secretion was not significantly altered during the hour after the administration of NMA.

**Effect of LHRH on LH and FSH Secretion**

Mean plasma LH and FSH concentrations before and after the i.v. injection of 500 ng LHRH in sham-castrated and castrated rats pretreated with oil or LHRH antagonist are depicted in Figures 3 and 4. The administration of LHRH caused 4- to 5-fold (p < 0.01) increases in LH levels in oil-treated sham-castrated (Fig. 3A) and castrated (Fig. 3B) male rats within 20 min. The effect of LHRH on LH release was greatly blunted, but not completely blocked, by pretreatment with LHRH antagonist. The administration of LHRH also resulted in significant 50% and 30% (p < 0.01) increases in FSH levels, which peaked within 20 to 30 min of injection, in the oil-treated sham-castrated (Fig. 4A) and castrated (Fig. 4B) rats. Pretreatment with LHRH antagonist also blunted the FSH responses to LHRH in both surgical groups.

**Effect of NMA on PRL Secretion**

Circulating PRL levels in the sham castrates (39.6 ± 2.0 ng/ml) were approximately 2-fold higher than PRL levels in the castrated animals (22.3 ± 0.9 ng/ml). Mean plasma PRL concentrations in oil-treated sham-castrated and castrated male rats before and after the i.v. injection of 5 mg NMA are depicted in Figure 5. In oil-treated sham-castrated animals, the administration of NMA produced a nonsignifi-
A significant increment in mean plasma PRL levels, from 43.9 ± 3.0 ng/ml 10 min before injection to 60.4 ± 9.1 ng/ml 10 min after injection (Fig. 5A). In oil-treated castrated rats the administration of NMA resulted in a significant increase (p < 0.05) in mean PRL levels from 24.8 ± 5.4 ng/ml 10 min before injection (Fig. 5B) to 40.8 ± 4.7 ng/ml 10 min after injection. The increment in mean PRL levels after NMA injection was almost identical in the oil-treated sham-castrated (16.5 ng/ml) and castrated (16.0 ng/ml) male rats. Although missing PRL values from the LHRH antagonist-treated animals prevented a true statistical comparison of mean PRL levels between vehicle and antagonist groups, pretreatment with LHRH antagonist did not appear to have an effect on NMA-induced PRL secretion.

**Effect of Castration on Median-Eminence LHRH Levels**

The median-emience LHRH content in the castrates, 3.49 ± 0.48 ng/tissue, was found to be significantly reduced compared to the 5.84 ± 0.76 ng/tissue observed in the intact males (p < 0.025).

**DISCUSSION**

The present experiments demonstrate that a bolus i.v. injection of NMA, which stimulates a pulse of LH secretion that can be blocked by LHRH antagonist, fails to significantly alter the secretion of FSH. It is therefore concluded that NMA elicits secretion of an amount of LHRH that is capable of stimulating an LH pulse, but incapable of stimulating a FSH pulse. Since NMA-induced LH pulses (and presumably NMA-induced LHRH pulses) approximate physiological LH [17, 24] pulses, we therefore infer that under normal circumstances, endogenous LHRH pulses are themselves of insufficient magnitude to stimulate FSH pulses. Our findings are consistent with the hypothesis that endogenous, pulse-like increments in FSH secretion either are constitutive endocrine events or are stimulated by an LHRH-independent, neurosecretory mechanism [12–14, 17].

Several previous studies have suggested that FSH secretion is less dependent than LH secretion on pulsatile LHRH stimulation. In a push-pull perfusion study conducted in this laboratory [17] it was found that pulses of LH, but not of FSH, were temporally associated with LHRH pulses; in the same study, LH and FSH pulses were also not temporally correlated in animals sampled only through atrial catheters. Others have similarly reported lack of pulsatile LH and FSH coincidence in gonadectomized rats [13, 14] and hamsters [29]. To our knowledge, only one group has reported that LHRH, LH, and FSH pulses are temporally correlated in rats [30]; technical reasons were cited as possible...
be in the temporal characteristics of the FSH secretory response to LHRH. Indeed, it is well known that the metabolic clearance rate of FSH is quite slow [32]. Since it takes one half-life for the equilibrium serum concentration of a molecule to be attained after its secretion rate has increased or decreased abruptly, FSH requires about 100 to 200 min to reach equilibrium in vivo [32]. Thus, the lack of a clear temporal relationship between LHRH and/or LH pulses and FSH pulses in vivo may partially result from the long serum transients of the latter. This would also explain why pulsatile LHRH administration in vitro can elicit distinct pulses of both FSH and LH secretion from perfused pituitary fragments [31].

There may also be qualitative differences in the FSH versus the LH responses to LHRH. While LHRH in vivo may ultimately influence LH secretion by stimulating both secretory [33] and synthetic [34] processes, the actions of LHRH in regulating FSH secretion may be exerted primarily through the latter route. Rather than directly stimulating the FSH secretory process, LHRH may regulate some subcellular process leading to accumulation of FSH in the releasable hormone pool. The most likely mechanism for this is transcriptional regulation, as it has recently been shown that pulsatile infusions of the decapeptide increase FSH-β mRNA levels in rats [35] and sheep [36], and that chronic LHRH antagonist and antisera administration prevent the postcastration rise in FSH-β mRNA levels and suppress FSH-β mRNA levels in intact male rats [37].

A second finding of interest in the present study was that LH responses to NMA were attenuated in male rats gonadectomized nine days earlier. This observation is in accord with results of a study by Estienne et al. [5], who found that NMA stimulates LH secretion only after steroid priming in ovariectomized sheep. There are several possible reasons for the apparent steroid dependency of NMA actions. One explanation is that castration may result in a decrease in pituitary sensitivity to LHRH, which would result in reduced amplification of the NMA-induced LHRH stimulus. This possibility, however, is not supported by our observation that an LHRH challenge induced even greater (absolute) elevations in gonadotropin secretion in castrated male rats. Furthermore, our group and others have previously noted that castration increases pituitary sensitivity to LHRH in vivo [38, 39] and that testosterone treatments in vivo [38] or in vitro [40] exert the opposite effect. A second explanation for attenuated NMA effects in castrates is that the number and/or affinity of NMA receptors may be altered by gonadectomy. In the present study, however, NMA elicited virtually identical increases in mean plasma PRL levels in sham-castrated and castrated animals; this suggests that NMA receptor populations are not generally affected by castration. Thus, it seems more likely that attenuated LH responses to NMA in castrates result from a decrement in the LHRH secretory response following NMA receptor activation. We suggest that the reason for this is that the readily

![Graph showing plasma PRL levels in oil-treated sham-castrated (A) and castrated (B) male rats at 10-min intervals 1 h before and 1 h after the i.v. injection of 5 mg NMA (arrow).](image-url)
releasable pool of LHRH is diminished in castrated rats. In agreement with this idea, we found that LHRH tissue levels in median eminence were reduced in rats castrated nine days before they were killed. Others have also found that LHRH content [19], LHRH release in vitro [20], LHRH immunocytochemical signal [21], and electrically stimulated LHRH release in vivo [23] and in vitro [22] are decreased in long-term (> 2 wk) castrated rats. A decrement in the releasable LHRH pool may, in turn, result from the increase in frequency of pulsatile LHRH release that occurs after removal of gonadal feedback suppression of the LHRH pulse generator [17, 25, 26]. That is, increased frequency of pulsatile LHRH release, in the absence of a compensatory increase in LHRH biosynthesis [41], may lead to the diminution of the readily releasable LHRH pool. Responses of the LHRH secretory apparatus to secretagogues such as NMA would diminish accordingly ([5, 28] and present paper). This hypothesis remains to be tested by examination of LHRH/LH responses to NMA in castrated, testosterone-treated rats.

In summary, we have used peripheral injections of the LHRH secretagogue, NMA, to provide evidence that 1) endogenous LHRH pulses that stimulate physiologically proportioned pulses of LH are not capable of stimulating FSH pulses and 2) LHRH responses to a secretagogue are diminished following castration.

ACKNOWLEDGMENTS

We gratefully acknowledge the NIDDK (Bethesda, MD) for supplying materials for LH, FSH, and PRL RIA's and Dr. Fred Bex of Wyeth-Ayerst Research for the LHRH antagonist.

REFERENCES


18. Wise PM, Rance N, Barr GD, Bardoughl CA. Further evidence that luteinizing hormone-releasing hormone also is follicle-stimulating hormone-releasing hormone. Endocrinology 1979; 104:946–947.


23. Wisse PM, Kowal KE, Barr GD. Further evidence that luteinizing hormone-releasing hormone also is follicle-stimulating hormone-releasing hormone. Endocrinology 1985; 104:55–43.


