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Direct Activation of Gonadotropin-Releasing Hormone Secretion Through Different Receptors to Neuroexcitatory Amino Acids

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Abstract. In order to evaluate the involvement of gonadotropin-releasing hormone (GnRH) in the effects of neuroexcitatory amino acids on luteinizing hormone (LH) secretion, N-methyl-*D,L*-aspartate (NMDA; 30 mg/kg s.c.) was administered to 50-day-old male rats. The in vitro release of GnRH from the hypothalamus showed a maximal increase (4.6-fold) in animals sacrificed 7.5 min after NMDA injection, while serum LH levels rose concomitantly. Incubation of rat hypothalami in vitro with kainate or NMDA concentrations > 0.1 m*M* resulted in a dose-related release of GnRH, NMDA being twofold more potent than kainate. Quisqualate (10 m*M*) did not affect the release of GnRH. On a molar basis, quinolinate (50 m*M*), a possible endogenous ligand for NMDA receptors, was the most effective in inducing GnRH secretion (34.9 ± 4.9 pg/7.5 min, mean increment $\pm$SEM, $n = 10$). The effects of kainate and NMDA were mediated through different types of receptors, since GnRH response to kainate was unchanged in the absence of glycine or in the presence of increased concentrations of Mg^{2+} (2 m*M*) or Ca^{2+} (5.8 m*M*). In contrast, the GnRH response to NMDA was reduced by Ca^{2+} (5.8 m*M*) and abolished in the absence of glycine or in the presence of Mg^{2+} (2 m*M*). In addition, *D,L*-amino-5-phosphonopentanoic acid (AP5), a competitive antagonist of NMDA receptors, prevented the NMDA-induced release of GnRH. The permissive effect of glycine on GnRH response to NMDA was 2.7-fold more important using glycine concentrations of 0.01 μ*M* than when concentrations ≥ 100 μ*M* were used. Intermittent incubation with NMDA in vitro (every other 7.5-min period) did not affect the amplitude of GnRH response, while continuous exposure to NMDA resulted in an initial and transient increase in GnRH release followed by a prolonged desensitization period. It is concluded that different neuroexcitatory amino acids acting through distinct receptor types may be involved in the hypothalamic control of LH release by regulating the secretion of GnRH.

In rats and monkeys in vivo administration of agonists of the neuroexcitatory amino acids glutamate and aspartate triggers the release of luteinizing hormone (LH) [14, 17, 21, 27, 30, 37]. These observation may be relevant to the hypothalamic mechanisms thought to be involved in pulsatile secretion of LH and its changes at onset of puberty. Such an hypothesis has been raised in recent studies using agonists and antagonists of neuroexcitatory amino acids in the immature monkey [14, 26] and in the rat [2, 34]. In these experiments, it has been assumed that the study of LH provided an estimate of changes in gonadotropin-releasing hormone (GnRH) secretion. This was based on indirect observations suggesting that the effects of neuroexcitatory amino acids are mediated in the hypothalamus [14, 21, 24, 30, 33]. In addition, most studies have been directed towards receptors to N-methyl-*D,L*-aspartate (NMDA), while in the central nervous system, at least two types of distinct amino acid receptors have been characterized using kainate and NMDA as specific ligands [35]. The aim of this study was to evaluate directly the putative changes in GnRH secretion induced by neuroexcitatory amino acids and to determine if different types of receptors are involved.

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Materials and Methods

Rats

Male Wistar rats aged 50 days at the time of the experiment were purchased from Janssens (Beerse, Belgium). They were housed 6 per cage under constant temperature and a light-dark schedule (lights on 06.00–20.00 h) with free access to food and

water. For each experiment, a group of 12 rats were killed around 13.00 h. The retrochiasmatic hypothalamus was rapidly dissected as previously described [6, 7] and incubated in vitro.

Incubation of Hypothalamic Explants

A static in vitro system was used, as previously described [6, 7], in order to study 12 hypothalami individually, each explant being in 0.5 ml culture medium which was renewed every 7.5-min period. Culture medium was Dulbecco's modified eagle's medium (Flow Laboratories, MacLean, Va., USA) which contained 25 m*M* glucose, 1.8 m*M* Ca^{2+}, 1 m*M* Mg^{2+}, and 0.4 m*M* glycine. Eagle's minimum essential medium (Flow Laboratories) was also used after being enriched with leucine 5.2 mg/dl and glucose 350 mg/dl in order to obtain a medium similar to Dulbecco's modified Eagle's medium except the absence of glycine. Culture medium contained bacitracin at a final concentration of 20 μ*M* in order to prevent degradation of GnRH by hypothalamic peptidases [29].

Radioimmunoassay of GnRH

From the 0.5-ml samples collected every 7.5 min, 0.1 ml was assayed in duplicate, according to a method decribed previously [5, 6], using the RR-5 anti-GnRH antiserum generously supplied by Dr. A. Root (St. Petersburg, Fla., USA). Since the sensitivity of the assay varied between 0.5 and 1.0 pg/tube, the detection limit of GnRH release was 5 pg/7.5 min. The standard curves were prepared in Dulbecco's modified Eagle's medium. The absence of nonspecific effects on the binding of radiolabelled GnRH and on the second antibody was checked in the presence of the different substances used.

Radioimmunoassay of LH

Serum concentrations of LH were determined in duplicate by double-antibody radioimmunoassay as previously described [5], using NIAMDD rat pituitary gonadotropin reagents kindly supplied by the Pituitary Agency of the National Institutes of Health. Results were expressed with reference to the rat standard IRP-1.

Study Protocols

Experiment 1. Intact rats (4–12/series) were injected subcutaneously with NMDA (Sigma, St. Louis, Mo., USA) 30 mg/kg body weight or with saline (controls). One, 3, 5, 7.5, and 10 min after the injection, the rats were sacrificed. Trunk blood was collected, and serum for the radioimmunoassay of LH was obtained after centrifugation. Immediately after decapitation, the hypothalamus was dissected and transferred to the incubator where the release of GnRH in culture medium was measured after a 7.5-min incubation period.

Experiment 2. The in vitro release of GnRH from 9 to 12 individual hypothalami was studied in the presence of increasing concentrations (0.001–100 m*M*) of kainate (Sigma) or NMDA applied for 7.5 min at 15-min intervals. Quisqualate (Sigma), a ligand to receptors distinct from those binding kainate and NMDA [32, 35], and quinolinate (Sigma), a possible endogenous ligand to NMDA receptors [31], were also tested. The results were expressed as increment in GnRH release, i.e., the difference between the levels of GnRH release observed immediately before and after the application of a test substance.

Experiment 3. The effect of kainate 50 m*M* or NMDA 50 m*M* on GnRH release in vitro was evaluated under different conditions. Controls were obtained in medium containing Ca^{2+} 1.8 m*M*, Mg^{2+}

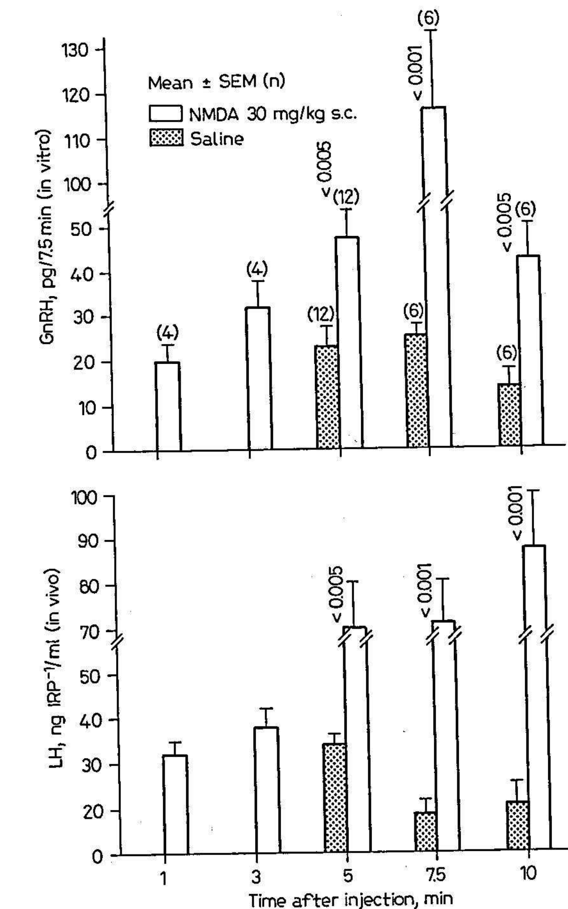

Fig. 1. Serum LH concentrations (lower panel) and levels of GnRH release from individual hypothalami in vitro (upper panel) in relation to time after subcutaneous injection of NMDA 30 mg/kg or saline in 50-day-old male rats. The significance of differences is indicated by p values.

1 m*M*, and glycine 0.4 m*M*. The effects of kainate and NMDA were studied in the presence of D-600 0.1 m*M* (Methoxy Verapamil; Knoll, Ludwigshafen, FRG), a calcium channel blocker [4], and in medium enriched with CA^{2+} 4 m*M* or Mg^{2+} 1 m*M* or using glycine-free medium. In addition, the effect of NMDA was studied in the presence of equimolar concentrations of AP5 (Tocris, Neuramin, Bukhurst Hill, UK), a competitive antagonist of NMDA receptors [16]. In these experiments, the basal level of GnRH release was determined under the same conditions as for NMDA or kainate stimulation.

Experiment 4. The in vitro GnRH release induced by NMDA 50 m*M* was studied in the absence of glycine or in the presence of increasing glycine concentrations (0.01–10,000 μ*M*).

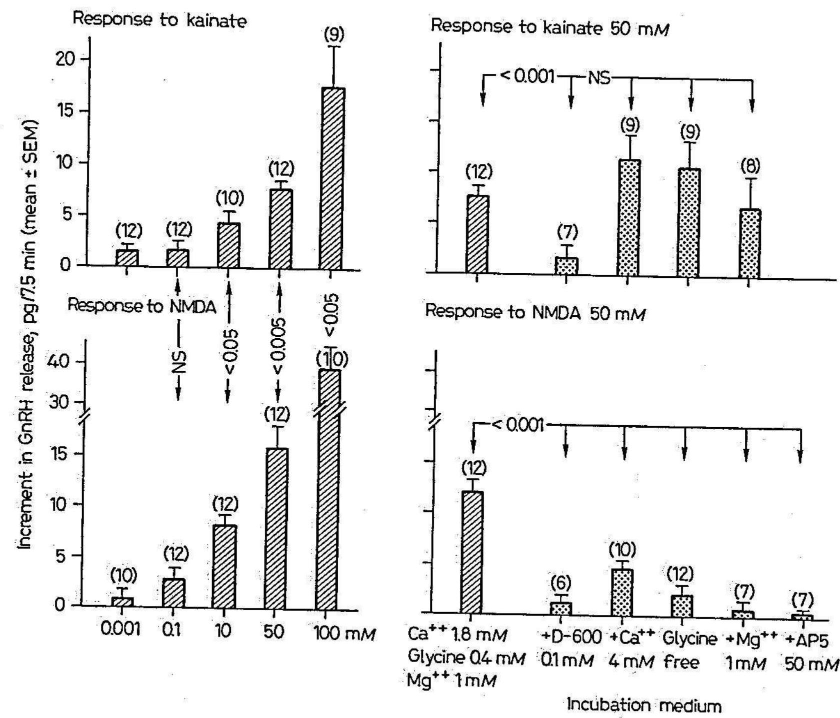

Fig. 2. Left panel: increment in GnRH release from rat hypothalami in vitro in relation to the concentrations of kainate or NMDA applied for 7.5 min. Right panel: increment in GnRH release induced by kainate or NMDA 50 mM under control conditions (light-hatched columns) or using culture media with several modifications (dark-hatched columns). The number of hypothalami studied is indicated in parentheses at the top of the bars. The p levels give the significance of differences between responses to kainate and NMDA (left panel) and between control and modified incubation medium (right panel).

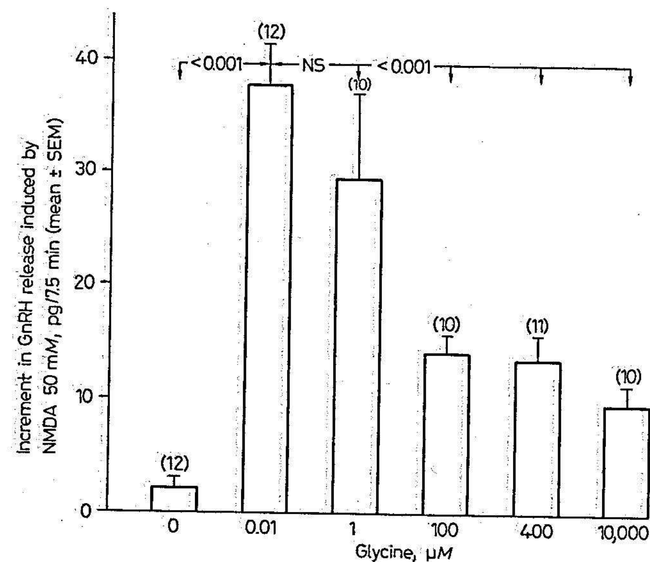

Fig. 3. Increment in GnRH release in vitro induced by NMDA 50 mM in relation to the glycine concentration in incubation medium. The significance of differences is indicated by p values.

Experiment 5. The effects of high frequency or continuous stimulation of GnRH release by NMDA were studied by incubating hypothalami in the presence of NMDA 50 mM applied every other 7.5-min period for 120 min or during continuous exposure to NMDA 50 mM for 75 min.

Statistical Analysis

The significance of differences was calculated using Student's t test.

Results

As shown in figure 1, in vivo administration of NMDA 30 mg/kg s.c. induced an increase in the secretion of LH in vivo and in the release of GnRH in vitro. When compared to the data obtained 1 min after NMDA injection or 5 min after saline injection, LH serum concentrations and GnRH release in vitro were significantly increased ($p < 0.005$) 5 min after NMDA injection. The peak level of GnRH release induced by NMDA was observed 7.5 min after NMDA injection, while the rise in LH serum concentrations occurred up to 10 min after NMDA injection.

Incubation of rat hypothalami in vitro in the presence of increasing concentrations of kainate or NMDA resulted in a dose-related stimulation of GnRH release (fig. 2, left panel). At concentrations of 10, 50 and 100 mM, NMDA was about twice as potent ($p < 0.05$) as kainate in eliciting the release of GnRH. At a 50-mM concentration, quinolinate, a putative endogenous ligand of NMDA receptors, induced a GnRH increment of 34.9 ± 4.9 (SEM) pg/7.5 min ($n = 10$) which was 2.2-fold greater than the response to 50 mM NMDA ($p < 0.001$). In contrast, 10 mM quisqualate did not affect the release of GnRH (increment 1.7 ± 1.0 pg/7.5 min; $n = 11$).

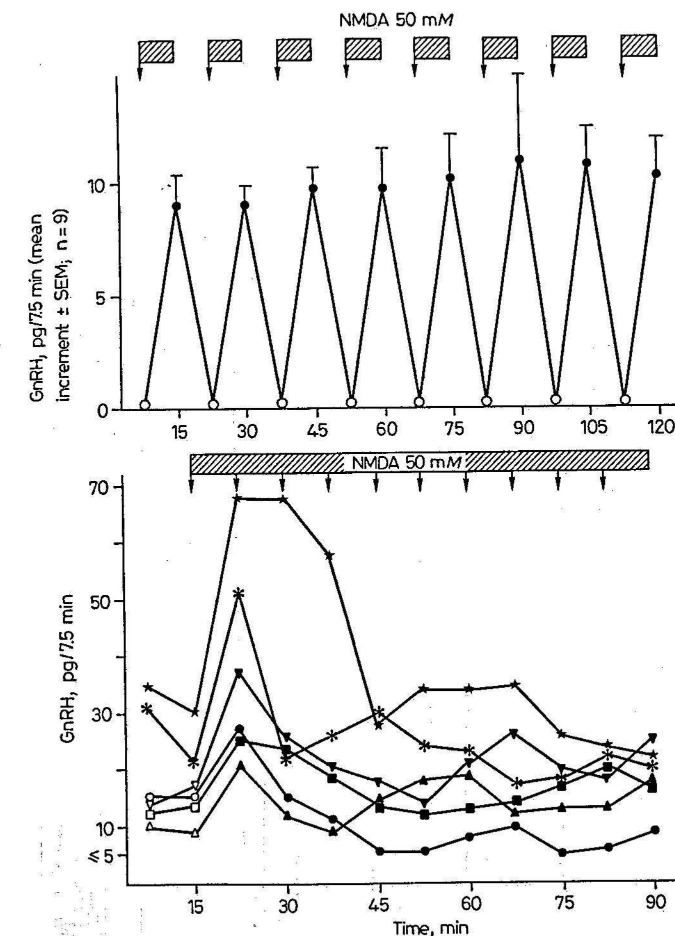

Fig. 4. GnRH release from individual rat hypothalami in vitro (upper panel: increment; lower panel: absolute levels) during intermittent (upper panel) or continuous (lower panel) exposure to NMDA 50 mM.

As shown in the right panel of figure 2, the response of GnRH to kainate or NMDA 50 mM was abolished ($p < 0.001$) in the presence of D-600, a Ca^{2+} channel blocker. When compared to controls obtained in medium containing 1.8 mM Ca^{2+}, 1 mM Mg^{2+}, and 0.4 mM glycine, the stimulatory effect of 50 mM kainate was not significantly affected by increased concentrations of Ca^{2+} (up to 5.8 mM) or Mg^{2+} (2 mM) or by using glycine-free culture medium. In contrast, the stimulatory effect of NMDA 50 mM was blunted in Ca^{2+}-enriched medium ($p < 0.001$) and abolished ($p < 0.001$) in the presence of Mg^{2+} 2 mM or when using glycine-free medium. In addition, equimolar concentrations of AP5, a competitive antagonist specific of NMDA receptors, suppressed ($p < 0.001$) the response of GnRH to NMDA (fig. 2).

While no response to 50 mM NMDA was observed in the absence of glycine (fig. 3), the use of low glycine concentrations (0.01–1 µM maximized the NMDA-induced release of GnRH which was significantly greater ($p < 0.001$) than the release seen in the presence of higher glycine concentrations (100–10,000 µM).

As shown in figure 4, the increment in GnRH release induced by NMDA 50 mM occurred repetitively when intermittent NMDA stimulation was performed every other 7.5-min period. The amplitude of GnRH response to NMDA remained similar during eight successive stimulations. In contrast, continuous exposure to NMDA 50 mM for 75 min resulted in an initial increase in GnRH release, immediately followed by a decrease back to the baseline level which was unchanged throughout the entire subsequent period of stimulation.

Discussion

In this paper, we provide the first direct evidence that neuroexcitatory amino acids trigger the release of GnRH. Several authors have previously suggested that the hypothalamus was the primary site where neuroexcitatory amino acids could affect the secretion of LH. After neonatal exposure of rodents to excito-toxic amino acids such as monosodium glutamate, which destroy the vast majority of perikarya in the arcuate nucleus [22], the response of LH to NMDA administration is abolished [23]. Although not tested at concentrations higher than 10 µM, NMDA does not affect the release of LH from rat and monkey pituitary cultures in vitro [30, 33]. In the rat, pituitary hormone secretion including LH is activated by intrahypothalamic injection of quinolinic acid, a ligand to NMDA receptors [21]. More recently, the LH-releasing effect of NMDA in monkeys was shown to by prevented by pretreatment with a GnRH receptor antagonist [14].

In the central nervous system, the receptors to excitatory amino acids have been classified in three groups according to the exogenous agonists which bind selectively to these receptors: NMDA, kainate, and quisqualate [32, 35, 36]. Receptors to NMDA and kainate have been demonstrated in the hypothalamus where the concentration of kainate-binding sites is predominant over NMDA-binding sites [9]. A subtype of NMDA receptors is bound by quinolinic acid which is derived endogenously from tryptophan [31, 32]. Among these ligands, NMDA [27, 37], kainate [27], and

quinolinate [17, 21] have been shown to induce the release of pituitary hormones, particularly LH. Our data indicate that both NMDA and kainate receptors may be involved in the mechanisms regulating GnRH secretion, while receptors to quisqualate do not seem to play any role. Quisqualate was not effective at 10 m*M*, while NMDA was consistently effective at the same concentration. A possible effect of quisqualate at higher concentrations has not been excluded. Our data confirm the observations of Price et al. [27] showing that NMDA and kainate receptors may mediate the stimulation of LH release. After the early report of Price et al. [27], the difference between effects mediated through kainate and NMDA receptors has been elucidated. In fact, a series of mechanisms are selectively involved in the activation of NMDA receptors: noncompetitive antagonism by Mg^{2+} [3, 12], inhibition by elevated Ca^{2+} concentrations [20], modulation by glycine which potentiates NMDA effects at low concentrations [18], competitive antagonism by AP5 [16], and desensitization during continuous exposure to NMDA [13]. In this paper, we have shown that the NMDA-induced release of GnRH bears all those characteristics of NMDA receptor mediated effects as evidenced in other systems. We have also shown that kainate-induced release of GnRH is mediated through non-NMDA receptors, since this effect is not affected by factors such as glycine or Mg^{2+} concentrations. While NMDA and kainate are potent exogenous activators of receptors to excitatory amino acids, quinolinic acid is of particular interest, since it represents a possible endogenous ligand to NMDA receptors [25, 31]. On a molar basis, the GnRH-releasing activity of quinolinate is greater than that of NMDA, although the potency of quinolinate has been originally reported to be one tenth of the NMDA potency [31].

A major unresolved issue is the significance of our observations for the mechanisms controlling the release of hypothalamic peptides under physiological conditions. In this respect, questions arise from the concentrations of agonists used which may be much higher than the concentrations of endogenous substrates activating NMDA and kainate receptors. For instance, quinolinate has been found at micromolar concentrations in most brain areas [19], while we have used millimolar concentrations in vitro . Furthermore, Nemeroff et al [21] have observed a stimulation of LH after intrahypothamalic injection of a quinolinate amount which was 100 times less than the one we have used in vitro. In contrast, molar concentrations of kainate or glutamate have been applied by some investigators to the hypothalamus in vivo [1] or in vitro [15] in order to obtain neuroexcitatory effects.

In our experiments, repeated exposure to very high concentrations of NMDA (50 m*M*) for 2 h failed to alter the response of GnRH. Thus, it may be argued that the neurotoxic effect which characterizes the use of high concentrations of excitatory amino acids [22, 28] did not occur during our experiments. This suggests either that, under our experimental conditions, only a small fraction of the amount of NMDA applied is effectively distributed to the receptors or that there is a low receptor sensitivity to the agonist. The first hypothesis is consistent with the high affinity of many acidic amino acids to divalent cations [3]. Thus, it is possible that NMDA or kainate are partly complexed with Ca^{2+} or Mg^{2+} and inactivated. The second hypothesis is supported by the recent findings of Cicero et al. [8]. These authors have found that the LH response of male rats to NMDA was maximal between 20 and 35 days of age, minimal at 40 days, and virtually absent at 60 days. This might explain the requirement of very high concentrations during our experiments in 50-day-old rats.

The concentrations of Mg^{2+} used in our experiments may be an alternative explanation to the high concentrations of NMDA required. In some systems, micromolar concentrations of Mg^{2+} inhibit the neuronal response to NMDA [3]. Since 1 m*M* Mg^{2+} has been used in our control experiments, the effect of NMDA may have been counteracted, resulting in the need for high concentrations of amino acid well above those physiologically active. However, other observations do not support that explanation. A 1-m*M* concentration of Mg^{2+} is physiologically present in the central nervous system and does not result in the requirement of very high NMDA or quinolinate concentrations during in vivo experiments [21]. In addition, Drouva et al. [11] have shown that the depolarization-induced release of GnRH from hypothalamic fragments in vitro is similar in the absence of Mg^{2+} or in the presence of 1 m*M* Mg^{2+}, while 7.5 m*M* Mg^{2+} have inhibitory effects. In fact, our data indicate that increasing Mg^{2+} concentrations from 1 to 2 m*M* may suppress the excitatory effects of NMDA. Complementary experiments are required to evaluate the neuroexcitatory and neurotoxic properties of the amino acids in the absence of Mg^{2+} or using very high Mg^{2+} concentrations.

Glycine is known to depress neuroexcitability [10], molar concentrations resulting in a decreased firing rate of tuberal hypothalamic neurons in vitro [15]. Recently, Johnson and Ascher [18] have demonstrated the facilitatory effect of nanomolar glycine concentrations on the electrophysiological response to NMDA. This led us to study different glycine concentrations. In the absence of glycine, no GnRH release is induced by NMDA 50 m*M*. However, glycine may not be a prerequisite to the effect of NMDA, since an increase of NMDA concentrations may overcome the reduced excitability caused by the absence of glycine [18]. While maximal responsiveness of GnRH to NMDA is observed in the presence of glycine 0.01–1 μ*M*, higher concentrations result in a reduced response, indicating a possible regulatory role of glycine on the NMDA-induced secretion of GnRH.

The desensitization of the GnRH response to continuous exposure to NMDA suggests that an intermittent activation

of receptors to neuroexcitatory amino acids is a prerequisite to the neurosecretory activity of GnRH neurons. This is interesting in the light of the pulsatile nature of GnRH release [6] and supports the concept that endogenous substrate binding to neuroexcitatory amino acid receptors plays a role in the mechanism controlling the pulsatile release of GnRH. Our experiments do not allow to delineate whether the desensitization occurs at the NMDA receptor level [13] or whether the desensitization results from the ultrashort loop negative feedback of GnRH on its own secretion [7].

In order to demonstrate the role of hypothalamic amino acid receptors in the control of gonadotropin secretion, NMDA has been administered intermittently to prepubertal female rats [34] or prepubertal male monkeys [26], at a frequency consistent with an adult hypothalamic GnRH pulse generator. This treatment results in a precocious pubertal development in both species. More recently, it has been shown that AP5 inhibits the pulsatile release of LH in the male rat [2]. This finding strongly supports the physiological role of NMDA receptors in the regulation of GnRH secretion. Following our previous observations on the pulsatile pattern of GnRH release in vitro [6, 7] with an increasing frequency around 3 weeks of age in male rats [6], important questions are to be addressed regarding the effects of amino acid receptor antagonists in relation to age and sexual maturation.

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References

1 Arnaud, E.; Layton, B.S.; Padjen, A.A.; Renaud, L.P.: Actions of acidic amino acids on the excitability of medial hypothalamic neurons in the rat. Neuroendocrinology *37:* 184–192 (1983).
2 Arslan, M.; Pohl, C.R.; Plant, T.M.: *DL*-2-Amino-5-phosphonopentanoic acid, a specific N-methyl-*D*-aspartic acid receptor antagonist, supresses pulsatile LH release in the rat. Neuroendocrinology *47:* 465–468 (1988).
3 Ascher, P.; Nowak, L.: The role of divalent cations in the N-methyl-*D*-aspartate responses of mouse central neurones in culture. J. Physiol. *399:* 247–266 (1988).
4 Baker, P.F.; Meves, H.; Ridgway, E.B.: Calcium entry in response to maintained depolarization of squid axons. J. Physiol. *231:* 527–531 (1973).
5 Bourguignon, J.P.; Gérard, A.; Franchimont, P.: Age-related differences in the effect of castration upon hypothalamic LHRH content in male rats. Neuroendocrinology *38:* 376–381 (1984).
6 Bourguignon, J.P.; Franchimont, P.: Puberty-related increase in episodic LHRH release from rat hypothalamus in vitro. Endocrinology *114:* 1941–1943 (1984).
7 Bourguignon, J.P.; Gérard, A.; Debougnoux, G.; Rose, J.; Franchimont, P.: Pulsatile release of gonadotropin-releasing hormone (GnRH) from the rat hypothalamus in vitro: Calcium and glucose dependency and inhibition by superactive GnRH analogs. Endocrinology *121:* 993–999 (1987).
8 Cicero, T.J.; Meyer, E.R.; Bell, R.D.: Characterization and possible opioid modulation of N-methyl-*D*-aspartic acid induced increases in serum luteinizing hormone levels in the developing male rat. Life Sci. *42:* 1725–1732 (1988).
9 Cotman, C.W.; Monaghan, D.T.; Ottersen, O.P.; Storm-Mathisen, J.: Anatomical organization of excitatory amino acid receptors and their pathways. Trends Neurosci. *10:* 273–280 (1987).
10 Dreifuss, J.J.; Matthews, E.K.: Antagonism between strychnine and glycine and bicuculline and GABA in the ventromedial hypothalamus. Brain Res. *45:* 599–603 (1972).
11 Drouva, S.V.; Epelbaum, J.; Hery, M.; Tapia-Arancibia, L.; Laplante, E.; Kordon, C.: Ionic channels involved in the LHRH and SRIF release from rat mediobasal hypothalamus. Neuroendocrinology *32:* 155–162 (1981).
12 Evans, R.H.; Francis, A.A.; Watkins, J.C.: Selective antagonism by magnesium of amino acid induced depolarization of spinal neurones. Experientia *33:* 489–491 (1977).
13 Fagni, L.; Baudry, M.; Lynch, G.: Classification and properties of acidic amino acid receptors in hippocampus. Electrophysiological studies of an apparent desensitization and interactions with drugs which block synaptic transmission. J. Neurosci. *3:* 1538–1546 (1983).
14 Gay, V.L.; Plant, T.M.: N-methyl-*D,L*-aspartate elicits hypothalamic gonadotropin-releasing hormone release in prepubertal male rhesus monkeys *(Macaca mulatta).* Endocrinology *120:* 2289–2296 (1987).
15 Geller, H.M.: Effects of some putative neurotransmitters on unit activity of tuberal hypothalamic neurons in vitro. Brain Res. *108:* 423–430 (1976).
16 Harrison, N.L.; Simmonds, M.A.: Quantitative studies on some antagonists of NMDA in slices of rat cerebral cortex. Br. J. Pharmacol. *84:* 381–391 (1985).
17 Johnson, M.D.; Whetsell, W.O.; Crowley, W.R.: Quinolinic acid stimulates luteinizing hormone secretion in female rats – evidence for involvement of NMDA preferring receptors. Exp. Brain Res. *59:* 57–61 (1985).
18 Johnson, J.W.; Ascher P.: Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature *325:* 529–531 (1987).
19 Lombardi, G.; Carla, V.; Moneti, G.; Moroni, F.: Developmental changes of the content and synthesis of quinolinic acid in the rat brain. Acta pharmac. tox. *53:* suppl. 1, P. 24 (1983).
20 Mayer, M.L.; Westbrook, G.L.: The physiology of excitatory amino acids in the vertebrate central nervous system. Prog. Neurobiol. *28:* 197–276 (1987).
21 Nemeroff, C.B.; Mason, G.A.; Bissette, G.; Parks, D.A.; Schwarcz, R.: Effects of intrahypothalamic injection of quinolinic acid on anterior pituitary hormone secretion in the anaesthetized rat. Neuroendocrinology *41:* 332–336 (1985).
22 Olney, J.W.: Brain lesions, obesity and other disturbances in

mice treated with monosodium glutamate. Science *164:* 719–721 (1969).
23 Olney, J.W.; Price, M.T.: Neuroendocrine interactions of excitatory and inhibitory amino acids. Brain Res. Bull. *5:* 361 (1980).
24 Perez, V.J.; Olney, J.W.: Accumulation of glutamic acid in the arcuate nucleus of the hypothalamus of the infant mouse following subcutaneous administration of monosodium glutamate. J. Neurochem. *19:* 1777–1781 (1972).
25 Perkins, M.N.; Stone, T.W.: An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. Brain Res. *247:* 184–187 (1982).
26 Plant, T.M.; Gay, V.L.; Marshall, G.R.; Arslan, M.: Chronic intermittent chemical excitation of the hypothalamic GnRH system of the prepubertal monkey with N-methyl-*D,L*-aspartate prematurely reactivates the pituitary-Leydig cell axis. in: 69th annual meeting of the Endocrine Society, Indianapolis. Abstr. 65 (1987).
27 Price, M.T.; Olney, J.W.; Cicero, T.J.: Acute elevations of serum luteinizing hormone induced by kainic acid, N-methyl aspartic acid or homocysteic acid. Neuroendocrinology *26:* 352–358 (1978).
28 Rothman, S.M.; Olney, J.W.: Excitotoxicity and the NMDA receptor. Trends Neurosci. *10:* 299–302 (1987).
29 Rotsztejn, W.H.; Charli, J.L.; Pattou, E.; Epelbaum, J.; Kordon, C.: In vitro release of LHRH from rat mediobasal hypothalamus: Effects of potassium, calcium and dopamine. Endocrinology *99:* 1663–1665 (1976).
30 Schainker, B.A.; Cicero, T.J.: Acute central stimulation of luteinizing hormone by parenterally administered N-methyl-*D,L*-aspartic acid in the male rat. Brain Res. *184:* 425–437 (1980).
31 Stone, T.W.; Perkins, M.N.: Quinolinic acid: A potent endogenous excitant at amino acid receptors in rat CNS. Eur. J. Pharmacol. *72:* 411–412 (1981).
32 Stone, T.W.; Burton, N.R.: NMDA receptors and ligands in the vertebrate CNS. Prog. Neurobiol. *30:* 333–368 (1988).
33 Tal, J.; Price, M.T.; Olney, J.W.: Neuroactive amino acids influence gonadotrophin output by a suprapituitary mechanism in either rodents or primates. Brain Res. *273:* 179–182 (1983).
34 Urbanski, H.K.; Ojeda, S.R.: Activation of luteinizing hormone-releasing hormone release advances the onset of female puberty. Neuroendocrinology *46:* 273–276 (1987).
35 Watkins, J.C.; Evans, R.H.: Excitatory amino acid transmitters. Annu. Rev. Pharmacol. Toxicol. *21:* 165–204 (1981).
36 Watkins, J.C.; Olverman, H.J.: Agonists and antagonists for excitatory amino acid receptors. Trends Neurosci. *10:* 265–272 (1987).
37 Wilson, R.C.; Knobil, E.: Acute effects of N-methyl-*D,L*-aspartate on the release of pituitary gonadotropins and prolactin in the adult female rhesus monkey. Brain Res. *248:* 177–179 (1982).

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