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Pulsatile Release of Gonadotropin-Releasing Hormone from Hypothalamic Explants Is Restrained by Blockade of *N*-Methyl-D,L-Aspartate Receptors*

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ABSTRACT. We have shown previously that *N*-methyl-D,L-aspartate (NMDA) and kainate, two neuroexcitatory amino acids acting through distinct receptors, may induce the release of GnRH from hypothalamic explants. However, that effect could have no physiological significance, since very high concentrations (50 mM) of NMDA and kainate were required. Here, using agents blocking the activation of receptors to neuroexcitatory amino acids, we evaluated their possible physiological involvement in the pulsatile release of GnRH from the hypothalamus of 50-day-old male rats *in vitro*. In control conditions (10 nM glycine and 1 mM Mg^{2+}), the release of GnRH in 7.5-min fractions collected for 2–4 h showed an obvious pulsatile pattern. The mean (±1 SD) interval between pulses, identified by PULSAR program, was 34.3 ± 11.4 min. The stimulation of GnRH release by NMDA (50 mM) added to the medium for 7.5 min could be blocked reversibly in the presence of MK-801 (100 μM) using medium without glycine or enriched with Mg^{2+} (2 mM). The endogenous pulses of GnRH secretion were abolished in the presence of MK-801 or using increased Mg^{2+} concentrations as well as in the absence of glycine. In contrast, pulsatile release of GnRH was not affected in the presence of 6,7-dinitroquinoxaline-2,3-dione (0.1 mM), a selective inhibitor of kainate and quisqualate receptors which suppressed the increase in GnRH release induced by kainate (50 mM) without affecting the response to NMDA. These data indicate that the physiological mechanism of pulsatile GnRH secretion in the hypothalamus may involve endogenous neuroexcitatory factors acting through NMDA-sensitive receptors. (*Endocrinology* **125:** 1090–1096, 1989)

THE AGONISTS of neuroexcitatory amino acids (NEAA), such as *N*-methyl-D,L-aspartate (NMDA) and kainate, activate hypothalamic receptors possibly involved in the control of anterior pituitary hormone secretion, including LH (1–5). Chronic intermittent administration of NMDA mimics the effects of pulsatile GnRH secretion on LH and induces sexual maturation (6, 7). While those observations could result from pharmacological effects, the suppression of pulsatile LH secretion *in vivo* by a specific antagonist of NMDA receptors (8) has provided indirect evidence of a possible physiological role for those receptors.

Recently, we showed that the release of GnRH from hypothalamic explants *in vitro* could be directly stimulated in the presence of kainate and NMDA, although very high concentrations were required (9). Since the release of GnRH *in vitro* had been shown to be pulsatile in our experimental conditions (10, 11), the present study was designed to examine directly if endogenous neuroregulatory substrates acting through NMDA or kainate receptors might control pulsatile GnRH secretion. Therefore, antagonists of NMDA- and kainate-sensitive receptors were used to block a putative physiological mechanism involving those receptors.

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Materials and Methods

Animals and incubation procedure

The retrochiasmatic hypothalamus of 50-day-old male Wistar rats (Janssens, Beerse, Belgium) was dissected and studied individually *in vitro*, as previously described (9–11). For each experiment, 12 static incubation chambers were used, each containing 1 hypothalamic explant in 0.5 ml culture medium, which was renewed every 7.5 min for 2.5–4 h.

While our previous experiments (9) were carried out using Dulbecco's Modified Eagle's Medium, the present data were obtained using Eagle's Minimum Essential Medium modified with Earle's salts (Flow, MacLean, VA), which is available without glycine, aspartate, and glutamate and contains 1.8 mM Ca^{2+}. In control conditions, Minimum Essential Medium was enriched with glycine, magnesium, and glucose to achieve final concentrations of 10 nM, 1 mM, and 25 mM, respectively. Also,

bacitracine 20 μM (Sigma, St. Louis, MO) was added to prevent degradation of GnRH by hypothalamic peptidases.

RIA of GnRH and pulse analysis

Using the highly specific RR-5 anti-GnRH antiserum generously supplied by Dr. A. Root (St. Petersburg, FL), GnRH was measured in duplicate on 0.1-ml samples from the 0.5-ml fractions collected every 7.5 min, as described previously (10, 11). The intra- and interassay coefficients of variations of both the explant incubation and the RIA procedure were 14% and 18%, respectively. The sensitivity of the assay varied between 0.5 and 1 pg/tube, so that the limit of detection was 5 pg/7.5-min fraction, which was the value assigned to data below that limit. The standard curves for the RIA of GnRH were prepared in the medium used for incubation of the hypothalamic explants, and no interaction was found between the test substances used and the binding of radiolabeled GnRH to the antiserum. The pH was controlled using an indicator (phenol red) in the medium and brought to neutrality (7.4) when necessary.

The occurrence of significant pulses of GnRH release was estimated using an IBM-PC compatible version (12) of the Pulsar program (13). The 14% coefficient of variation described above was used as the B coefficient to determine the assay SD. The cutoff criteria (G) for peak identification were calculated empirically. G_1 was 2.5, and G_2 2.0. The vast majority of pulses observed were single data point peaks. Pulse amplitude was calculated by the difference between the peak value and the smoothed baseline value determined at the same time by the computer program. Pulse frequency was calculated using the intervals between pulses observed within the study period of each single hypothalamus. To estimate pulse frequency when no pulse or only one pulse was released from an explant, the interval was considered to be at least equal to the total length or half the length of the period studied, respectively.

Test substances

Two different agonists of NEAA, NMDA and kainate (Sigma), were used at a 50-mM concentration because it consistently resulted in a submaximal release of GnRH which was less likely to be neurotoxic than at the plateau of the response (9). MK-801, a noncompetitive inhibitor of NMDA receptors (14), was generously supplied by Merck, Sharp, and Dohme Research Laboratories (Rahway, NJ) and used at concentrations of 0.01 and 100 μM. Increased Mg^{2+} concentrations (2 mM) were also used as a noncompetitive inhibition to NMDA receptors (15, 16). Glycine-free medium was used to suppress the facilitatory role played by glycine at the allosteric site of the NMDA receptor (17). DNQX (Tocris Neuramin, Buckhurst Hill, United Kingdom), a selective antagonist of kainate and quisqualate receptors (18), was used at a 0.1-mM concentration. Veratridine (50 μM; Sigma), a depolarizing agent, was used to evaluate the GnRH release induced by depolarization (10, 11).

Study protocols

Using six individual hypothalami, veratridine-induced GnRH release was evaluated before and after exposure to NMDA. The agonist was used twice at the same concentration of 50 mM or at increasing concentrations (50 and 100 mM).

In a second set of experiments, GnRH release from 12 individual hypothalami was studied before and after incubation for 7.5 min in the presence of kainate or NMDA (50 mM). This study was repeated using medium enriched with MK-801 (0.01 or 100 μM), Mg^{2+} (2 mM), 6,7-dinitro-quinoxaline-2,3-dione (DNQX; 0.1 mM), or glycine-free culture medium. The hypothalami were exposed to those modified culture medium for two consecutive 7.5-min periods, immediately before and during stimulation by NMDA or kainate. After using MK-801 or Mg^{2+} as antagonists, the effect of NMDA on GnRH secretion was reevaluated in control conditions to study the reversibility of the antagonistic effects. The GnRH release induced by veratridine was also studied before, during, and after exposure to MK-801 or increased Mg^{2+} concentrations in order to rule out a nonspecific effect of those antagonists on tissue viability.

In a third set of experiments, the spontaneous pattern of GnRH secretion was studied for 2.5–4 h in the absence of NMDA or kainate. During each experiment, 6 hypothalami were incubated in the control conditions described above, while 6 hypothalami were incubated in the presence of MK-801 (0.01 or 100 μM), Mg^{2+} (2 mM), or DNQX (0.1 mM) or using glycine-free medium. Since these experiments were performed twice, a total of 60 explants were studied in control conditions, while 12 explants were studied using each of the antagonists of NEAA receptors. After having discarded the data obtained using explants that happened to be fragmented or accidentally shaken before termination of the experiment, a total of 42 control explants were studied, while 9–10 explants were studied in each of the antagonist conditions.

Statistical analysis

Paired Student's *t* test was used to calculate the significance of differences in basal and NMDA- or veratridine-induced GnRH release, evaluated twice contiguously within an *in vitro* experiment. The significance of differences in distribution of GnRH interpulse intervals and differences in pulse amplitude between the antagonist conditions were calculated, respectively, using Kolmogorov-Smirnov's test and Scheffé's test for multiple comparison with analysis of variance (19).

Results

As shown in Fig. 1 (*left panel*), veratridine induced an increment in GnRH secretion. This increment (mean ± 1 SD) was, respectively, 18.8 ± 8.7 and 21.1 ± 12.1 pg before and after repeated exposure to 50 mM NMDA, which elicited two pulses of GnRH release. In contrast, after a challenge using 100 mM NMDA, there was a significant reduction (4.7 ± 3.2 *vs.* 16.2 ± 8.4 pg; $P < 0.01$) of the responsiveness to veratridine (Fig. 1, *right panel*).

As shown in Fig. 2 both kainate and NMDA (50 mM) elicited a significant increase in GnRH secretion *in vitro*, confirming our previous observations (9). At a 0.01-μM concentration, MK-801 prevented the stimulatory effect

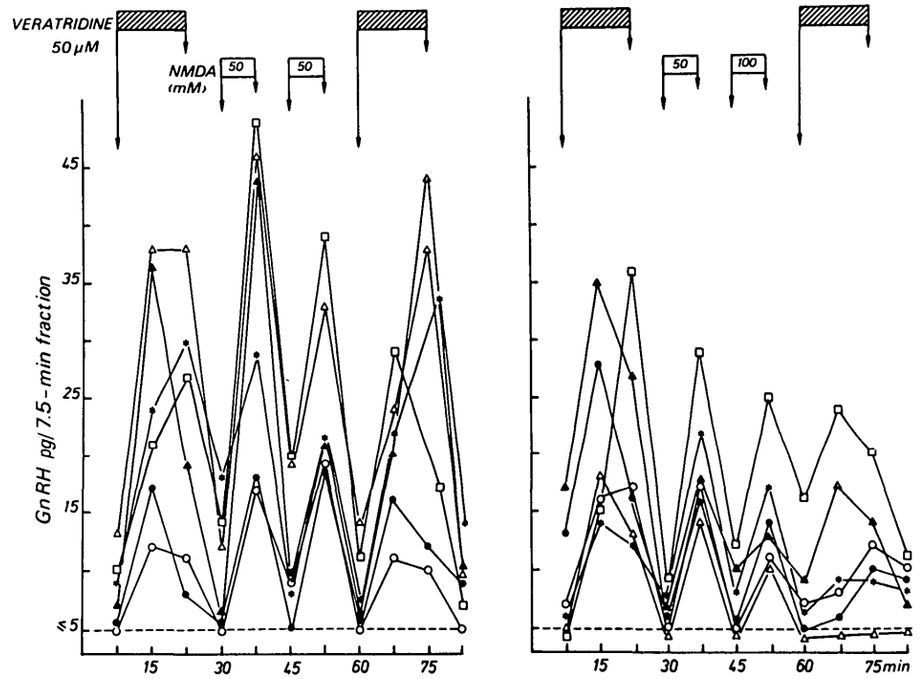

FIG. 1. Individual profiles of GnRH release from six hypothalamic explants challenged using veratridine before and after exposure to NMDA, twice at the same concentrations (*left panel*) or at increasing concentrations (*right panel*). The *dashed lines* indicate the detection limit of the assay.

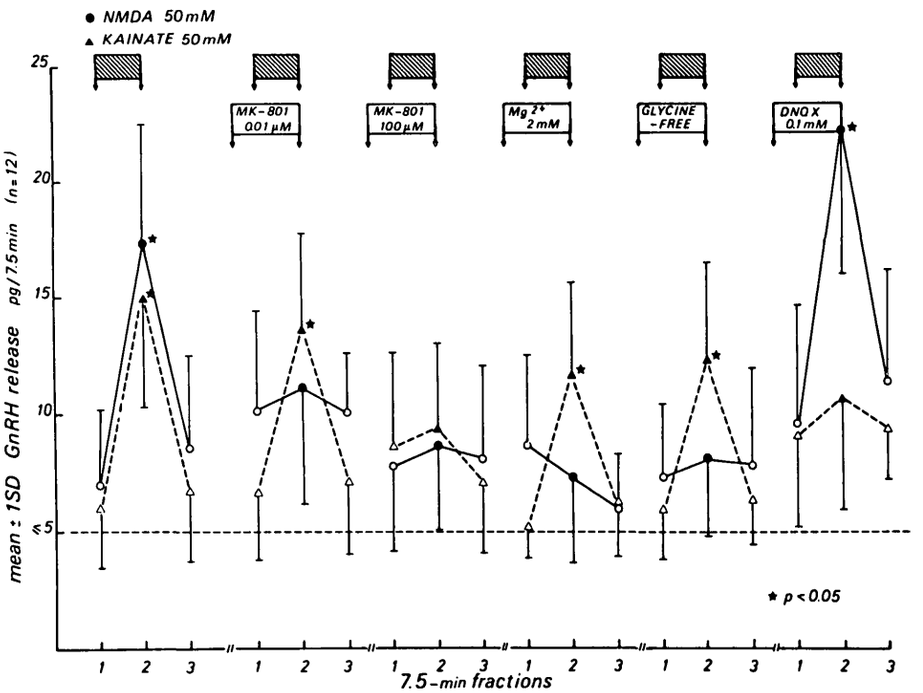

FIG. 2. Release of GnRH (mean ± 1 SD) from 12 hypothalamic explants *in vitro* induced by a 7.5-min exposure to 50 mM NMDA (●) or kainate (▲) in control conditions (*left panel*) or in the presence of inhibitors of the receptors to those NEAA. The *dashed line* indicates the detection limit of the assay.

of NMDA, while the response to kainate was still present. At a higher concentration (100 μM), MK-801 blocked the effects of both NMDA and kainate. Increased concentrations of Mg^{2+} (2 mM) or glycine-free medium resulted in a selective suppression of the response to NMDA without affecting the response to kainate. On the contrary, the increase in GnRH release induced by kainate was no longer observed in the presence of 0.1 mM DNQX, which did not affect the response to NMDA.

As shown in Table 1, the increment in GnRH release induced by 50 mM NMDA as well as that induced by 50 μM veratridine were significantly reduced in the presence of 2 mM Mg^{2+} or 100 μM MK-801. When retested under control conditions, the response to NMDA or veratridine was not significantly different from pretreatment values.

Under control conditions, GnRH release from hypo-

TABLE 1. Reversibility of the inhibitory effects of antagonists of NMDA receptors on NMDA- and veratridine-induced release of GnRH from hypothalamic explants *in vitro* (n = 12)

	Increment in GnRH release (pg/7.5 min) induced by	
	NMDA (50 mM)	Veratridine (50 μM)
Before exposure to Mg^{2+} (2 mM)	15.3 ± 4.4	12.4 ± 2.8
In the presence of Mg^{2+} (2 mM)	0.6 ± 0.8[a]	8.3 ± 4.2[b]
In control conditions after Mg^{2+} (2 mM)	13.8 ± 4.8[c]	13.2 ± 5.5[c]
Before exposure to MK-801 (100 μM)	12.8 ± 4.3	14.9 ± 4.1
In the presence of MK-801 (100 μM)	1.3 ± 1.5[a]	3.8 ± 3.2[a]
In control conditions after MK-801 (100 μM)	13.3 ± 6.3[c]	17.8 ± 6.0[c]

Values are the mean ± 1 SD.
[a] $P < 0.001$ *vs.* pretreatment value.
[b] $P < 0.005$ *vs.* pretreatment value.
[c] P = NS *vs.* pretreatment value.

thalamic explants was pulsatile (Figs. 3 and 4, *upper panels*). The mean ± 1 SD interval between GnRH pulses was 34.3 ± 11.4 min (n = 164), and the mean amplitude of GnRH pulses was 14.0 ± 5.8 pg (n = 236). Similar profiles of GnRH secretion were observed regardless of the basal secretory level of GnRH; the trough values were below the limit of detection (5 pg/7.5 min) in some explants or above 10 pg/7.5 min in others.

In the presence of 0.01 μM MK-801 (Fig. 3, *middle panel*), the frequency of GnRH secretory pulses (mean interval ± 1 SD, 49.4 ± 27.6 min; n = 17) was significantly lower ($P < 0.01$) than that under control conditions. There was an important variability in the frequency of GnRH pulses, ranging between one and six pulses during a 3-h period. In the presence of 100 μM MK-801 (Fig. 3, *lower panel*), pulsatile GnRH release was almost completely abolished. Increased concentrations of Mg^{2+} or the absence of glycine in culture medium resulted in a suppression of the pulsatile pattern of GnRH release (Fig. 4). In the presence of 0.1 mM DNQX (Fig. 4, *lower panel*), the interval between GnRH pulses was 39.3 ± 9.9 min, not significantly different from the control value. It can also be seen in Figs. 3 and 4 that the basal secretory level of GnRH was different from one explant to the other under control conditions as well as in the presence of antagonists of NMDA receptors.

As shown in Fig. 5, the frequency distribution of GnRH interpulse intervals was similar in control conditions and in the presence of DNQX, which antagonized kainate and quisqualate receptors. A significantly greater interval between GnRH pulses was observed in all the conditions antagonizing NMDA receptors. There was no significant difference in GnRH pulse amplitude between the different conditions studied.

Discussion

Our observations provide a direct demonstration that the hypothalamic mechanism of pulsatile GnRH secretion may physiologically involve activation of the receptors to NMDA. The physiological role of receptors to NEAA in the control of LH secretion in the rat was

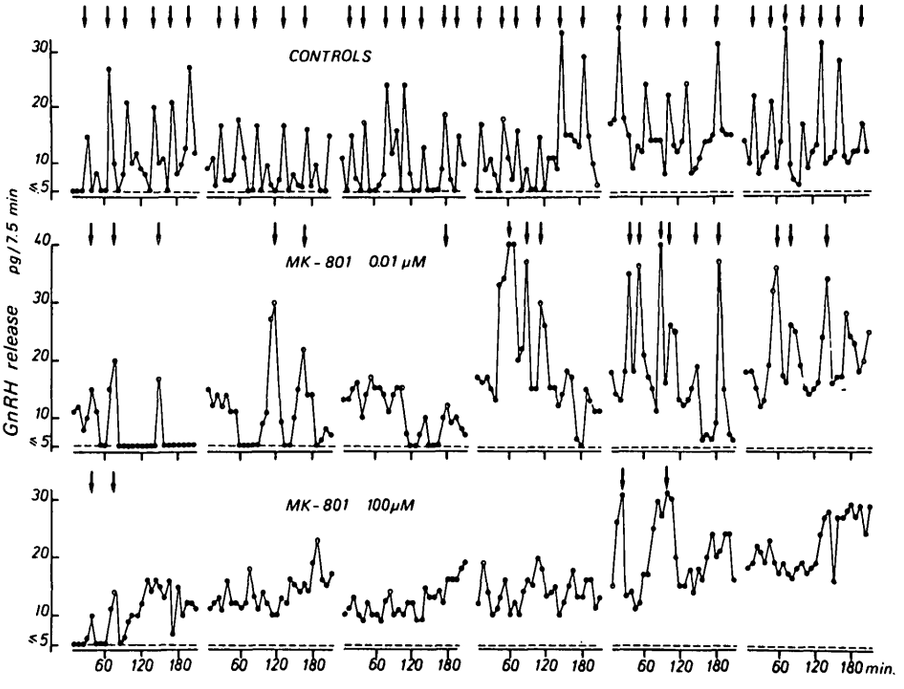

FIG. 3. Release of GnRH in 7.5-min fractions collected for 3.5 h during *in vitro* incubation of individual hypothalamic explants in control conditions or in the presence of MK-801 at two different concentrations. The *arrows* denote significant GnRH secretory pulses. The *dashed lines* indicate the detection limit of the assay.

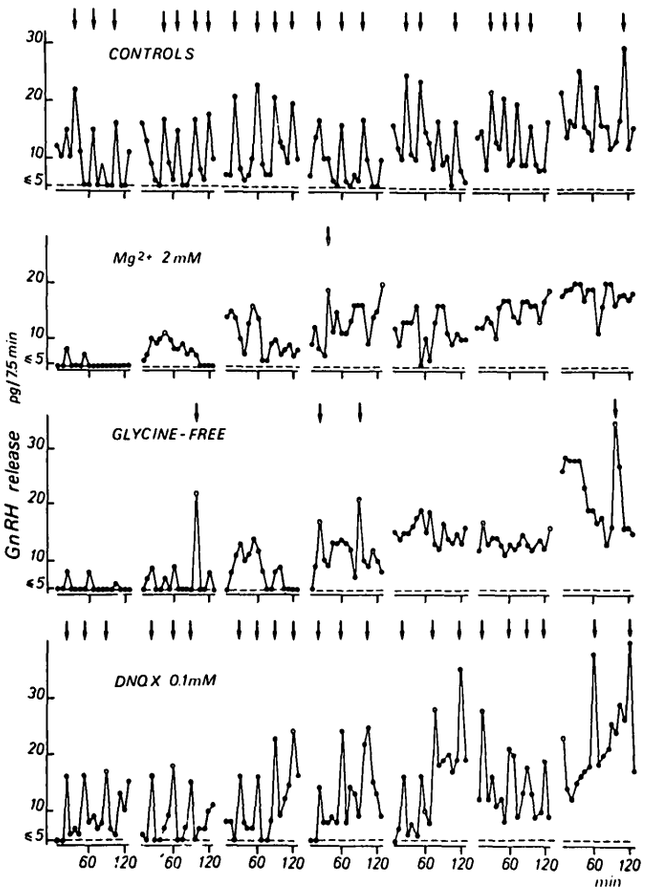

FIG. 4. Release of GnRH in 7.5-min fractions collected for 2.1 h during *in vitro* incubation of individual hypothalamic explants in control conditions, in the presence of DNQX or increased Mg^{2+} concentrations, or in the absence of glycine. The *arrows* denote significant GnRH secretory pulses. The *dashed lines* indicate the detection limit of the assay.

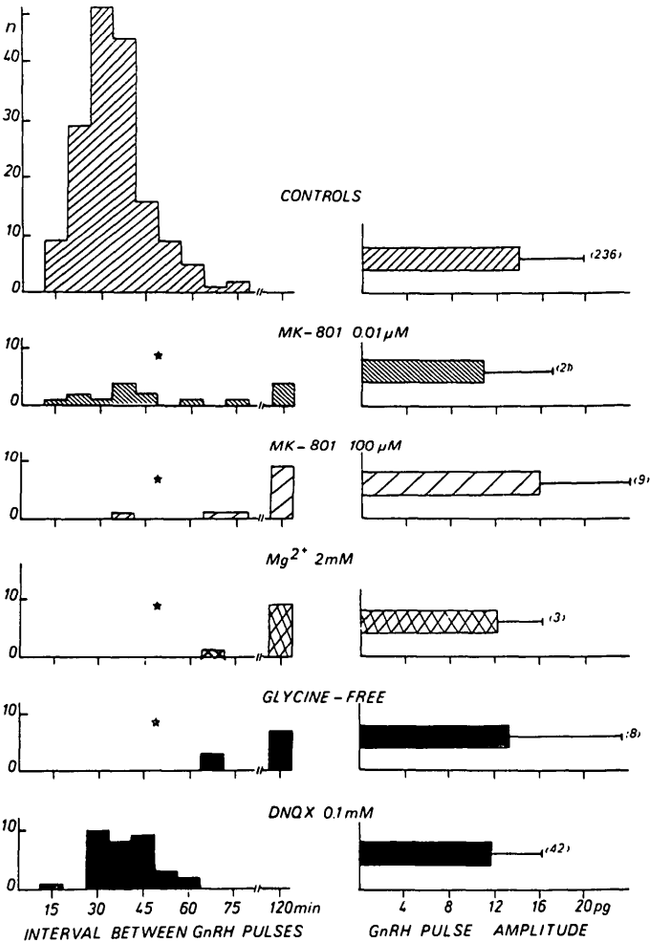

FIG. 5. Frequency distribution of the intervals between GnRH pulses (*left panel*) and mean ± 1 SD amplitude of GnRH pulses (*right panel*) in control conditions or in different conditions blocking the activation of receptors to NEAA. The number of pulses used to calculate the mean amplitude is given in *parentheses*. The *stars* denote significant differences ($P < 0.05$) *vs.* controls.

demonstrated recently by the suppression of LH pulsatile release *in vivo* using AP5, a selective competitive antagonist of NMDA receptors (8). This study took advantage of the finding that retrochiasmatic hypothalami exhibit pulsatile GnRH *in vitro* (10, 11). This enabled us to directly evaluate the role of NEAA in GnRH release. Since we showed previously that exogenous agonists such as kainate and NMDA stimulated GnRH release (9), emphasis was put on the effects of antagonists on the endogenous mechanism controlling GnRH secretion.

The question of whether antagonists of NEAA would affect GnRH secretion was particularly important, because extremely high concentrations of kainate or NMDA were required to elicit an increase in GnRH secretion *in vitro* (9). A tentative explanation for the very high concentrations of agonists required was the age-related variations in the sensitivity of LH increase in response to NMDA, which was shown to increase at 20 days of age and to decrease dramatically between 35 and 60 days of age (20). The concentration of agonist was a matter of great concern, because kainate and NMDA were shown to induce neuronal lesions at concentrations much lower than those used in our experiments (21). Since we found previously that the response to 50 mM NMDA could be obtained repetitively in our experimental conditions (9), we assumed that no neurotoxic effect occurred at the submaximal concentration. As shown in this paper, the unaltered capacity of veratridine to elicit GnRH release after exposure to 50 mM NMDA provides further support for that hypothesis. However, a 2-fold higher NMDA concentration (100 mM) resulted in a reduction of the subsequent response to veratridine. This indicated that a 100-mM concentration of NMDA could have neurotoxic effects under our conditions. In accordance with studies on LH *in vivo* (1), we found only a slight difference between neuroexcitatory

and neurotoxic concentrations of NEAA *in vitro*.

According to our previous observations using agonists of NEAA (9), both kainate and NMDA receptors could be involved in the control of GnRH release. Using selective antagonists, such as DNQX, for kainate receptors (18), it has been shown in this paper that NMDA receptors may play a physiological role, while kainate receptors have no obvious importance to the physiological mechanism controlling GnRH secretion. The data obtained using MK-801, a noncompetitive antagonist of NMDA receptors (14), were somewhat conflicting, since a 0.01-μM concentration blocked the effect of 50 mM NMDA, while a 100-μM concentration was required to suppress pulsatile GnRH release. This may be explained by the use-dependent property of MK-801 (22), which means that the antagonistic activity is a direct function of the concentration of the agonist acting at the receptor level. It is conceivable that the concentrations of endogenous NEAA activating the receptors to NMDA are far below the 50-mM concentration of NMDA used in our experiments. The very high concentrations of kainate used may also account for the inhibition of kainate-induced GnRH release by high concentrations of MK-801, which is known as a specific antagonist of NMDA receptors (14). A mediation of kainate effects through the receptors sensitive to NMDA is unlikely, since kainate effects were abolished by DNQX but not by increased Mg^{2+} concentrations or the absence of glycine.

The reversibility of the blocking effect of Mg^{2+} or MK-801 on the GnRH release induced by NMDA or veratridine indicates that their antagonistic effect does not result from an impaired viability of the explant. The significant reduction of the GnRH response to veratridine by antagonists of NMDA receptors is particularly interesting because it suggests that the interaction between NEAA and the GnRH axon is located at a presynaptic site more distal than those depolarized by veratridine.

The NMDA receptors exhibit the unique feature of being regulated by Mg^{2+} and glycine concentrations. Increased Mg^{2+} concentrations block the NMDA ionic channel in a noncompetitive and voltage-sensitive manner (15, 16). While such an inhibitory effect may occur at micromolar concentrations of Mg^{2+} in some experimental conditions (23), pulsatile GnRH release *in vitro* could be observed in the presence of a physiological concentration of 1 mM Mg^{2+}. However, a 2-fold increase in Mg^{2+} concentration was sufficient to obtain a marked inhibition of pulsatile GnRH secretion. It cannot be excluded that variations in intrahypothalamic Mg^{2+} concentrations participate in the regulation of GnRH secretion.

It was shown recently that glycine, at concentrations as low as 10 nM, could potentiate the response to NMDA in a voltage-independent manner through an allosteric site at the NMDA receptor level (17). We found previously that the GnRH release elicited by NMDA was maximal in the presence of 10 nM glycine and less using higher glycine concentrations (9). A possible influence of glycine on the GnRH pulse-generating system in the hypothalamus was also suggested by the regular occurrence of GnRH secretory pulses shown here in the presence of 10 nM glycine, while using medium containing 0.4 mM glycine, a more variable pattern of pulsatile GnRH release was observed in our previous studies (10, 11). When the facilitatory role of glycine at the NMDA receptor level was abolished by using glycine-free medium, pulsatile GnRH release was inhibited. This might suggest that glycine plays a regulatory role on the mechanism of pulsatile GnRH release. Also, our data emphasize the importance of the nature of culture medium for studying pulsatile GnRH release *in vitro*.

Recently, Cicero *et al.* (20) showed that the sensitivity of LH to NMDA administration *in vivo* increased markedly between 15 and 25 days of age in the male rat. Interestingly, this period is coincident with an increase in the frequency of pulsatile GnRH release *in vitro* (10). The possible role of NMDA receptors in the central mechanism driving sexual development is also suggested by the induction of puberty during chronic intermittent administration of NMDA to prepubertal female rats (7) and male monkeys (6). Our experimental model provides a direct means of studying the involvement of NMDA receptors in the developmental changes in pulsatile GnRH secretion.

This study provides additional evidence that the hypothalamus, deafferented from the preoptic area and the rest of the brain, is capable of generating GnRH secretory pulses. Since most cell bodies of GnRH neurons are located in the prechiasmatic region (24), our observations support the concept that the GnRH pulse generator would be 1) one or several neuronal systems distinct from the GnRH neuron itself, 2) located in the retrochiasmatic hypothalamus, 3) capable of autonomous activity independent of extrahypothalamic structures, and 4) capable of modulating presynaptically the secretory activity of the GnRH axon through receptors sensitive to NMDA.

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References

1. Price MT, Olney JW, Cicero TJ 1978 Acute elevations of serum luteinizing hormone induced by kainic acid, *N*-methyl aspartic acid

or homocysteic acid. Neuroendocrinology 26:352
2. Schainker BA, Cicero TJ 1980 Acute central stimulation of Luteinizing hormone by parenterally administered *N*-methyl-D,L-aspartic acid in the male rat. Brain Res 184:425
3. Wilson RC, Knobil E 1982 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
4. Nemeroff CB, Mason GA, Bissette G, Parks DA, Schwarcz R 1985 Effects of intrahypothalamic injection of quinolinic acid on anterior pituitary hormone secretion in the anasthetized rat. Neuroendocrinology 41:332
5. Gay VL, Plant TM 1987 *N*-Methyl-D,L-aspartate elicits hypothalamic gonadotropin-releasing hormone release in prepubertal male rhesus monkeys (*Macaca mulatta*). Endocrinology 120:2289
6. Plant TM, Gay VL, Marshall GR, Arslan M, Chronic intermittent chemical excitation of the prepubertal monkey with *N*-methyl-D,L-aspartate prematurely reactivates the pituitary-Leydig cell axis. 69th Annual Meeting of The Endocrine Society, Indianapolis IN, 1987 (abstract 65)
7. Urbanski HK, Ojeda SR 1987 Activation of luteinizing hormone-releasing hormone release advances the onset of female puberty. Neuroendocrinology 46:273
8. Arslan M, Pohl CR, Plant TM 1988 DL-2-Amino-5-phosphonopentanoic acid, a specific *N*-methyl-D-aspartic acid receptor antagonist, suppresses pulsatile LH release in the rat. Neuroendocrinology 47:465
9. Bourguignon JP, Gerard A, Franchimont P 1989 Direct activation of GnRH secretion through different receptors to neuroexcitatory amino acids. Neuroendocrinology 49:402
10. Bourguignon JP, Franchimont P 1984 Puberty-related increase in episodic LHRH release from rat hypothalamus *in vitro*. Endocrinology 114:1943
11. Bourguignon JP, Gerard A, Debougnoux G, Rose J, Franchimont P 1987 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
12. Gitzen JF, Ramirez VD 1987 PC Pulsar-Pulsar pulse analysis for the IBM-PC, Psychoneuroendocrinology 12:3
13. Merriam GR, Wachter KW 1982 Algorithms for the study of episodic hormone secretion. Am J Physiol 249:E310
14. Wong EF, Kemp JA, Priestley T, Knight AR, Woodruff GN, Iversen LL 1986 The anticonvulsant MK-801 is a potent *N*-methyl-D-aspartate antagonist. Proc Natl Acad Sci USA 83:7104
15. Mayer M, Westbrook GL, Guthrie PB 1984 Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. Nature 309:261
16. Nowak L, Bregestovski P, Ascher P, Herbert A, Prochiantz A 1984 Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462
17. Johnson JW, Ascher P 1987 Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325:529
18. Honore T, Davies SN, Drejer J, Fletcher EJ, Jacobsen P, Lodge D, Nielsen FE 1988 Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. Science 241:701
19. Zar JH 1984 Biostatistical Analysis, ed 2. Prentice-Hall, Englewood-Cliffs
20. Cicero TJ, Meyer ER, Bell RD 1988 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
21. Rothman SM, Olney JW 1987 Excitotoxicity and the NMDA receptor. Trends Neurosci 10:299
22. Honey CR, Miljkovic Z, Mac Donald JF 1985 Ketamine and phencyclidine cause a voltage dependent block of responses to L-aspartic acid. Neurosci Lett 61:135
23. Ascher P, Nowak L 1988 The role of divalent cations in the *N*-methyl-D-aspartate responses of mouse central neurones in culture. J Physiol 399:247
24. Barry J, Hoffman GE, Wray S 1985 LHRH-containing systems. In: Bjorklund A, Hokfelt T (eds) Handbook of Chemical Neuroanatomy, Vol 4, part I. Elsevier, Amsterdam, p 166