The Anterior Pituitary Gland as a Possible Site of Action of Kainic Acid (43783)

MARIAROSA ZANISI,[1] MARIARITA GALBIATI, ELIO MESSI, AND LUCIANO MARTINI
Institute of Endocrinology, University of Milano, 20133 Milano, Italy

Abstract. **The purpose of the present study was to analyze the direct effect of kainic acid (KA), an agonist of L-Glutamate, on the secretion of LH and FSH from anterior pituitary (AP) of male rats perifused *in vitro*. At low concentrations (1 μM), KA was able to stimulate the release of both gonadotropins from AP of 50-day-old male rats, but the response to subsequent stimuli was markedly impaired. This, however, was not due to a neurotoxic action of KA, but seemed rather suggestive of a down-regulation or desensitization of KA receptors.**

The stimulatory action of KA on LH and FSH secretion was age-dependent, since the agonist was completely ineffective on the AP of 75-day- and 18-month-old male rats. DNQX (6,7-dinitroquinoxaline-2,3-dione), a specific antagonist of the KA receptor subtype, was able to block the KA-induced gonadotropin secretion; similarly, AP-5 (2-amino-5-phosphonovalerate), a competitive NMDA receptor antagonist, prevented the stimulatory effect of KA on LH and FSH release. An interaction between the opiatergic and the excitatory aminoacid (EAA) systems emerged from the observation that pulses of KA applied to AP of 50-day-old male rats during a continuous perifusion with a medium containing morphine (5 μM) failed to increase gonadotropin secretion.

These results indicate that KA can, at low concentrations, directly stimulate LH and FSH secretion by acting at AP level; this effect disappears with progression of age, and might be exerted both through NMDA and non-NMDA receptor subtypes. Finally, the results provide evidence that opioids and excitatory aminoacids might influence gonadotropin secretion from AP by acting in opposite directions.

[P.S.E.B.M. 1994, Vol 206]

Although the luteinizing hormone-releasing hormone (LHRH) is the key regulator of gonadotropin secretion, a variety of other agents can influence the release of luteinizing hormone (LH) and/or follicle-stimulating hormone (FSH) by acting directly on the anterior pituitary gland (AP). The effect of these agents is directed either to the control of the synthesis and/or the release of the gonadotropins, or to the induction of modifications in the sensitivity of the anterior pituitary to LHRH. *In vitro* studies have shown that neuropeptide Y (1, 2), oxytocin and vasopressin (3), angiotensin II (4), thymosin α-1 (5), interleukin-6 (6), endothelin-1 (7), etc., stimulate LH release by a direct effect on the AP, and that their activity is steroid-dependent (6). In addition, classical neurotransmitters are known to act as gonadotropin secretagogues at the AP level. In fact, β-receptors have been identified in the gland, and their number has been shown to fluctuate following alterations in the levels of gonadal steroids both in male and female rats (8, 9).

Recently, a lot of interest has been devoted to the possible facilitatory effect of excitatory aminoacids (EAAs) on the function of the hypothalamic-pituitary-gonadal axis (10–12). A variety of data suggests that activation of EAA pathways might be important in the control of gonadal function and may play a role in the neural regulation of sexual maturation both in the rat and in the monkey (13, 14). Based on these observations, it has been assumed that EAAs might represent an additional class of neuromodulators controlling LH secretion. EAAs exert their effect by interacting with

[1] To whom requests for reprints should be addressed at Institute of Endocrinology, Via G. Balzaretti 9, 20133 Milano, Italy.

Received December 2, 1993. [P.S.E.B.M. 1994, Vol 206]
Accepted April 14, 1994.

0037-9727/94/2064–0431$10.50/0

a variety of receptors, pertaining both to the ionotropic and to the metabotropic type. These have been characterized by their selective interactions with different synthetic agonists and antagonists. *In vitro* experiments have shown that NMDA (N-methyl-D-aspartate), a classical EAA agonist, does not seem to affect the release of LH by acting at AP level; moreover, NMDA-induced gonadotropin release *in vivo* is abolished by pretreatment with an LHRH antagonist; these data suggest that the action of NMDA, and possibly of other EAA agonists, might be exerted at hypothalamic level, via the stimulation of LHRH release (10, 15–19). In *in vitro* perifusion studies, we have recently shown that NMDA is able to induce LHRH release from the mediobasal hypothalamus (MBH) of young male rats, at least at rather elevated doses, while kainate (KA), another EAA agonist, appears to be ineffective in modifying LHRH release in the same experimental conditions. However, KA stimulates LH and FSH release directly from the perifused AP, an action which is markedly reduced by castration (20).

KA binds to a specific receptor type (KA-receptor), that recognizes also quisqualate and L-Glutamate (Glu), and whose activation induces Na^+ and K^+ permeability at the membrane level; KA actions are antagonized specifically by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6,7-dinitro-quinoxaline-2,3-dione (DNQX). The aim of this work was to analyze the ability of KA to release LH and FSH directly from the AP, using tissue obtained from normal 50-day-old male rats, perifused *in vitro*, and to evaluate the receptor subtype involved in this effect (by using DNQX as antagonist). In addition, in view of the observation that the effects of EAAs are particularly evident around the time of puberty and that Glu binding sites and Glu synthesis seem to decrease in the brain of aging rats and monkeys (21), we have also studied the AP responses to KA at different ages (i.e. in adult [75-day-old] and aging [18-month-old] animals). Since interactions between the EAA and the opiatergic system have been proposed (22), the modulatory action of morphine on the effect of KA on LH and FSH release was also studied.

Materials and Methods

Animals. All experiments were performed on Sprague-Dawley male rats Crl:CD BR (Charles River Italia). Animals were maintained in rooms with controlled temperature (22 ± 1°C), relative humidity (60%), and with a lighting schedule of 14 hr of light and 10 of darkness (lights-on, 06:00–20:00 hr); standard diet and water was available *ad libitum*. At sacrifice, the anterior pituitary was sectioned and halved, and each single AP was placed in a perifusion chamber.

Chemicals. Kainic acid (KA), dl-2-amino-5-phosphonovaleric acid (AP5) were obtained from Sigma Chemical Co. (St. Louis, MO); 6,7-dinitro quinoxaline-2,3-dione (DNQX) has been obtained from RBI (Natick, MA). Morphine chloridrate was obtained from C. Erba (Milan, Italy). Merck compounds (Merck, Darmstadt, Germany) were used for preparation of perifusion medium, in which test substances were directly dissolved (pH 7.4).

***In Vitro* Perifusion System.** The perifusion system consisted of four independent chambers (made from a 3-ml glass syringe), which could be used simultaneously. In each chamber, the tissue was held between two nylon nets (130 μ pores). Perifusion medium was delivered to each chamber by means of a peristaltic pump (Microperpex; LKB, Bromma, Sweden) at a flow rate of 6 ml/h. The perifusion medium or the test substances were delivered to the chambers through a system consisting of two 4-way valves, one fastened under the chamber and the other placed outside the water bath. This device allowed the filling up of the loading circuit through which it was possible to inject the test substances without interrupting the flow of the medium. The chambers and the medium reservoir were submerged in a constant temperature water bath at 37°C. Both the medium delivered and that contained in the chambers were saturated with a mixture of O_2–CO_2 (95%:5%). Medium was drained out of the chambers through polyethylene tubing, collected every 10 min (1 ml), by a fraction collector (Ultrarac 7000; LKB, Bromma, Sweden), and quickly frozen and kept so until performance of LH and FSH radioimmunoassay (RIA). The perifusion system used in these experiments was adapted from that previously described (23). The perifusion medium consisted of (in millimolar concentrations): NaCl, 154; KCl, 5.6; $CaCl_2$, 0.8; $NaHCO_3$, 6; HEPES, 2; Glucose, 6; (pH 7.4).

Hormone Assays. RIA-LH was performed using an antiovine LH antiserum provided by G. D. Niswender (Colorado State University, Fort Collins, CO) and an ovine LH (LER-1056-C2) for iodination provided by L. E. Reichert (Albany Medical College, Albany, NY). Values are expressed in terms of NIH-LH-S26. RIA-FSH was performed using materials provided by the NIADDK National Hormone and Pituitary Program through Dr. A. F. Parlow. Values are expressed in terms of NIDDK-rFSH-RP-2.

Statistical Analysis. The results of the perifusion experiments were expressed as ng of LH or FSH per ml/10 min, and Δ was calculated (Δ represents the total amount of hormone released during the whole secretory response to the stimulus, minus the amount of hormone released over the same period of time under basal conditions). The data were statistically evaluated by Repeated Measures with Grouping Factors-ANOVA to compare the responses between and within the groups. Statistical tests have been calcu-

lated using the statistical package, SYSTAT (Wilkinson, Leland. SISTAT: The system for statistics, Evanston, IL: SYSTAT, Inc., 1989), on a Macintosh SE/30, Apple computer.

Experimental Protocol. *Experiment 1.* Anterior pituitary obtained from 50-day-old male rats were perifused *in vitro,* and the release of LH and FSH was measured, both in basal conditions and during perifusion with medium containing graded concentrations of kainate. After an adaptation period of 30 min, pulses of KA of 10-min duration at concentrations either 1, 10, 100 μM (low) or 1, 10, 50 mM (high) were applied to the tissue. Each pulse was separated by a 90-min interval, during which control medium was perifused. The flow rate of the perifusion was 6 ml/hr.

Experiment 2. In vitro LH and FSH release induced by kainate was evaluated as a function of age using AP obtained from 75-day- and 18-month-old male rats. Concentrations of KA ranging from 1 to 100 μM were applied as pulses of 10 min. Perifusion parameters as in Experiment 1.

Experiment 3. The effect of kainate (1–100 μM) on LH and FSH release was tested on AP obtained from 50-day-old male rats continuously perifused *in vitro* with a medium containing a specific antagonist of KA (DNQX) or the NMDA receptor antagonist AP-5 at 1 μM concentration.

Experiment 4. The possible interplay between EAA and opiatergic systems was evaluated by stimulating (with doses ranging from 1 μM to 100 μM) AP obtained from 50-day-old male rats during continuous perifusion with a medium containing morphine cloridrate at the concentration of 5 μM.

Results

Effect of KA at AP Level in Normal 50-Day-Old Male Rats. A 10-min pulse of KA 1 μM (i.e., an extremely low dose) was very active in stimulating LH release from the AP of 50-day-old male rats perifused *in vitro;* a good parallelism between LH and FSH secretion was also observed (Fig. 1). The stimulatory effect on both gonadotropins disappeared when successive pulses of KA at the dose of 10 and 100 μM were applied. When the AP was submitted to pulses of higher concentration (1 and 10 mM) the KA-induced secretion of LH and FSH were evident following the first pulse; stimulation with 10 mM concentration of KA had only a very modest action on the release of the two gonadotropins, but the tissue was still very responsive to a 10-min pulse of KA 50 mM.

Effect of KA at AP Level in 75-Day- and 18-Month-Old Male Rats. In order to evaluate whether progression of age could influence the response to KA, we tested the agonist on the AP of normal male rats of different ages; in particular, attention was focused on the action exerted by low concentrations (1–100 μM) of KA. The effect of KA on the release of LH and FSH seemed to be restricted to peripuberal age, since the AP of 75-day- and 18-month-old rats was completely unresponsive to the three doses tested (Fig. 2).

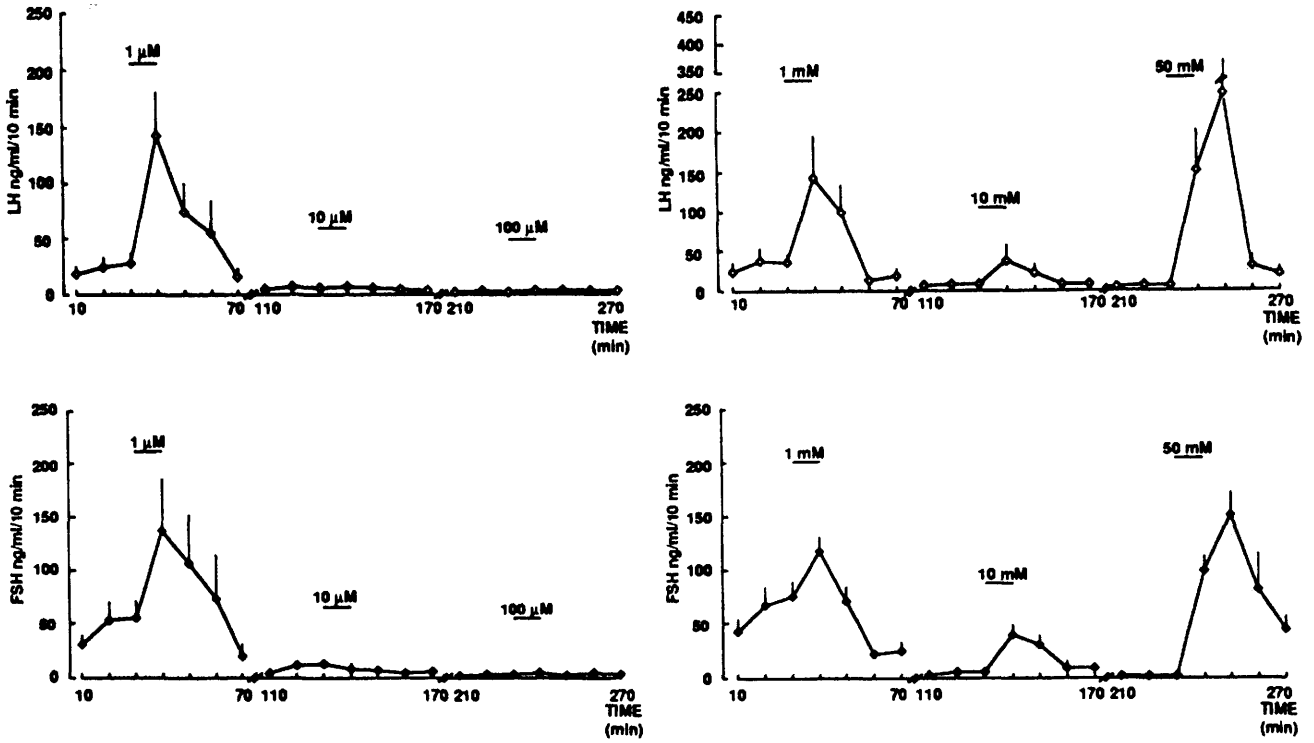

Figure 1. Effect of graded concentrations of KA on LH and FSH secretory patterns from perifused anterior pituitary of 50-day-old male rats. Pulses of 1 μM–50 mM (10 min on, 90 min off) are indicated by plain lines. Half brackets indicate SE of the mean (n = 4).

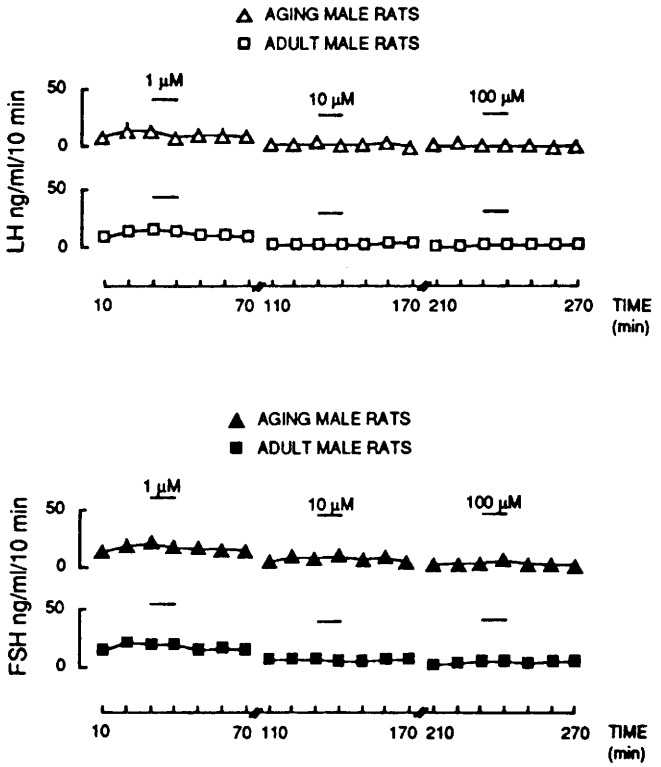

Figure 2. Effect of graded concentrations of KA on LH and FSH secretory patterns from perifused anterior pituitary of 75-day- and 18-month-old male rats. Pulses of 1–100 μ*M* (10 min on, 90 min off) are indicated by plain lines. Half brackets indicate SE of the mean (n = 4).

Effect of KA at AP Level in Normal 50-Day-Old Male Rats in the Presence of EAA Antagonists. Receptor mediation of KA-induced LH and FSH release was evaluated by employing the selective KA-receptor antagonist DNQX. In this experiment, attention was focused again on the action of the low concentrations of KA (1–100 μ*M*). Continuous perifusion of the AP of 50-day-old male rats with a medium containing DNQX (1 μ*M*) almost completely abolished the peaks of LH and FSH secretion induced by a 10-min pulse of KA (1 μ*M*). Also, a continuous perifusion with AP-5, a competitive NMDA-receptor antagonist, abolished the stimulatory effect of 1 μ*M* KA on the release of both gonadotropins (Fig. 3). These results suggest that KA might act both through a specific DNQX sensitive receptor, as well as through a NMDA receptor. In the presence of either antagonist, KA remained ineffective on AP of 75-day-old rats (data not shown).

Effect of KA at AP Level in Normal 50-Day-Old Male Rats in the Presence of Morphine. Finally, we evaluated the possible interactions between the opiatergic and the EAA systems; these interactions seem to play a relevant role particularly at the time of the onset of puberty. AP obtained from 50-day-old male rats was perifused for 5 hr with a medium containing morphine chloridrate (5 μ*M*), and exposed to 10-min pulses of graded concentrations of KA (1–100 μ*M*). In

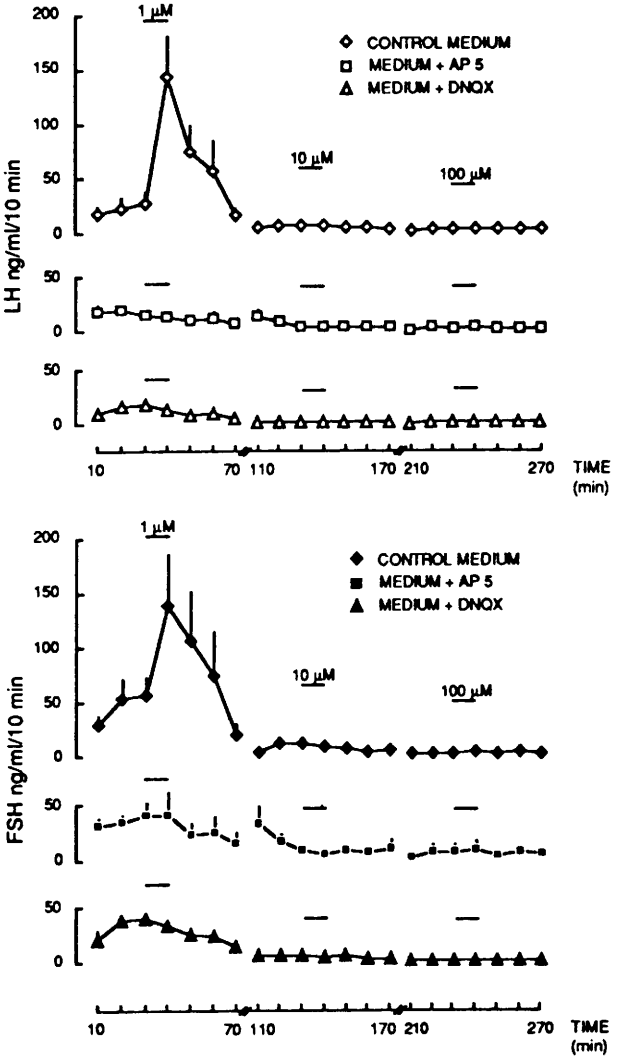

Figure 3. Effect of graded concentrations of KA on LH and FSH release from AP of 50-day-old male rats applied during continuous perifusion with DNQX or AP-5 at equimolar concentration. Pulses of 1–100 μ*M*. Half brackets indicate SE of the mean (n = 4).

the presence of morphine, which was ineffective in itself (data not shown), AP failed to respond to the 1 μ*M* concentration of KA both in terms of LH and of FSH release (Fig. 4).

Discussion

The present study provides the first evidence that KA might affect gonadotropin secretion directly at AP level. Furthermore, the study provides novel information on the types of receptors involved in the effect of KA and on its possible interactions with the opiatergic system.

The data reported here show that KA, at very low concentration (1 μ*M*), is able to stimulate the release of both LH and FSH from the AP of 50-day-old male rats perifused *in vitro*. An action of EAAs directly on AP has been shown by Login (24), who demonstrated

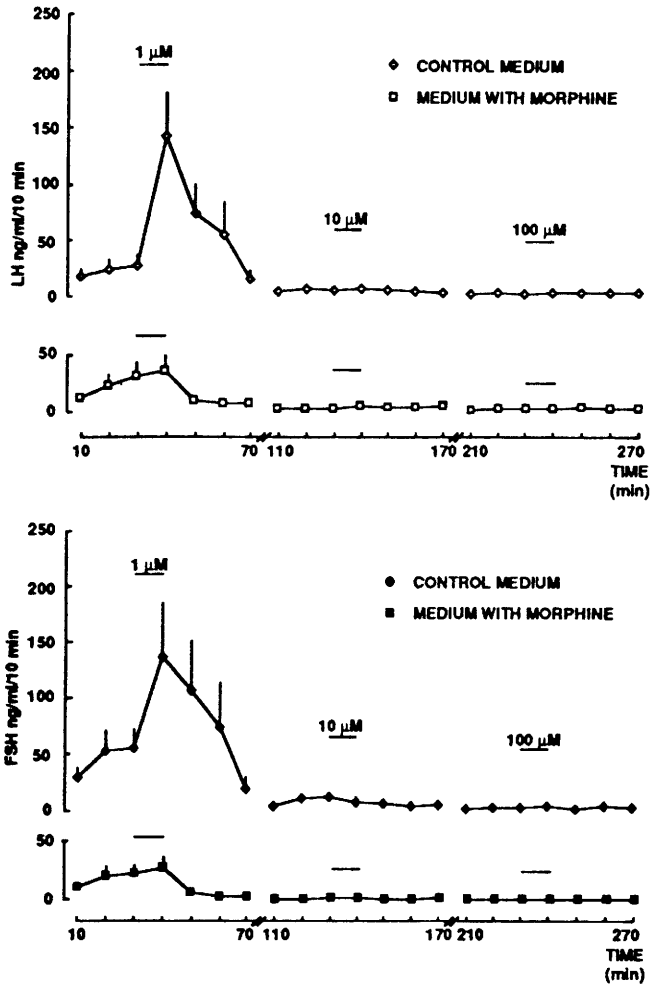

Figure 4. Effect of graded concentrations of KA on LH and FSH release from AP of 50-day-old male rats applied during continuous perifusion with morphine at the concentration of 5 μ*M*. Pulses of 1–100 μ*M*. Half brackets indicate SE of the mean (n = 4).

a stimulation of prolactin release by Glu in primary cultures of dispersed adult female rat AP cells, studied in a perifusion protocol. This action of Glu is abolished by the noncompetitive antagonist MK 801 and exposure to glutamate does not influence the subsequent responses to physiological hypothalamic secretagogues. Recently, Lindstrom and Ohlsson (25), have shown that both KA (100 μ*M*) and NMDA (1 μ*M*) can directly stimulate GH secretion from perifused pituitary somatotropes.

All these results seem to contradict most of the studies performed so far, which have indicated the hypothalamus, rather than the AP, as the site of action through which Glu and a variety of its agonists induce LH release. This conclusion has been based either on *in vivo* studies, in which the effects of EAAs were abolished by the administration of an LHRH antagonist, or on *in vitro* studies performed by means of static incubations of AP (see above). As far as KA is concerned, *in vitro* incubation studies have shown that this agonist is considerably more potent than Glu or NMDA in enhancing LHRH release from arcuate nucleus-median eminence fragments obtained from adult male rats (12, 27); however this effect was observed at a concentration (1 m*M*) higher than those effective, in the present studies, at pituitary level.

Obviously, the physiological significance of the action of KA at AP level needs to be supported by identifying KA receptors in AP tissue, which apparently has not yet been done; on the contrary, the presence of KA binding sites has been extensively confirmed by autoradiography in the CNS (28–30). However, Yoneda and Ogita (31) succeeded in solubilizing the binding activity of ^{3}H-glutamic acid from membranous homogenates of the rat AP. Recently, Kiyama and coworkers (32) have shown by immunocytochemistry and *in situ* hybridization the presence of Glu Rec 1- and Glu Rec 2/3-positive cells in the anterior lobe of the pituitary.

In our experimental conditions, the stimulatory effect exerted by KA (1 μ*M*) on LH and FSH secretion from the perifused AP disappeared when repeated stimulations either with low (10 and 100 μ*M*) or with high (1–10 m*M*) doses were applied. The lack of action observed after repeated stimulations was not due to tissue damage, since the 50-m*M* pulse applied at the end of the perifusion period could induce a sharp increase in both LH and FSH release. In previous studies, we have shown that repetitive pulses of NMDA applied to the perifused MBH of 50-day-old male rats, resulted in dose-related responses of LHRH secretion; in contrast, KA pulses of increasing concentrations resulted in a progressive decline of LHRH release (20). Similarly, Abbud and Smith (33) reported that the stimulatory effect of KA on LH secretion declines progressively *in vivo* after repetitive injections, a phenomenon which was not observed with NMDA. A decay in the response to KA and to Glu has been observed also by Medhamurthy *et al.* (34) in the prepubertal male monkey. Moreover, it has been reported that intermittent microiontophoretic applications of KA to hypothalamic neurons in anesthetized rats result in progressive decreases in single unit activity, and in an eventual loss of excitability to this agonist (35). The explanations of our result, and of the literature quoted above, may reside in a mechanism of down-regulation or desensitization of KA receptor. In the same line of thinking, Gall *et al.* (36) have shown that successive activations of the KA receptor in the CNS bring about homologous modifications of receptorial mRNA levels, suggesting that the down-regulation phenomenon might represent a means through which activity levels affect the functional properties of neuronal circuits.

The evaluation of the receptor type involved in the effect of KA on LH and FSH secretion was performed using a specific antagonist (DNQX) known to bind to

KA receptors. When pulses of KA (1 μM) were applied to AP perifused with medium containing DNQX in equimolar concentration, the stimulatory effect of KA on gonadotropin secretion was completely prevented. In the present studies, it was also shown that the effect of KA was inhibited by AP-5, a specific competitive antagonist of NMDA receptors. There are few considerations that must be made in order to explain these results. Recent electrophysiological evidence and receptor studies have shown a more complex classification of EAA receptor subtypes. Bettler and coworkers (37) have isolated the cDNAs encoding for four Glu receptor subunits. More recently, seven different subunits (Glu R1-7) of non-NMDA ionotropic receptors have been cloned (38) while NMDA receptors seem to consist of several subtypes (39). Single protein subunits are sufficient to form functional receptor ion-channel complexes that are activated by KA, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) or quisqualate but not by NMDA; this indicates that KA, AMPA, and quisqualate can activate the same ion channels. Expression studies have also demonstrated that receptor ion-channel complexes might be either homo-oligomeric or ethero-oligomeric, and made up by recombinant subunits to form channels opened by both KA and AMPA. Henley *et al.* (40) have shown, in the CNS of *Xenopus laevis,* a receptor series comprising interchangeable subunits, namely an unitary KA/AMPA-receptor, a NMDA-receptor, and a hybrid unitary KA/AMPA/NMDA-receptor. These data suggest that subunit-exchanges may account for the known multiplicity of EAA receptor subtypes, and for the possibility that the same agonist might bind to more than one subtype. The fact that the activity of KA may be inhibited both the DNQX and by AP-5 is probably explained by these considerations.

Another observation emerging from these studies is the age-related activity of KA on the AP. This effect is completely blunted in 75-day- and 18-month-old rats. This is probably not surprising, in view of the fact that EAAs seem to exert their effects in general in the pre- and peripubertal animal (both rat and monkey), and that their effects decline already in adulthood (13, 14, 16). In the case of aging animals, it has also been shown that the brain levels of EAAs are markedly decreased (41), while it is known that senescence impairs most, if not all, neuroendocrine processes (i.e., long feed-back mechanisms).

With regard to the inhibitory action of morphine on KA-stimulated LH and FSH secretion, it must be underlined that an interaction between the opiatergic and the EAA systems has been hypothesized (22), with emphasis on the onset of puberty; at this time the opiatergic tone is known to be relatively low, while the EAA action is at its zenith; these two processes, going in the opposite directions, may explain the increased frequency of LHRH pulsatility at the time of puberty. A direct action of opioids at AP level has been shown by studies *in vitro* on cultured cells (42, 43); in addition, evidence for the presence of opioid receptors in the pituitary is also available (44–46). Finally, naloxone, a specific opioid antagonist, is able to stimulate LH and FSH release directly at AP level (47). To explain the inhibitory effect morphine exerts on the gonadotropin-stimulating activity of KA one might postulate the intervention of paracrine mechanisms; it is possible for instance that the stimulatory effect of KA does not take place directly on the gonadotropes, but that it is due to release of secretatogues from other pituitary cell populations; morphine might intervene on these paracrine mechanisms.

In conclusion, we have presented evidence that KA induces LH and FSH release by acting directly at the AP level. Taken together with previous observations on the effect of other EAA agonists at hypothalamic level, the data reinforce the hypothesis that the EAA system might represent an important mechanism of regulation of gonadotropin secretion.

This work was supported by CNR funds through the project BTBS 92.01.273PF70 and 92.02.843CT04, and by MURST grants.

Thanks are due to NIADDK, and Dr. L. E. Reichert and G. D. Niswender for supplying the RIA materials for LH and FSH. The skillful technical assistance of G. Micciché is gratefully acknowledged.

1. McDonald JK, Lumpkin MD, Samson WK, McCann SM. Neuropeptide Y affects secretion of luteinizing hormone and growth hormone in ovariectomized rats. Proc Natl Acad Sci USA **82**:561–564, 1984.
2. Crowley WR, Hassid A, Kalra SP. Neuropeptide Y enhances the release of luteinizing hormone (LH) induced by LH releasing hormone. Endocrinology **120**:941–945, 1987.
3. Evans JJ, Catt KJ. Gonadotrophin-releasing activity of neurohypophysial hormones: II. The pituitary oxytocin receptor mediating gonadotrophin release differs from that of corticotrophs. J Endocrinol **122**:107–116, 1989.
4. Steele MK, Negro-Vilar A, McCann SM. Effect of angiotensin II on *in vivo* and *in vitro* release of anterior pituitary hormones in the female rat. Endocrinology **109**:893–899, 1981.
5. Milenkovic L, McCann SM. Effect of thymosin alpha-1 on pituitary hormone release. Neuroendocrinology **55**:14–19, 1992.
6. Ohmichi M, Hirota K, Koike K, Kurachi H, Ohtsuka S, Matsuzaki N, Yamaguchi M, Miyake A, Tanizawa O. Binding sites for interleukin-6 in anterior pituitary gland. Neuroendocrinology **55**:199–203, 1992.
7. Stojilkovic SS, Iida T, Cesnjaj M, Catt KJ. Differential actions of endothelin and gonadotropin-releasing hormone in pituitary gonadotrophs. Endocrinology **131**:2821–2828, 1992.
8. Petrovic SL, Bedran de Castro JC, McCann SM. β-adrenergic agonists increase the amplitude of luteinizing hormone in orchidectomized rats. Am J Physiol **251**:E316–322, 1986.
9. Petrovic SL Bedran de Castro JC, McCann SM. β-adrenergic blockers decrease levels of luteinizing hormone in plasma of orchidectomized rats. Biol Reprod **37**:539–545, 1987.
10. Donoso AO, Lopez FJ, Negro-Vilar A. Glutamate receptors of

the non-N-methyl-D-aspartic acid type mediate the increase in luteinizing hormone-releasing hormone release by excitatory amino acids *in vitro*. Endocrinology **126**:414–420, 1990.
11. Brann DW, Mahesh VB. Endogenous excitatory amino acid regulation of the progesterone-induced LH and FSH surge in estrogen-primed ovariectomized rats. Neuroendocrinology **53**:107–110, 1991.
12. Bourguignon JP, Gerard A, Alvarez-Gonzales ML, Fawe L, Franchimont P. Gonadal independent developmental changes in activation of N-methyl-D-aspartate receptors involved in gonadotropin releasing hormone secretion. Neuroendocrinology **55**:634–641, 1992.
13. Gay VL, Plant TM. N-methyl-D,L-aspartate elicits hypothalamic gonadotropin-releasing hormone release in prepubertal male rhesus monkeys. Endocrinology **120**:2289–2296, 1987.
14. Urbanski HF, Ojeda SR. Activation of luteinizing hormone-releasing hormone release advances the onset of female puberty. Neuroendocrinology **46**:273–276, 1987.
15. Schainker BA, Cicero TJ. 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.
16. Bourguignon JP, Franchimont P. Puberty related increase in episodic LHRH release from rat hypothalamus *in vitro*. Endocrinology **114**:1943–1945, 1984.
17. Olney JW, Price MT, Fuller TA, Labruyere J, Samson L, Carpenter M, Mahan K. The anti-excitotoxic effects of certain anesthetics, analgesics and sedative hypnotics. Neurosci Lett **68**:29–34, 1986.
18. Bourguignon JP, Gerard A, Franchimont P. Direct activation of gonadotrophin-releasing hormone secretion through different receptors of neuroexcitatory amino acids. Neuroendocrinology **49**:402–408, 1989.
19. Carbone S, Szwarcfarb B, Losada MEO, Moguilevsky JA. Effects of ovarian steroids on the gonadotropin response to N-Methyl-D-Aspartate and on hypothalamic excitatory amino acid levels during sexual maturation in female rats. Endocrinology **130**:1365–1370, 1992.
20. Zanisi M, Messi E. Sex steroids and the control of LHRH secretion. J Steroid Biochem Mol Biol **40**:155–163, 1991.
21. Wenk GL, Walker LC, Price DL, Cork LC. Loss of NMDA, but not GABA-A, binding in the brains of aged rats and monkeys. Neurobiol Aging **12**:93–98, 1991.
22. Cicero TJ, Meyer ER, Bel RD. 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.
23. Zanisi M, Messi E, Motta M, Martini L. Ultrashort feedback control of luteinizing hormone-releasing hormone secretion *in vitro*. Endocrinology **121**:2199–2204, 1987.
24. Login IS. Direct stimulation of pituitary prolactin release by glutamate. Life Sci **47**:2269–2275, 1990.
25. Lindstrom P, Ohlsson L. Effect of N-methyl-D,L-aspartate on isolated rat somatotrophs. Endocrinology **131**:1903–1907, 1992.
26. Stojilkovic SS, Dufau ML, Catt KJ. Receptors and secretory actions of sigma-phencyclidine agonists in anterior pituitary cells. Endocrinology **121**:2044–2054, 1987.
27. Lopez FJ, Donoso AO, Negro-Vilar A. Endogenous excitatory amino acids and glutamate receptor subtypes involved in the control of hypothalamic luteinizing hormone-releasing hormone secretion. Endocrinology **130**:1986–1992, 1992.
28. Foster AC, Mena EE, Monaghan DT, Cotman CW. Synaptic localization of kainic acid binding sites. Nature **289**:73–75, 1981.
29. Monaghan DT, Cotman CW. The distribution of (3H) kainic acid binding sites in rat CNS as determined by autoradiography. Brain Res **252**:91–100, 1982.
30. Hollmann M, O'Shea-Greenfield A, Rogers SW, Heinemann S. Cloning by functional expression of a member of the glutamate receptor family. Nature **342**:643–648, 1989.
31. Yoneda Y, Ogita K. Solubilization of stereospecific and quisqualate-sensitive activity of (H-3)glutamate binding in the pituitary of the rat. Neuropharmacology **28**:611–616, 1989.
32. Kiyama H, Sato K, Tohyama M. Characteristic localization of non-NMDA type glutamate receptor subunits in the rat pituitary gland. Mol Brain Res **19**:262–268, 1993.
33. Abbud R, Smith MS. Differences in the luteinizing hormone and prolactin responses to multiple injections of kainate, as compared to N-Methyl-D,L-Aspartate, in cycling rats. Endocrinology **129**:3254–3258, 1991.
34. Medhamurthy R, Gay VL, Plant TM. Repetitive injections of L-Glutamic acid, in contrast to those of N-Methyl-D,L-Aspartic acid, fail to elicit sustained hypothalamic GnRH release in the prepubertal male Rhesus Monkey (Macaca-Mulatta). Neuroendocrinology **55**:660–666, 1992.
35. Arnauld E, Layton BS, Padjen AL, Renaud LP. Actions of acidic amino acids on the excitability of medial hypothalamic neurons in the rat. Neuroendocrinology **37**:184–192, 1983.
36. Gall C, Sumikawa K, Lynch G. Levels of messenger RNA for a putative kainate receptor are affected by seizures. Proc Natl Acad Sci USA **87**:7643–7647, 1990.
37. Bettler B, Boulter J, Hermans-Borgmeyer I, O'Shea-Greenfield A, Deneris ES, Moll C, Borgmeyer U, Hollmann M, Heinemann S. Cloning of a novel glutamate receptor subunit, Glu R5: Expression in the nervous system during development. Neuron **5**:593–595, 1990.
38. Dingledine R. New wave of non-NMDA excitatory aminoacid receptors. Trends Pharmacol Sci **12**:360–362, 1991.
39. Moyer K, Sprenhel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakman B, Seeburg PH. Heterometric NMDA receptors: Molecular and functional distribution of subtypes. Science **256**:1217–1221, 1992.
40. Henley JM, Ambrosini A, Rodriguez-Iithurralde D, Sudan H, Brackley P, Kerry C, Mellor I, Abutidze K, Usherwood PNR, Barnard EA. Purified unitary kainate/α-amino-3-hydroxy-5-methylisooxazole-propionate (AMPA) and kainate/AMPA/N-Methyl-D-Aspartate receptors with interchangeable subunits. Proc Natl Acad Sci USA **89**:4806–4810, 1992.
41. Meites J. Changes in neuroendocrine control of anterior pituitary function during aging. Neuroendocrinology **34**:151–156, 1982.
42. Lien EL, Fenichel RL, Garsky-Sarantakis D, Grant NH. Enkephalin-stimulated prolactin release. Life Sci **19**:837–840, 1976.
43. Cacicedo L, Sanchez-Franco F. Direct action of opioid peptides and naloxone on gonadotropin secretion by cultured rat anterior pituitary cells. Life Sci **38**:617–625, 1986.
44. Simantov R, Solomon HS. Opiate receptor binding in the pituitary gland. Brain Res **124**:178–184, 1977.
45. Atweh SF, Kuhar MJ. Distribution and physiological significance of opioid receptors in the brain. Br Med Bull **39**:47–52, 1983.
46. Lightman SL, Ninkovic M, Hunt SP, Iversen LL. Evidence for opiate receptors on pituicytes. Nature **305**:235–237, 1983.
47. Chao CC, Moss GE, Malven PV. Direct opioid regulation of pituitary release of bovine luteinizing hormone. Life Sci **39**:527–534, 1986.