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Excitatory amino acids as modulators of gonadotropin secretion

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Summary. The effects of quinolinic acid (QUIN) and quisqualate (QA) on the secretion of GnRH from MBH and LH and FSH from AP of 50 day old male rats have been evaluated by means of an "in vitro" perifusion technique.

QUIN (100 μM) is able to increase GnRH secretion with an action mediated by an NMDA receptor type, as shown by the inhibitory effect exerted by both a competitive (AP-5) and a non-competitive (MK-801) specific antagonist.

QA "per se" at the concentrations tested (1–100 μM) does not modify GnRH and gonadotropin secretion, but in the presence of a specific KA/QA receptor antagonist (DNQX) exerts a stimulatory effect at both levels.

This observation might indicate that of the two QA receptor subtypes (ionotropic and metabotropic), this agonist binds to the metabotropic one with very low affinity: thus it is likely that a higher dose is required in order to have any effect on gonadotropin secretion. However, in the presence of DNQX, which binds to the ionotropic receptor, all the available QA can bind to the metabotropic one and can exert its action at MBH AP levels.

Keywords: Amino acids – EAA – GnRH – LH – FSH – Hypothalamus – Pituitary

Introduction

Hypothalamic peptidergic neurons involved in the control of anterior pituitary (AP) function are regulated by several circuits that utilize a variety of neuromodulators. Pertinent to gonadotropin secretion, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH), it has been assessed that their synthesis and secretion is mainly regulated, at AP level, by the appropiate pulsatile secretion of Gonadotropin Releasing Hormone (GnRH) produced by neurons scatterly distributed in the preoptic area (POA)-anterior hypotalamic region.

Most of this neurons project their axons to the median eminence upon the capillary bed of the portal blood vessels through which GnRH reaches the AP, while axons of other neurons project either to other brain regions where GnRH

functions as neurotrasmitter or to the POA where they constitute an intrinsic GnRH neural circuit. Many afferents arising from all major brain regions reach the POA where they synapse on GnRH neurons (Leranth et al., 1988).

Thus, the activity of GnRH neurons can be modulated by a variety of factors including gonadal steroids, neuropeptides and bioamines that can act directly on these neurons or via interneurons which in turn affect their biosynthetic and/or releasing activity.

Recent data suggest that L-Glutamate (L-Glu) and other amino acids, in addition to their function of synaptic transmitters, have a relevant role as modulator of responses to other chemical mediators such as monoamines and acetylcholine. Furthermore, evidence do exist indicating that endogenous excitatory amino acids (EAA) activate hypothalamic receptors possibly involved in the control of anterior pituitary function. "In vivo" treatment with L-Glu and with the more potent analog NMDA induces acute elevations of plasma LH levels in prepubertal and adult rats (Ondo et al., 1976; Price et al., 1978; Arslan et al., 1988) and Rhesus monkeys (Gay and Plant, 1987). Chronic intermittent administration of NMDA has been shown to mimic the effect of pulsatile GnRH secretion on LH release and to induce sexual maturation (Urbanski and Ojeda,

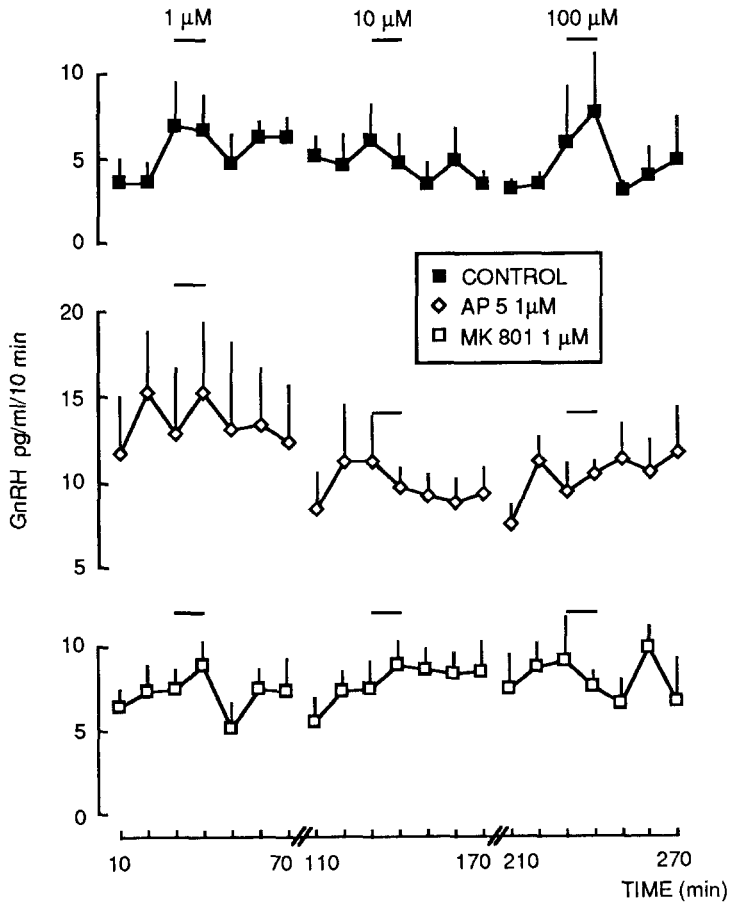

Fig. 1. Effect of QUIN either alone or in the presence of AP-5 or MK801 on GnRH secretion (See text for details)

1987; Bourguignon et al., 1989). Suppression of pulsatile LH secretion "in vivo" has been observed following injection of a specific antagonist of NMDA receptors (Arslan et al., 1988), providing evidence for a possible physiological role of this receptor type. In addition, Kainic Acid (KA), a non-NMDA glutamate receptor agonist, has been shown to possess clear LH-releasing activity (Price et al., 1978, 1979). By "in vitro" studies utilizing pituitary incubation, Schainker and Cicero (1980) failed to show any direct effect of NMDA on LH release. This data together with the observation that the effects of NMDA can be blocked by the administration of a GnRH antagonist (Cicero et al., 1988) has brought to propose that the action of EAA on LH secretion might be exerted via the central nervous system, through the increased release of GnRH.

However, we have recently shown that different exogenous EAA agonists might affect LH and FSH secretion by acting at different levels (hypotalamic and/or anterior pituitary): while NMDA at rather elevated doses is able to increase GnRH secretion, KA induces a sharp rise in both LH and FSH secretion by a direct action on the AP, at very low doses (Zanisi and Messi, 1991).

These results have been obtained by an "in vitro" perifusion technique which allows to differentially examine the site of action of a given substance that is directly applied to the tissue (hypotalamus and/or AP) perifused in different chambers and at variance with incubation procedure, avoids the accumulation of secretion or degradation products.

All these observations, suggest that EAAs might represent an additional class of neuromodulators influencing GnRH/LH secretion.

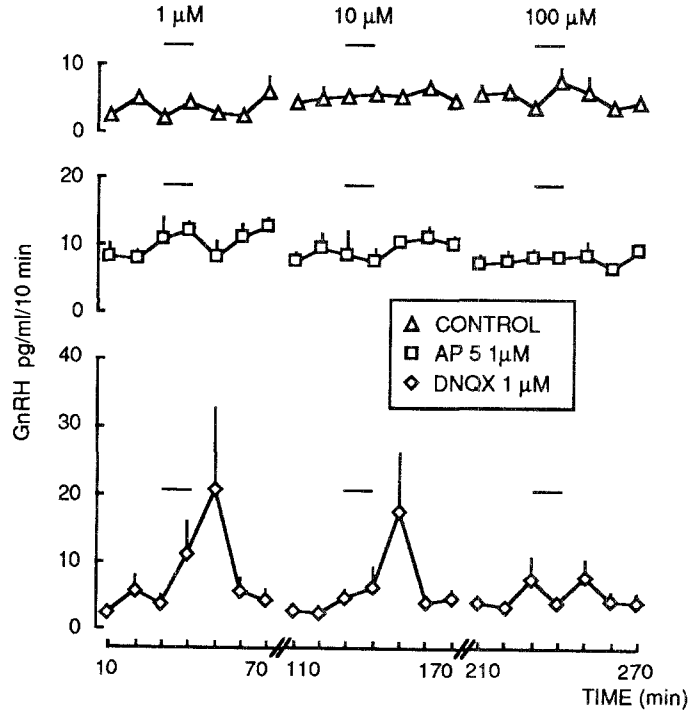

Fig. 2. Effect of QA either alone or in the presence of AP-5 or DNQX on GnRH secretion (See text for details)

In addition to glutamate, another endogenous amino acid is quinolinic acid, a metabolite of L-triptophan in the liver and a product of kynurenin metabolism in the rodents' brain. This amino acid is considered a possible candidate as an endogenous activator of the NMDA receptor (Perkins and Stone, 1983).

The recent identification of a metabotropic type of EAA receptor can offer some insight for clarifying the mechanism of action of the EAAs on the neuroendocrine system.

Two subtypes of metabotropic receptor have been identificated: one is activated by Quisqualate and coupled via a G-protein to phosphoinositide second messenger system, whereas the other is activated by L-2-amino-4-phosphonobutirrate and increases c-GMP via a G-protein mediated process (Nawy and Jahr, 1990). However, it must be recalled that the quisqualate receptor class includes, in addition to the metabotropic one, an ionotropic receptor subtype which does not clearly differ from the kainate one (Thio et al., 1991).

The rational behind these experiments is from one side that of verifying whether other endogenous EAAs, besides Glu, can influence the neuroendocrine system acting either at hypothalamic or AP level and, from the other side, of evalutating which type of receptor such an effect might be mediated through.

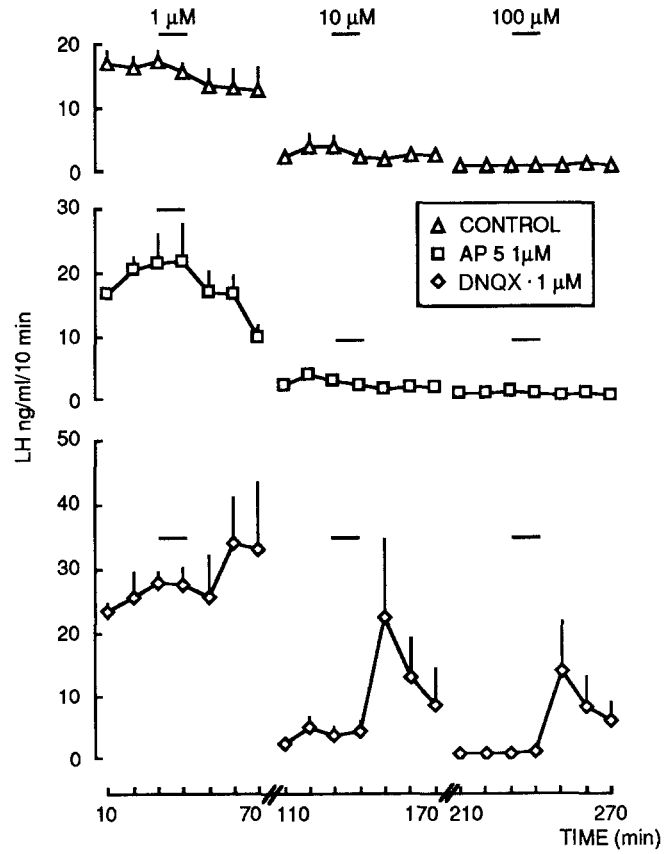

Fig. 3. Effect of QA either alone or in the presence of AP-5 or DNQX on LH secretion (See text for details)

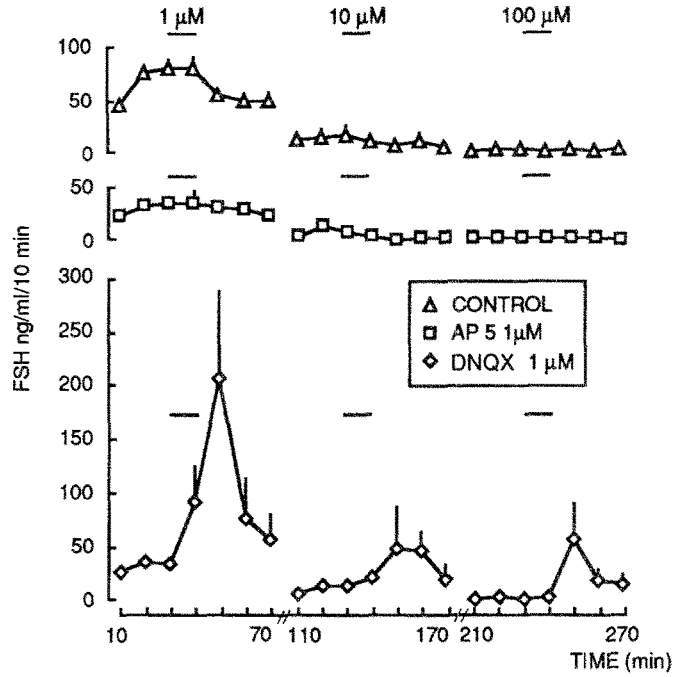

Fig. 4. Effect of QA either alone or in the presence of AP-5 or DNQX on FSH secretion (See text for details)

For these reasons, Quinolinic acid (QUIN) and Quisqualate (QA) have been administrated in pulsatile manner to MBH and AP obtained from 50 day old male rats, by means of a perifusion system.

Measurements by specific RIA of GnRH and LH and FSH secreted both in basal and stimulated conditions, in the effluents collected from the chambers containing the different tissues, have been performed.

Materials and methods

Animals

All the experiments have been performed in Sprague Dawley 50 day old male rats Crl:CD® BR (Charles River Italia, Como). Animals have been maintained in rooms with controlled temperature (22 ± 1°C) and relative humidity (60%), standard diet and water ad libitum and with a lighting schedule of 14 h of light and 10 h of darkness (light on, 06.00–20.00 h). At sacrifice, the mediobasal hypothalamus (MBH), defined anteriorly by the optic chiasm, posteriorly by the mammillary bodies and laterally by the hypothalamic sulci, has been cut longitudinally along the third ventricle and transferred to the perifusion chamber. The AP has been dissected, halved and placed in the perifusion chamber. In this series of experiments, single MBH or AP fragments have been placed in each chambers.

Chemicals

Different agonists and antagonists of EAA have been tested: Quinolinic acid (QUIN), Quisqualic acid (QA), dl-2-amino-5-phosphonovaleric acid (AP5) (obtained from Sigma Chemical Co., St Louis, Mo.); 6,7-dinitro quinoxaline-2,3-dione (DNQX) and (+)-MK-801

hydrogen maleate (obtained from RBI, Natick, Ma.). Merck (Darmstadt, Germany) substances have been used for preparation of perifusion medium.

Perifusion system

The perifusion system consists of four independent chambers, each made from a 3-ml glass syringe, which can be used simultaneously. In each chamber the tissue is held between two nylon nets with 130μ pores, fastened to the bottom and top end pieces. Perifusion medium is 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 are delivered to the chambers through a system consisting of two 4-way valves, one fastened under the chamber and the other placed out of the water bath. This device allows the filling up of the loading circuit through which it is possible to inlet the test substances in the chamber without interruption of the medium flow. The chambers and the medium reservoir are submerged in a constant temperature water bath at 37°C. Both the medium delivered and that contained in the chambers are saturated with a mixture of O_2–CO_2 (95%:5%). Medium is drained out of the chambers through polyethylene tubing, collected every 10 minutes (1 ml), by a fraction collector (Ultrarac 7000, LKB, Bromma, Sweden) and quickly frozen until RIAs. The perifusion system used in these experiments has been adapted from that previously described (Zanisi et al., 1987). 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). The same medium serves also as solvent for the different substances to be tested.

Hormone assays

GnRH content of the perifusion effluent from the chamber containing MBH has been measured by RIA using reagents supplied by Amersham International plc (Buckinghamshire, England) and using reference preparations obtained from Sigma Chemical Co. (St Louis, Mo.). LH and FSH contents of the perifusion effluent obtained from the chamber containing the AP have been determined by RIAs. The materials for RIA-FSH has been provided by the NIADDK National Hormone and Pituitary Program through Dr. A. F. Parlow; LH concentrations have been measured using an antiovine LH anti-serum provided by Dr. G. D. Niswender (Colorado State University, Fort Collins, CO) and ovine LH provided by Dr. L. E. Reichert Jr. Values are expressed in terms of NIH-LH-S26 and NIDDK-rFSH-RP2 respectively.

Statistical analysis

The results of the perifusion experiments have been expressed as picograms (GnRH) or nanograms (LH and FSH) per ml/10 min. For the statistical analysis the data have been expressed as Δ (where 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) and evaluated by Repeated Measures with Grouping Factors-ANOVA to compare the responses between and within the groups. Statistical tests were calculated using the statistical package "SYSTAT".

Results

Two agonists for different EAA receptor types have been tested on their ability to modulate GnRH release from the hypothalamus and LH and FSH from the anterior pituitary of 50 day-old male rats, by means of an "in vitro" perifusion system.

1) Pulses of QUIN at concentrations of 1 or 10 μM are not able to modify the releasing activity of either tissue. However, at 100 μM, QUIN exhibits a

weak stimulatory effect on GnRH release from the MBH. This GnRH-releasing effect of QUIN appears to be exerted through the NMDA receptor subtype since it is completely abolished by the presence in the perifusion medium of NMDA antagonists, both competitive (AP-5) and non-competitive (MK-801). At the AP level, no effects of QUIN, either alone or in the presence of the antagonists, have been observed.

2) Activation of the non-NMDA receptors of the QA subtype does not influence gonadotropin secretion at any of the concentrations tested (1–100 μM). When the pulses of KA have been applied to the tissues during continuous perifusion with a medium containing the NMDA antagonist AP5 (1 μM) no changes on GnRH, LH or FSH secretion has been observed.

The presence, in the perifusion medium of DNQX (1 μM), a specific non-NMDA receptor antagonist, enhances the release of GnRH from the MBH and of LH and FSH from the AP, an effect that is in inverse relation to the dose tested (maximal at 1 μM, minimal at 100 μM).

Discussion

The present results underline once more that Glu agonists (both endogenous and exogenous) can affect the neuroendocrine system and in particular the hypothalamic-pituitary-gonadal axis. In addition, this study provides some pharmacological characterization of the receptor subtype involved in this action.

QUIN, an endogenous agonist supposed to activate the NMDA receptor, is able to increase gonadotropin secretion by an action exerted at hypothalamic level through the stimulation of GnRH release. This effect is detectable only at the highest concentration used in our studies (100 μM). However, it must be underlined that the data on the action of QUIN on gonadotropin secretion have been obtained with much higher concentration (on the mM range). Lopez and co-workers (1992) have shown a dose-dependent increase in GnRH release from arcuate nucleus-median eminence (AN-ME) fragments incubated "in vitro" only at concentrations ranging from 10–50 mM. Furthermore, in an "in vivo" study Nemeroff and co-workers (1985) after directly infusing QUIN bilaterally into the hypothalamus, observed an increased release of LH only at high (50 μg) but not at low (0.5–15 μg) doses. The hypothesis that QUIN might be considered a putative modulator of endocrine function is corroborated by the identification of this EAA agonist in rat and human brain tissue (Moroni et al., 1988; Wolfensberger et al., 1983) and by the presence of metabolic enzymes for both QUIN synthesis and degradation in the rat hypothalamus (Schwarcz et al., 1983; Foster and Schwarcz, 1984).

In respect to the subtype of Glu receptors involved in QUIN action some discrepancy do exist QUIN has been postulated to act as an agonist of L-Glu and in addition is considered an endotoxin involved in both the neuropathology of hypoglycemia and in the pathogenesis of Huntington's disease (Schwarcz et al., 1984). While the neuronal activation and convulsant effects of QUIN are reversed by AP-7 and by other NMDA antagonists, its neurotoxic effects show important differences from those of NMDA and similarities with those of KA. The data on the blockade of QUIN effect on GnRH release by either AP-5 or

MK801 indicate the presence of specific QUIN-sensitive receptors on neurons which are intimately associated with reproductive functions and confirm that this effect of QUIN is mediated, at least in part, through the NMDA receptor type. Further studies utilizing antagonists of the non-NMDA receptor subtypes (and in particular KA) are needed to exclude that the endocrine action of QUIN is solely mediated by NMDA receptor.

Quisqualate "per se" is not able to affect GnRH or LH and FSH secretion from the MBH or the anterior pituitary respectively, at the concentrations tested (1–100 μM). This lack of effect might mainly be due to the low affinity of MBH receptor sites for this agonist. Lopez and co-workers (1992) have reported a sharp increase on GnRH release in an incubation system of AN-ME fragments in response to higher concentrations (1 mM). Previously the same group (Donoso et al., 1990) had shown in the same experimental conditions, that the threshold of QA-induced GnRH release is reduced in the presence of K^+-enriched medium. As for the lack of action of QA at the AP level it must be recalled that no data are available on the kind of EAA receptor subtypes on the gland.

An interesting result of our experiment is the marked ability of QA to release gonadotropins when applied in pulses during continuous perifusion with a selective non-NMDA antagonist (DNQX). The QA receptor class includes two distinct subtypes: an ionotropic receptor linked to a cationic conductance and a metabotropic receptor associated with the activation of the metabolism of phosphoinositides (Yool et al., 1992). Quinoxalinedions have been found to be potent and competitive antagonists at non-NMDA Glu-receptors. These compounds have been very useful in determining the structure-activity relations of QA and KA receptors and the role of such receptors in synaptic transmission in the mammalian brain (Honoré et al., 1988).

The antagonist DNQX is a strong blocker of ionic currents triggered by QA ionotropic receptor activation (Honoré et al., 1988) but does not inhibit QA-elicited accumulation of IPs (Récasens et al., 1988). Yool and coworkers (1992) on the basis of current clamps studies on Purkinje neurons have suggested that the complex nature of the response of these cells to QA indicates that multiple ionic mechanisms are involved, perhaps linked to different receptor subtypes.

In addition, recent studies have shown that receptor-ion channel complexes might be the result of recombinant interchangeable subunits, suggesting that this might account for the multiplicity of EAA receptor subtypes and for the possibility that the same agonist might bind to more than one subtype (Bettler et al., 1990).

On this basis, the observed stimulatory effect that QA exerts only in the presence of DNQX might be explained by the low binding affinity of QA for the metabotropic receptor, thus, at the low doses employed in our studies, no effect can be evidentiated. The blockade of the ionotropic QA receptor by DNQX, would leave the metabotropic receptor available for the binding with QA (even if present in low concentration) that in such a way can exert its effect on GnRH secretion from the MBH. Studies utilizing higher QA concentrations are in progress in order to clarify this issue. It is rather difficult to establish whether such a mechanism is also the basis of the effect of QA at the AP level. Actually,

our knowledge on the existence and on the type of receptors present on the AP is scanty.

The data confirm that glutamate agonists are able to influence the neuroendorine system and can be considered as an additional class of modulators of the hypothalamic-pituitary-gonadal axis.

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