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Control of Puberty by Excitatory Amino Acid Neurotransmitters and its Clinical Implications

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Excitatory amino acids, glutamate in particular, have a marked stimulatory effect on the reproductive axis, particulary at puberty. Glutamate, *N*-methyl-D-aspartate (NMDA), and kainite-stimulate gonadotropin-releasing hormone (GnRH) secretion in immature mammals and NMDA receptor stimulation results in precocious puberty in rats and monkeys. Puberty is characterized by an increased sensitivity of GnRH to glutamate as well as an increase in glutaminase activity in the hypothalamus. Glutamatergic and GABAergic regulation of GnRH secretion seem strongly interdependent around puberty. In addition to the transsynaptic glutamatergic regulation of GnRH secretion, a coordinated activity of glutamatergic neurons and astroglial cells has been shown to play an active role in puberty. The participation of kainate receptors in the estradiol-induced advancement of puberty suggest that these receptors may be involved in the estradiol-mediated activation of GnRH secretion at puberty. A case of precocious puberty associated with hyperglycinemia illustrates the NMDA involvement in puberty in humans. In this patient, the occurrence of precocious puberty was thought to result from excessive stimulation by glycine of the NMDA receptors linked to the GnRH neurons. Glutamate plays several roles in the hypothalamic mechanism of puberty as it has been shown in animal models, but there are still few clinical data supporting the role of glutamate in human puberty.

Key Words: Glutamate; NMDA; kainate; GnRH; estradiol; glia; hypothalamus.

Introduction

Excitatory amino acids (EAA), glutamate in particular, play a preeminent role in the control of brain functions. Glutamate and its receptors are expressed in the hypothalamus and are involved in key reproductive and neuroendocrine processes such as puberty, preovulatory surge, reproductive behavior, and stress. Glutamate receptors are either metabotropic, G protein–coupled receptors or ionotropic, ligand-gated ion channels. The three main classes of glutamate ionotropic receptors have been named according to their agonists: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5- methylisoxazole-4-propionate (AMPA), or kainate.

The onset of puberty results from the reactivation of the gonadotropin-releasing hormone (GnRH) pulse generator as a result of functional changes occurring in neuronal and astroglial networks connected to the GnRH neuron. Glutamate neurotransmission is the predominant mode of transsynaptic excitation used by hypothalamic neurons *(1)*, and several studies in various species have shown a marked stimulatory effect of EAAs on the reproductive axis, particularly at the time of puberty. Although the data discussed in this review have been obtained in different species, the vast majority of data come from the rodent and few from the primate, including humans. Also, the mechanisms and their developmental sequence may vary across species. Therefore, the clinical implications are rather limited and animal data cannot be extrapolated to human conditions.

Transsynaptic Regulation of GnRH Secretion by Glutamate at Puberty

GnRH neurons receive direct glutamatergic innervation *(2,3)*, and it has been shown that glutamate stimulates GnRH release from the adult hypothalamus in several species via activation of NMDA and kainate receptors *(4)*. Hypothalamic glutamate content increases during prepubertal and peripubertal development and reaches its maximum after the onset of puberty *(5)*. In addition, the release of glutamate from preoptic area/medial basal hypothalamus explants or from the preoptic area of the female rat in vivo increases during the peripubertal period *(6–8)*. Glutamate, NMDA, and kainate stimulate GnRH secretion in immature rats *(9–11)*, monkey *(12,13)*, and sheep *(14)*. Moreover, NMDA receptor stimulation results in precocious puberty in rats *(11, 15)* and monkeys *(16)* and administration of the NMDA receptor blocker MK-801 delays the timing of puberty in rats *(17,18)*.

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Sensitivity of the GnRH System to Glutamate in Relation to Puberty

Developmental changes in NMDA receptors were observed in several extrahypothalamic regions such as cortex, hippocampus, and cerebellum *(19–21)*. In the preoptic area, the ratio of NR1 and NR2a and 2b receptor subunits was reported to change as well *(22)* and could explain developmental variations in sensitivity to NMDA receptor activation. The most dramatic increase in expression of NR1, a subunit that is necessary for a functional NMDA receptor, seemed to take place between fetal d 18 and postnatal d 10. In the rat gray matter, genes encoding for the kainate receptor subunits were expressed from fetal d 14 *(23)*. Their expression went through a peak in the late fetal/early postnatal period, when the contribution of non-NMDA receptors specifically involved in GnRH secretion was found to be predominant in our in vitro model *(24)*. The mRNA editing of different subunits of the non-NMDA receptors, which determines their gating characteristics, is developmentally regulated *(25)* and may also contribute to the ontogenetic changes in glutamate sensitivity.

Because glutamate receptors are ubiquitous in the central nervous system (CNS) including the hypothalamus where they play important roles in many processes throughout development, the above observations raise the issue whether the changes in glutamate receptor activity that affect GnRH secretion are part of a global maturational process in the CNS or result from a regionally specific mechanism. The facilitatory control exerted by glutamate on GnRH neurons increases gradually during the infantile–juvenile periods as evidenced by the increased ability of glutamate receptor stimulation to induce GnRH release during this time *(26, 27)*. Gore et al. showed that NMA treatment started on d 25 resulted in accelerated elevation of GnRH mRNA levels and sexual precocity. In addition, the percentage of GnRH neurons double-labeled for NMDA-R1 subunit was found to increase from 2–3% before or during puberty to 19% in adulthood, whereas hypothalamic NMDA-R1 mRNA levels increased before puberty between d 10 and 20 *(28)*. This indicates the possible discrepancies between global changes and those specific of the GnRH neurons. In our in vitro conditions where the developmental acceleration of pulsatile GnRH secretion occurs between 10 and 25 d of age, the NMDA receptor sensitivity was assessed by the sensitivity to the use-dependent antagonist MK-801. Such a sensitivity was found to increase dramatically in a gonad-independent manner between d 10 and 25 *(29,30)* when a peak sensitivity is observed in consistency with the data obtained in vivo by Cicero et al. *(10)*.

Glutamate Production During Puberty

Glutamate concentrations in the rat hypothalamus increase during prepubertal development *(5)*. A major pathway for glutamate biosynthesis is from glutamine under the action of glutaminase *(31)*. The mechanism of glutamate recycling in the brain involves reuptake by astroglial cells of the glutamate released at the synapse level and transformation into glutamine, which is delivered to glutamatergic neurons where glutaminase causes transformation into glutamate. Using hypothalamic explants of 15-d-old rats, we found that glutamine was able to elicit GnRH release in a dose-dependent manner similar to glutamate *(32)*. In the presence of 6-diazo-5-oxo-norleucine (DON), an inhibitor of glutaminase, the glutamine-evoked GnRH secretion was prevented while the glutamate-evoked secretion was not altered. DON also significantly inhibited spontaneous GnRH secretion as well as the veratridine-induced GnRH secretion. This indicates that glutamate biosynthesis from glutamine is a prerequisite to the physiological mechanism of pulsatile GnRH secretion. The DON concentrations required to inhibit the veratridine-evoked GnRH secretion was lower at 15 d of age than 50, suggesting that glutaminase shows increased activity around the onset of puberty when the frequency of pulsatile GnRH secretion is increased as well. In a more recent study, the glutamine-evoked secretion of GnRH compared with that evoked by glutamate was used to provide an indirect assessment of glutamate biosynthesis from glutamine. In such conditions, a critical increase in glutamate biosynthesis could be observed during the early postnatal period between birth and 15 d *(24)*, indicating that the increase in glutaminase activity was an early developmental event preceding puberty. Roth et al., however, did not find any ontogenetic change in glutaminase mRNA in the mediobasal hypothalamus and the preoptic area *(33)*. Still, a limit in the significance of such studies is the regional specificity of changes in face of the ubiquity of the enzyme. If an increase in glutamate biosynthesis from glutamine partly accounts for the developmental increase in pulsatile GnRH secretion at puberty, such a mechanism could be rather specific of the neuronal–glial apparatus controlling GnRH secretion.

GnRH Inhibitory Autofeedback Through Glutamate Receptors

We have described an autofeedback of GnRH on pulsatile GnRH secretion in the rat hypothalamus. This inhibitory autofeedback is mediated by the GnRH_{1-5} subproduct following degradation of the decapeptide by the prolyl endopeptidase (PEP) and involves the NMDA receptors but not the kainate receptor subtype *(34)*. This mechanism operates already in the fetal hypothalamus, but with minimal impact due to the prominent role of kainate receptors at that age *(24)*. Postnatally, the restricting effect of PEP on pulsatile GnRH secretion appears to be maximal during prepuberty, whereas it is reduced at 25 d and subsequently *(35)*. In the monkey, however, no evidence of such an inhibitory autofeedback could be obtained because intracerebroventricular administration of GnRH or a GnRH antagonist did not alter the electrophysiological correlates of the GnRH pulse generator *(36)*.

Coordination of Glutamate and GABA Regulation of GnRH Secretion at Puberty

Glutamatergic and GABAergic neurotransmissions are critical opposing components of the onset of puberty. Several observations suggest that they are strongly interdependent. Because glutamate is the natural precursor of GABA, any change in glutamate availability could affect GABA synthesis. Moreover, besides their direct action on GnRH neurons, glutamatergic neurons control GABAergic neurons. They inhibit GABA transmission through AMPA and kainate receptors *(37–39)*. The kainate receptor–mediated stimulation appears to be exerted directly on GnRH neurons, while the NMDA-mediated stimulation may require interneurons *(28)*. It is conceivable that the decrease in GABA transmission preceding puberty results from an increase of kainate receptor–mediated inputs to hypothalamic GABAergic neurons. An additional glutamate–GABA interaction can lie on NMDA receptors with differential effects related to molecular subunits of the receptors because we showed, through the use of antisense oligonucleotides, that the NR2A subunit mediated a facilitatory effect on GnRH secretion in vitro, whereas the NR2C subunit was involved in a GABAergic inhibitory pathway *(40)*. In this paradigm of rat hypothalamic explant, we also found that onset of puberty was preceded by a concomitant increase in NMDA receptor–mediated stimulation and GABA receptor–mediated inhibition of pulsatile GnRH secretion in vitro *(41)*.

Neuron-to-Glia Signaling Mediated by EAA During Puberty

Traditionally, astrocytes have been considered as having a limited structural role in the brain, but evidence has been obtained during the past few years that they play an active role in synaptic communication by exchanging information with the synaptic elements. The increase in GnRH secretion at puberty requires an increase in transsynaptic glutamatergic inputs as well as the activation of a glia-to-neuron signaling pathway mediated by erbB receptors and their ligands operating in the hypothalamic astrocytes. Transforming growth factor alpha (TGF-α) and neuregulin (NRG) stimulate GnRH secretion indirectly via the following sequence of events: activation of the erbB-1 and erbB-4 receptors, respectively, release of bioactive substances such as prostaglandin E2 (PG E2) and stimulation of GnRH by these substances *(42–44)*. Astrocytes are endowed with glutamate receptors *(45,46)* and respond to glutamate stimulation. It has been shown that hypothalamic astrocytes express the metabotropic glutamate receptor mGluR5 and the AMPA receptors GluR2 and GluR3 subunits. The mechanisms relating the glutamatergic neurotransmission and the erbB-mediated signaling have been described recently *(47)*. The combined activation of ionotropic and metabotropic glutamate receptors located on astrocytes enhances the functional capability of the erbB system by recruitement of the erbB receptors to the cell membrane, physical approximation of the receptors to their membrane-bound ligands, phosphorylation of the erbB receptors, and increase of erbB receptor gene expression. Glutamate receptor–induced erbB transphosphorylation requires a processing of the membrane-bound erbB ligands by metalloproteases. This mechanism may be used to coordinate the activation of glutamatergic neurons and astroglial cells during puberty, in addition to the coordination of neuron-to-neuron communication requiring NMDA and kainate receptors.

Nell-2

Nell-2, a neuron-specific gene, has recently been reported to be selectively expressed in glutamate neurons throughout the brain. Nell-2 is a positive regulator of glutamatergic transmission and appears to be a required component of the glutamate-dependent activational process leading to the initiation of puberty because its blockade using antisense oligonucleotides results in delayed puberty *(48)*.

Sex Steroid Interaction with Glutamate Receptor–Mediated Release of GnRH

Among the systems regulating the hypothalamic–pituitary–gonadal axis, the estradiol-mediated feedback on the hypothalamus has been extensively studied and its importance during the reproductive cycle of the adult female has been clearly demonstrated *(49)*. Estradiol can act through two nuclear receptors (called ERα and ERβ) that, upon activation, will act as transcriptional enhancers or repressors by binding to specific DNA sequences called ERE or estrogen responsive element *(50)*. These effects are known as long-term or genomic effects and are responsible for the positive and negative feedback effects of estradiol on the hypothalamus *(49,51)*. In addition to these genomic effects, estradiol was also shown to act rapidly and directly on cells or through the activation of second messengers such as protein kinases *(52)*.

The common concept that estradiol should act on GnRH neurons through intermediate cells *(49)* was challenged after the expression of functional ERβ in GnRH neurons has been reported and confirmed *(53–55)*. The possible role of estrogen in the onset of puberty has not been widely explored. There is an increased risk of precocious puberty in girls exposed to environmental estrogens *(56)*. In the rat, Ramirez et al. have reported that an injection of estradiol in the immature female induces precocious puberty *(57)*. This advancement of the pubertal onset could be due to an increased stimulation of the GnRH secretion as it has been reported that estradiol increased the responsiveness of the immature hypothalamus to stimulatory peptides *(58)*. The effects of estradiol on the onset of puberty could also be mediated by the activation of the neuregulin receptors that have been involved in the onset of puberty and can be regulated by estradiol *(43)*. In addition, we also reported that estradiol increased the GnRH pulse frequency after administration in vitro and in vivo *(59)*. Our in vitro studies indicate that estradiol increased the pulsatile GnRH secretion through a mechanism involving the ERα subtype and the kainate receptor. Although the implication of the glutamatergic system in triggering the onset of puberty is well known, only the NMDA receptor subtype has been involved in this process in vivo in the monkey *(60)* and in vitro in the rat *(61)*. The participation of the kainate receptor in the estradiol-induced advancement of puberty is interesting and leads to the hypothesis that, during development, as the gonads increase their hormonal secretion, the kainate receptors get activated and are responsible for the estradiol-mediated activation of the GnRH system. As the immature female goes into adulthood, these specific interactions between the estrogen and the kainate receptor might be integrated in the regulatory mechanisms of the estrous cycle. This hypothesis is supported by previous studies showing that, in the afternoon of proestrus, nearly half of activated GnRH neurons express kainate receptor subunits *(62)*. In addition, the AMPA/kainate receptor antagonist DNQX can block the estradiol-induced LH surge in ovariectomized rats *(63)*.

Precocious Puberty Associated with Hyperglycinemia: Illustration of NMDA Involvement in Puberty in Humans

Until recently, there was no evidence of NMDA receptor involvement in the regulation of puberty in human. Bourguignon et al. reported a case of precocious puberty in a 11-mo-old girl suffering from nonketotic hyperglycinemia (NKH) *(64)*, which is a severe genetic disease caused by an inherited defect in the enzymatic system cleaving glycine. This results in an increase of glycine concentration in the cerebrospinal fluid. Some nonketotic hyperglycinemia symptoms involve the inhibitory strychnine-sensitive glycine receptors *(65)*, whereas the pathogenesis of seizures involves the excitatory strychnine-insensitive glycine receptors belonging to the NMDA–receptor complex *(66)*. The occurrence of precocious puberty in this patient was thought to result from excessive stimulation by glycine of the NMDA receptors linked to the GnRH neurons. This hypothesis was supported by in vitro observations. Glycine increased the pulse frequency of GnRH secretion from hypothalamic explants of immature female rats and this effect was prevented by 7-chlorokynurenic acid, a glycin antagonist at the NMDA receptor complex. Regression of pubertal development under anticonvulsive treatment with GABA agonists suggested that the stimulatory effect of glycine could be overcome by GABA receptor–mediated inhibition. Those GABA agonists suppressed the stimulatory effect of glycine in vitro as well as the developmental increase in the frequency of pulsatile GnRH secretion.

Conclusion

Glutamate is an excitatory amino acid that can play several roles in the hypothalamic mechanism of puberty. The evidence, convincing in rodents, is however scarce in humans. Glutamate is likely to be more generally a critical neuromediator in the different neuroendocrine functions as suggested by the panhypopituitarism associated with disordered control of energy balance following neonatal insult of glutamate-sensitive cells by monosodium glutamate *(67)*. Although such a paradigm could be related to some clinical conditions with early and generalized failure of hypothalamic homeostatic mechanisms, there are still few clinical data supporting the role of glutamate in human neuroendocrine physiology and pathophysiology.

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