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Hypothalamic Excitatory Amino Acid System During Sexual Maturation in Female Rats

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The present results indicate that during sexual maturation the APOA–MBH from rats of 30 days of age released significantly higher quantities of GnRH than the tissue from 16-day-old rats ($P < 0.01$). The addition of NMDA, an agonist of the excitatory amino acids system (EAAs), to the medium after 30 min of incubation significantly increased ($P < 0.01$) the GnRH release in normal rats of both ages and this increase was significantly ($P < 0.01$) higher in 30-day-old rats (to 661%) than in rats of 16 days of age (to 273%). The administration of estrogen–progesterone (EP) to rats of 16 days of age did not modify the GnRH release response to NMDA. On the contrary, at 30 days of age EP administration significantly potentiated the GnRH release response to NMDA since while in the control group NMDA increased the GnRH release to 630%, in the EP-pretreated group this was to around 4700% ($P < 0.01$). EP pretreatment of prepubertal rats decreases the hypothalamic release of aspartate and glutamate, the excitatory amino acids involved in NMDA neurotransmission and glycine but increases EAAs release in peripubertal rats. On the basis of these results it is proposed that the increase in EAAs release by the hypothalamus is directly connected with the onset of puberty and that the maturation of the positive feedback effect of ovarian hormones on gonadotropin secretion is related to the maturation of the capacity of EP to increase hypothalamic EAAs. Before this maturational event EP inhibits EAAs release as well as gonadotropin release (prepubertal rats). NMDA receptor stimulation leads to a positive mechanism which increases the release of Asp and Glu from APOA–MBH both in prepubertal and peripubertal rats, but EP potentiates this mechanism only in peripubertal rats. This could be an additional neuroendocrine mechanism involved in the increase of gonadotropin during sexual maturation which induces the onset of puberty and the preovulatory discharge of these pituitary hormones.

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INTRODUCTION

Excitatory amino acids (EAA) appear to play an important role in the neurotransmission pathway that regulates gonadotropin secretion [1]. The effects of EAA on gonadotropin secretion seem to be exerted via the central nervous system since L-glutamate, a physiological neurotransmitter of the EAA system (EAAs) and *N*-methyl-D-aspartate (NMDA), an exogenous agonist of EAA receptors, stimulate release of gonadotropin releasing hormone (GnRH) from hypothalamic fragments *in vitro* [2–4]. On the other hand, the EAA appear to contribute to the enhancement of gonadotropin secretion at the time of puberty [5, 6].

The onset of puberty is the consequence of complex central mechanisms which induce changes in the secretion of gonadotropins and the consequent stimulation of gonads which results in increased sex hormone secretion [5]. In mammalian females sexual maturation and puberty also involves the maturation of the positive feedback effect of ovarian hormones on gonadotropin secretion, a physiological event related to the onset of sexual cycles [7–9].

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In the present experiments we determined in female rats the *in vitro* effect of NMDA on the anterior preoptic area and medial basal hypothalamus (APOA–MBH) release of GnRH and the hypothalamic content and *in vitro* release of aspartate (Asp), glutamate (Glu) and glycine (Gly) the EAA involved in NMDA neurotransmission, as well as the actions of ovarian hormones on these effects during sexual maturation. On the other hand, we also determined the *in vitro* effect of NMDA on hypothalamic EAA release.

The experiments were performed in Wistar female rats aged 16 (prepubertal) and 30 days (peripubertal) at the time of sacrifice. In these rats vaginal opening takes place around 32–34 days of age whereas the first cycle occurs between 35–37 days.

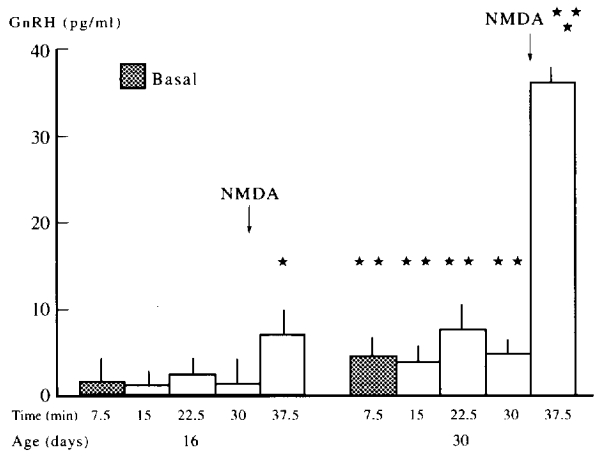

Fig. 1. Effect of NMDA on GnRH release by APOA–MBH in prepubertal and peripubertal rats. *$P < 0.001$ vs basal and **vs 16 days of age.

EFFECTS OF NMDA AND EP ON GNRH RELEASE BY APOA–MBH

As can be seen in Fig. 1, GnRH release to the medium from APOA–MBH was constant during the incubation time. On the other hand the APOA–MBH from rats of 30 days of age (peripubertal) release significantly higher quantities of GnRH than the tissue from 16-day-old rats ($P < 0.01$). The addition of NMDA to the medium after 30 min of incubation significantly increased ($P < 0.01$) GnRH release in normal rats of both ages and this increase was significantly ($P < 0.01$) higher in 30-day-old rats (to 661%) than in rats of 16 days of age (to 273%).

Pretreatment with estrogen–progesterone (EP) to rats of 16 days of age did not modify the GnRH release response to NMDA (Fig. 2) since the increase from the basal values of GnRH release induced by NMDA in control rats was 262% while that in EP pretreated rats was 245%. On the contrary, at 30 days of age, EP administration significantly potentiated the GnRH release response to NMDA (Fig. 2), since, while in the control group NMDA increased the GnRH release by 680%, in the EP-pretreated group this increase was around 4700% ($P < 0.001$).

EFFECTS OF EP AND NMDA ON APOA–MBH AMINO ACID CONTENT AND RELEASE

Prepubertals

As can be seen in Table 1, EP treatment significantly ($P < 0.01$) increases the Asp, Glu, and Gly content in the APOA–MBH. Pretreatment with ovarian hormones decreased ($P < 0.01$) the release of Asp, Glu, and Gly into the medium [Fig. 3(A)]. The maximal inhibition of release was about 80% for Asp, 50% for Glu, and 77% for Gly. The addition of NMDA to the incubation media [Fig. 4(A)] significantly increased ($P < 0.01$) the release of Asp, by around 140%, and of Glu by 100%. NMDA did not modify the hypothalamic release of Gly to the incubation medium. Pretreatment with EP [Fig. 4(A)] did not either modify the Asp and Glu release response to NMDA or the lack of response to Gly.

Peripubertals

EP treatment did not modify the hypothalamic concentration of the EAA (Table 1). Contrary to the observations in prepubertal rats, the ovarian hormones significantly ($P < 0.01$) increased release into the medium [Fig. 3(B)] of Asp by 150% and of Glu and of Gly by 100%. On the other hand NMDA significantly increased the *in vitro* release of Asp and Glu by 133 and 153%, respectively [Fig. 4(B)]. Pretreatment with EP potentiated the release of these amino acids (Asp to 200% and Glu to 500%) induced by NMDA. The ovarian hormones induced a releasing effect of NMDA on Gly (to 180%) that was not observed in controls.

These results confirm previous reports [1–5] indicating that NMDA stimulates GnRH secretion at the hypothalamic level and show that the stimulation of GnRH release by NMDA neurotransmission is significantly different during sexual maturation.

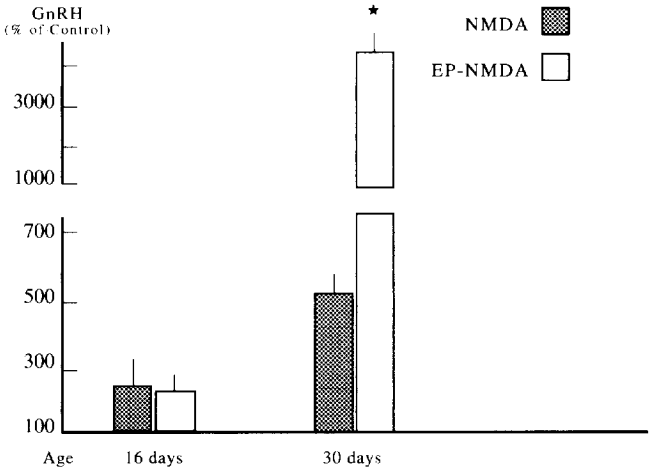

Fig. 2. Effect of EP administration on GnRH release by NMDA *in vitro*. *$P < 0.01$.

Table 1. Effects of EP and NMDA on the hypothalamic content of amino acids in prepubertal rats

	Prepubertal		Peripubertal	
	Control	EP	Control	EP
Aspartate	920 ± 73	1245 ± 101*	1200 ± 11†	930 ± 93
Glutamate	650 ± 56	980 ± 73*	950 ± 98†	960 ± 96
Glycine	120 ± 13	180 ± 12*	140 ± 12	133 ± 14

Mean ± SE (μg/g tissue) of 6–10 determinations. *$P < 0.01$ vs control. †vs control prepubertal.

Whereas in 16-day-old prepubertal rats the addition of NMDA to the incubation media increases GnRH release from APOA–MBH 3.7-fold, in the peripubertal group this stimulation was 7.6-fold (Figs 1 and 2).

EP did not modify the stimulatory effect of NMDA on GnRH release in 16-day-old rats. On the contrary, in peripubertal rats, while the GnRH release in the controls was increased by NMDA around 6-fold, in EP pretreated rats the increase was around 48-fold (Fig. 2). These results clearly indicate that in peripubertal rats of 30 days of age, EP potentiated, at the hypothalamic level, the GnRH release response to NMDA neurotransmission.

There are several reports indicating that the activation of NMDA neurotransmission is one of the neuroendocrine mechanisms involved in the increase of gonadotropin secretion during sexual development which induces the onset of puberty [5–7, 10]. In this respect and on the basis of the present results it could

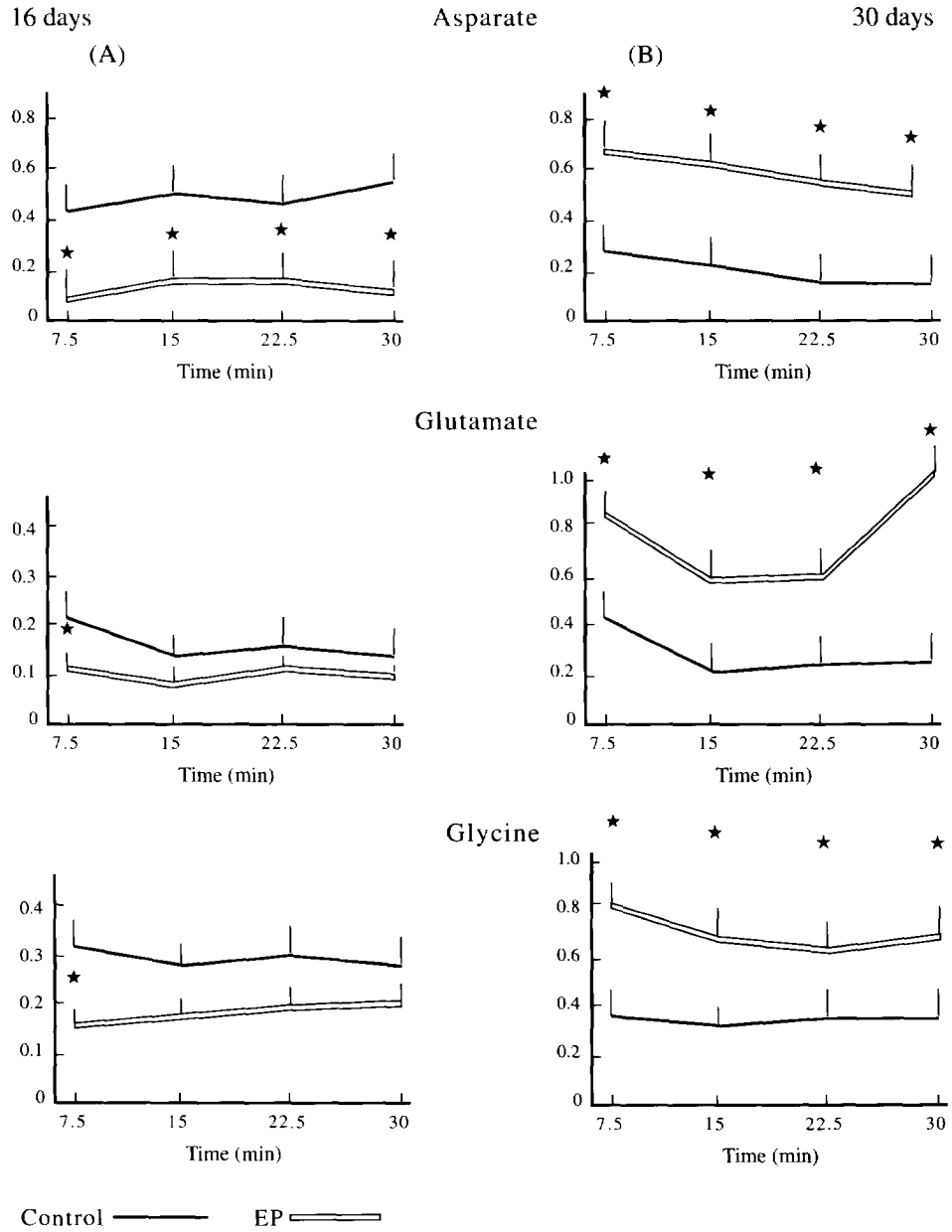

Fig. 3. Effect of EP treatment on the release of amino acids (ng/g tissue/ml medium) by anterior preoptic and medial basal hypothalamic areas in prepubertal (A) and peripubertal (B) female rats Each point represents the mean ± SEM of 10–15 determinations. *$P < 0.01$ vs control. **$P < 0.01$ vs prepubertal controls.

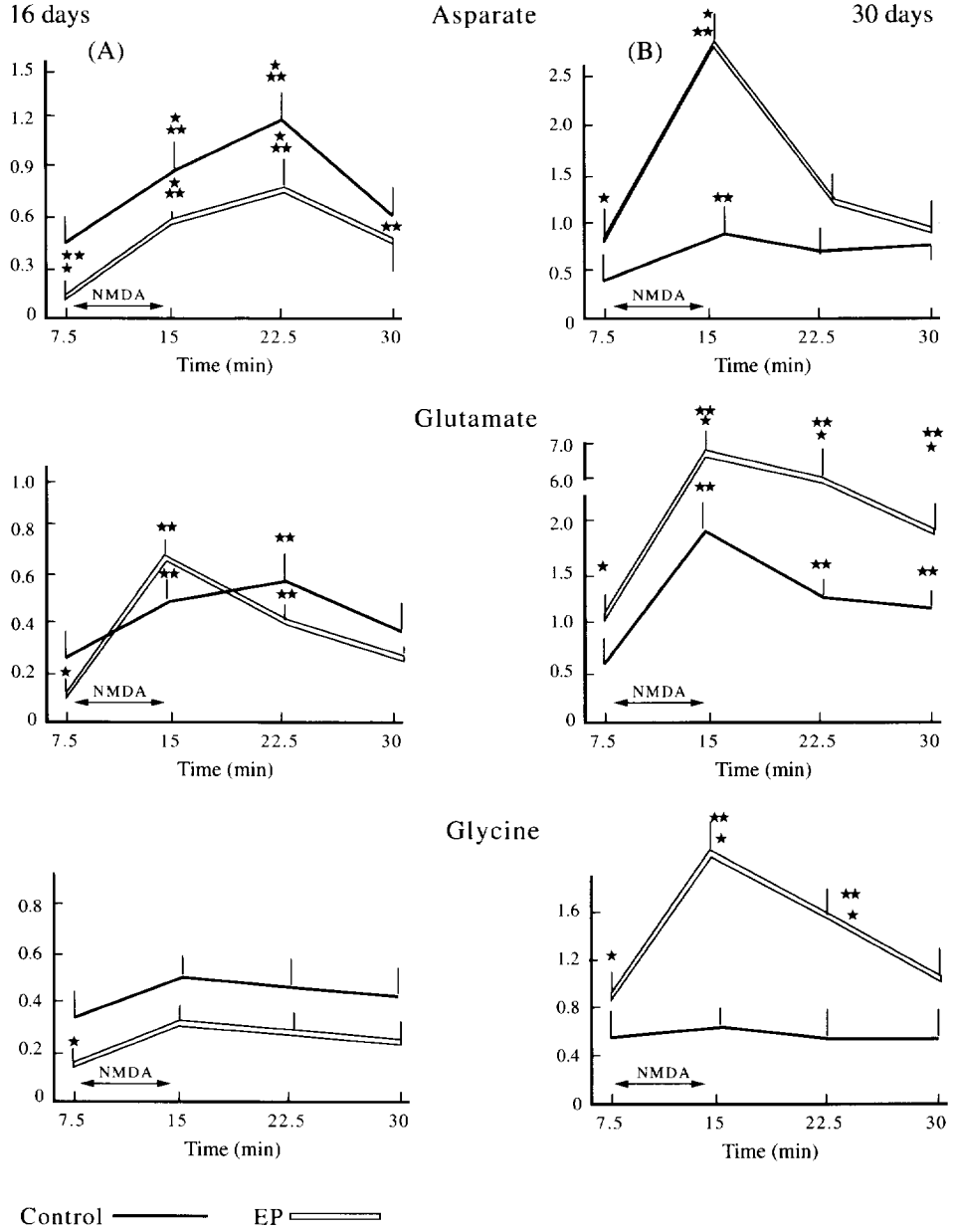

Fig. 4. Effect of EP pretreatment on the amino acids release (ng/g tissue/ml medium) by NMDA in prepubertal (A) and peripubertal (B) female rats. Mean ± SEM of 10–15 determinations. *$P < 0.01$ vs control and ** vs basal (7.5 min).

be considered that the potentiation by the ovarian hormones of the GnRH release response to NMDA that takes place during sexual maturation could be a mechanism directly connected with the changes of gonadotropin secretion that induce the onset of puberty and the sexual cycles of female rats. It is interesting to note that ovarian steroids exert a negative feedback effect on gonadotropin secretion in rats younger than 20–22 days of age and a stimulatory action after this age. The maturation of this positive mechanism of EP on gonadotropin secretion is one of the principal events connected with the onset of puberty and with the sexual cycles [7–9]. It is very probable that the maturation of the potentiating effect of EP on GnRH release induced by NMDA neurotransmission described here could be one of the mechanisms directly connected with the development of this positive mechanism of gonadotropin control. The fact that this mechanism of potentiation is not present in the prepubertal hypothalamus of 16-day-old rats at which age ovarian steroids induce a negative feedback on gonadotropin secretion further supports this point of view.

At around 20–22 days the positive feedback effect of sex hormones on gonadotropin secretion matures [7–9] which is a relevant mechanism involved in the onset of puberty of the female rats. Before this age, ovarian hormone administration induces a negative feedback effect, while after this age EP induces gonadotropin release. The results reported here in prepubertal rats show that EP administration decreases the *in vitro*

hypothalamic release of Asp, Glu and Gly, the EAA involved in NMDA neurotransmission, that stimulate GnRH-LH secretion [2–5, 7, 10]. It is interesting to note that the hypothalamic content of these EAAs was also increased by EP, indicating an inhibitory effect of ovarian hormones on the release rather than on the synthesis of the EAA. Conversely in peripubertal rats EP administration significantly increases the hypothalamic release of Asp, Glu and Gly without modification in the content, and these facts appear to indicate that at this age EP increases the synthesis of the hypothalamic EAA. In spite of the fact that it is difficult to extrapolate from *in vitro* observations to the conditions prevailing in the living animal, these results could explain, at least in part, the negative feedback of ovarian steroids on gonadotropin that is observed in prepubertal rats, on the basis of an inhibitory effect of EP on hypothalamic EAA release. In a similar way, the stimulatory effect of EP on gonadotropin in peripubertal rats appears to be connected with the stimulatory effect of ovarian hormones on hypothalamic EAA synthesis.

Consequently, it is very probable that the change from inhibitory to stimulatory effect of ovarian hormones on EAA release during sexual maturation induces the onset of the positive feedback effect of EP on the gonadotropin secretion mechanism directly involved in the initiation of puberty and sexual cycles in the female rats. It is difficult to explain the intimate mechanism by which EP changes its effect on the EAA during sexual maturation, but it is interesting to point out that we also have previously described qualitative changes in the effect of several neurotransmitters on GnRH secretion during sexual maturation, proposing that these modifications are related to hypothalamic maturation mechanisms that induce the onset of puberty and sexual cycles in the female rat [11–13].

In peripubertal rats, stimulation of NMDA neurotransmission by the NMDA receptor agonist stimulates the hypothalamic release of Asp and Glu and both effects are potentiated by EP. Moreover, NMDA that did not modify Gly release in control rats induced the release of this amino acid in peripubertal rats.

In prepubertal rats, NMDA also increases the hypothalamic release of Glu and of Asp but these effects are not modified by ovarian hormones. Thus, the probable mechanism of regulation (by which EAA induced gonadotropin release through stimulation of NMDA receptors which in turn increase the release of EAA by APOA–MBH) is potentiated by ovarian hormones in peripubertal but not in prepubertal rats. The maturation of this mechanism could also be a very important physiological event involved in the onset of puberty and sexual cycles in female rats since it could be one of the mechanisms involved in gonadotropin release.

In conclusion the present report indicates that during sexual development a hypothalamic mechanism by which ovarian steroids increase GnRH release response to NMDA neurotransmission matures, resulting in an increase in the hypothalamic synthesis of Asp, Glu and Gly, the EAA involved in NMDA neurotransmission. On the other hand, a mechanism by which ovarian steroids increase the hypothalamic release of EAA also matures. NMDA receptor stimulation leads to a positive mechanism which increases the release of Asp and Glu from APOA–MBH both in prepubertal and peripubertal rats, but EP potentiates this mechanism only in peripubertal rats. All of these findings appear to be connected with the increase of gonadotropin that characterizes the onset of puberty and the preovulatory discharge of gonadotropins.

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