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Activation of AMPA receptors inhibits prolactin and estradiol secretion and delays the onset of puberty in female rats

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Abstract

Previous experiments have evidenced the neuroendocrine role of AMPA receptors. Present studies were carried out to obtain information on the role of these receptors in the control of the onset of puberty. To this end, female rats were i.c.v. injected with vehicle or AMPA (agonist of AMPA receptors: 0.1 or 0.5 nmol/day) between 26 and 30 days (Experiment 1), or 30 and 34 days (Experiment 2) of age. Serum concentrations of PRL, LH and estradiol were measured before drug administration, 10 min after the last injection, at vaginal opening (VO) and at first estrus (FE) presentation. In both experiments, AMPA administration inhibited PRL and estradiol secretion without affecting LH release. When AMPA was administered between 26 and 30 days a significant delay in the day of vaginal opening was observed. These results confirmed the inhibitory effect of AMPA on PRL secretion and suggests a role of AMPA receptors in the control of puberty onset.

Keywords: Female puberty; Rat; LH; Prolactin; Estradiol; AMPA

1. Introduction

Excitatory aminoacids (EAAs) are the major activating transmitters in the brain [1]. In recent years, the role of EAA pathways in the control of neuroendocrine function has been firmly established (for a review see [2]). The actions of EAAs are mediated by different postsynaptic receptors which include *N*-methyl-D-aspartate (NMDA) receptors, kainate (KA) receptors, 2-amino-3-hydroxy-5 methylisoxazole-4-propionic acid (AMPA) receptors, and metabotropic receptors [2,3].

NMDA administered as 1-min pulse every 30 min for 5 h between 26 and 29 days of age advanced puberty in female rats by 7 days, while administration of MK801 (an antagonist of NMDA receptors) delayed significantly puberty onset [4,5]. Similar results have been obtained in male monkeys [6]. In contrast, administration of kainic acid (agonist of KA receptors) or DNQX (antagonist of KA receptors) failed to modify the puberty onset in female rats [7].

Recently, we have reported that activation of AMPA receptors stimulates secretion of GH and inhibits that of Prolactin (PRL) in prepubertal male and female rats, without affecting gonadotropin release [8,9]. Since the inhibitory action of AMPA on PRL secretion is limited to prepuberal age (Gonzalez et al., submitted) and considering the role of PRL on pubertal development [10], present experiments were carried out to analyse the possible involvement of AMPA receptors in the onset of puberty in female rats.

2. Materials and methods

2.1. Animals and drugs

Wistar female rats born in our laboratory were kept under controlled conditions of light (12 h light:12 h darkness, lights on at 07:00 h) and temperature (22°C), with free access to pelleted food (Pacsa Sanders, Seville, Spain) and tap water.

On day 1 of life, each dam was left with eight pups. Animals were implanted on days 25 or 29 with i.c.v cannulae under light ether anaesthesia. The i.c.v cannulae was lowered to a depth of 3 mm beneath the surface of the skull; the insert point was 1 mm posterior and

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1.2 mm lateral to bregma. (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, an agonist of AMPA receptors) was purchased from Research Biochemicals International (Natick, USA) and dissolved initially in a few drops of dimethylsulfoxide (DMSO). Thereafter, the drug was dissolved in saline up to the working concentration.

2.2. Experimental designs

In Experiment 1, the animals were i.c.v. injected with AMPA (0.1 or 0.5 nmol/day in 5 μl) or vehicle (5 μl) between 26 and 30 days (at 10.00–12.00 h). The age and body weights at vaginal opening and first estrous presentation were recorded. Animals were killed on day of first estrous. Uteri and ovaries were removed, dissected and weighed. Blood samples were obtained after light ether anaesthesia on day 26 (at the beginning of experiment), on day 30 (10 min after last vehicle or drug injection) and at vaginal opening and first estrous.

In Experiment 2, the animals were i.c.v. injected with AMPA (0.5 nmol/day in 5 μl) or vehicle (5 μl) between days 30 and 34 (at 10.00–12.00 h). Animals were studied similarly than in Experiment 1, except that they were maintained until adulthood to study vaginal cycles.

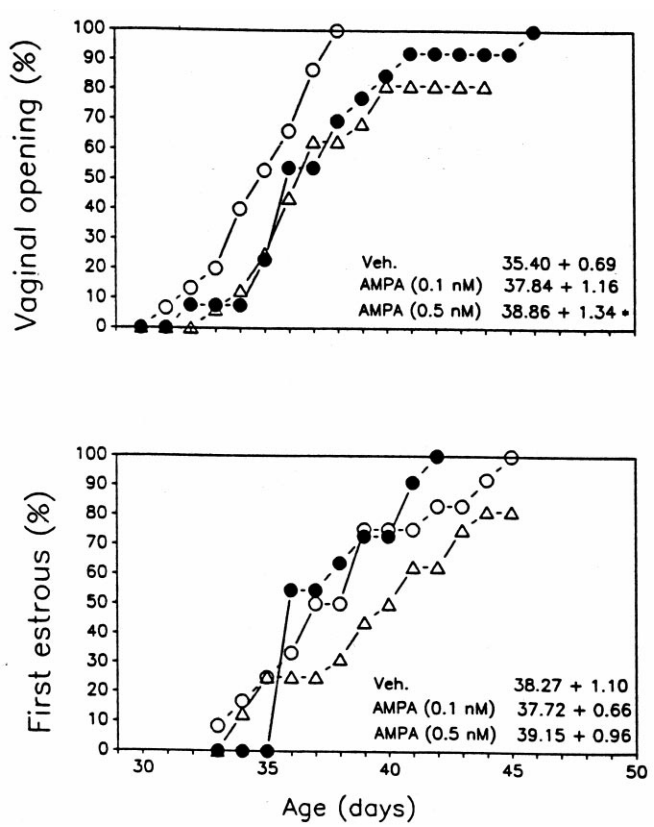

Fig. 1. Effects of AMPA injected i.c.v. bewteen 26 and 30 days of age on the presentation of vaginal opening (VO) and first estrous occurrence. Open circles: animals treated with vehicle; solid circles: animals treated with 0.1 nmol of AMPA; triangles: animals treated with 0.5 nmol of AMPA. * $P \leq 0.05$ vs vehicle injected group (ANOVA followed by Tuke's test).

2.3. Hormone measurements

After centrifugation ($1600 \times g$ at 4°C for 20 min), serum was collected, frozen and stored at −20°C until use. The concentrations of LH and PRL were measured in duplicate in 25–50 μl using a double-antibody RIA method and using kits supplied by NIH (Bethesda, MD). Rat-LH I-9 and Rat-PRL-I-6 were labelled with I^{125} by the Chloramine T method [11] and hormone concentrations were expressed using reference preparation (RP) LH-2 and RP-PRL-3 as standards. Intra- and inter-assay coefficients of variation were below 7 and 10%, respectively. The sensitivities of the assays were 20 and 10 pg per tube, respectively. Estradiol was measured using a kit from Diagnostics Products Corp. (Los Angeles, CA) with a sensitivity of 1 pg per tube.

2.4. Statistics

Results are expressed as means ± SEM. Differences between groups were determined by ANOVA followed by Tukey's test (Experiment 1) or Student's test (Experiment 2).

3. Results

3.1. Experiment 1

Females treated with AMPA showed a delay in the vaginal opening which was significant in the animals injected daily with 0.5 nmol (Fig. 1). In this experimental group, 3 out of 16 animals did not exhibit complete canalization of vaginal duct on day 50. However, the vaginal opening occured at the same body weight in animals injected with vehicle (116 ± 3.8 g) or AMPA (females injected with 0.1 nmol: 116 ± 6.1 g; females injected with 0.5 nmol: 114 ± 3.5 g). The age at first estrous was not affected by AMPA treatment, except in the three animals injected with 0.5 nmol/day in which vaginal opening was not observed.

Control and experimental animals sacrificed on the first estrous showed similar weights of ovaries (vehicle: 42 ± 2.3 mg/100 g body weight; AMPA 0.1 nmol: 43 ± 2.1; AMPA 0.5 nmol: 44 ± 1.4) and uteri (vehicle: 151 ± 7.5 mg/100 g body weight; AMPA 0.1 nmol: 164 ± 7.1; AMPA 0.5 nmol: 158 ± 7.3).

During pubertal development (in this experiment, specifically between days 26 and the first estrous) vehicle-injected animals showed a significant increase in serum concentrations of PRL and estradiol, while serum LH concentrations remained unchanged. Pro-

Table 1
Serum LH, PRL and E_2 concentrations at different ages in female rats injected between 26 and 30 days with vehicle or AMPA (0.1 or 0.5 nmol i.c.v.)[a]

	Day 26		Day 30	Day V.O.	Day 1st estrous
LH		Vehicle	0.71 ± 0.11 (10)	0.52 ± 0.07 (13)	0.57 ± 0.11 (12)
	0.88 ± 0.15 (36)	AMPA 0.1 nmol	0.63 ± 0.12 (8)	0.97 ± 0.10 (9)	0.70 ± 0.12 (8)
		AMPA 0.5 nmol	1.23 ± 0.22 (12)	0.63 ± 0.12 (12)	0.57 ± 0.09 (11)
PRL		Vehicle	17.43 ± 2.23 (13)	99.33 ± 19.27 (13)[c]	20.83 ± 5.73 (11)
	12.03 ± 0.89 (49)	AMPA 0.1 nmol	7.94 ± 1.63(10)[c,d]	108.43 ± 27.07 (9)	17.21 ± 5.31 (9)
		AMPA 0.5 nmol	6.42 ± 0.96 (19)[c,d]	40.06 ± 8.84 (13)[b,d]	10.16 ± 2.08 (12)
E_2		Vehicle	53.95 ± 6.74 (13)[b]	111.06 ± 13.28 (12)[c]	91.72 ± 5.73 (11)[c]
	36.06 ± 2.88 (48)	AMPA 0.1 nmol	52.29 ± 5.25 (13)	63.82 ± 8.11 (9) [d]	86.10 ± 7.38 (10)
		AMPA 0.5 nmol	52.83 ± 7.33 (20)	78.91 ± 10.01 (11)	103.64 ± 10.76 (11)

[a] Values are given as means $\pm$ SEM. Number of animals were indicated in brackets.
[b] $P < 0.05$ vs. levels on day 26.
[c] $P < 0.01$ vs. levels on day 26.
[d] $P < 0.01$ vs. corresponding vehicle injected group (two-way ANOVA followed by Tukey's test).

Table 2
Age (days) and body weight (g) at vaginal opening (VO) and first estrous (FE) in female rats injected i.c.v. with vehicle or AMPA (0.5 nmol) on days 30–34[a]

Treatment	Age at VO	Body weight at VO	Age at FE	Body weight at FE
Vehicle ($n = 12$)	33.0 ± 0.38	116 ± 2.3	34.2 ± 0.7	123 ± 4.5
AMPA ($n = 13$)	33.5 ± 0.74	117 ± 4.4	33.4 ± 0.8	115 ± 4.0

[a] Values are given as means $\pm$ SEM. Number of animals were indicated in brackets.

lactin concentrations showed a significant increase ($P \leq 0.01$) at vaginal opening and estradiol increased significantly ($P \leq 0.01$) at vaginal opening and at first estrous (Table 1).

Serum LH concentrations were unaffected by i.c.v. injection of AMPA. In contrast, significant differences were observed in PRL and estradiol concentrations (Table 1). Serum PRL concentrations were decreased on day 30, 10 min after administration of AMPA (0.1 or 0.5 nmol). The decrease in serum PRL concentrations after administration of 0.5 nmol of AMPA was also observed on the day of VO ($P \leq 0.05$) and at first estrous (although at this time differences were in the limits of significance). Serum estradiol concentrations were significantly lower at VO in animals injected with AMPA (Table 1).

3.2. Experiment 2

Females treated with vehicle or AMPA (0.5 nmol) between 30 and 34 days exhibited vaginal opening and first estrous at the similar age and body weight (Table 2). The animals were checked daily after vaginal opening up to adulthood and control and AMPA-injected groups showed normal cycles and uteri and ovarian weights.

During pubertal development (in this experiment, specifically between days 30 and the first estrous) vehicle-injected animals showed a significant ($P \leq 0.01$) increase in serum concentrations of PRL and estradiol at vaginal opening, while the serum LH concentrations remained unchanged (Table 3).

Serum LH and estradiol concentrations were unaffected by i.c.v. injection of AMPA. In contrast, significant differences were observed in PRL concentrations (Table 3). Serum PRL concentrations were decreased on day 30, 10 min after administration of AMPA (0.5 nmol). [The decrease in serum PRL concentrations after administration of 0.5 nmol of AMPA was also observed on the day of VO ($P \leq 0.05$) (Table 3)].

4. Discussion

Puberty onset is the end-point of a tightly regulated developmental process that involves reciprocal interaction between stimulatory and inhibitory signals. Previous experiments have shown that activation of NMDA receptors advanced puberty in female rats and monkeys [4,6], while activation of kainate receptors was ineffective. Present results indicate that i.c.v. administration of AMPA inhibited PRL secretion and in a short window of pubertal development (between 26 and 30 days) inhibited also estradiol secretion and delayed vaginal opening. As a whole, the results obtained after administration of selective agonists of different receptor sub-

Table 3
Serum LH, PRL and E_2 concentrations at different ages in female rats injected between 30 and 34 days with vehicle or AMPA (0.1 or 0.5 nmol i.c.v.)[a]

	Day 30		Day 35	Day V.O.	Day 1st Estrous
LH		Vehicle	0.57 ± 0.03 (9)	0.64 ± 0.19 (8)	0.51 ± 0.12 (6)
	0.80 ± 0.07 (23)	AMPA 0.5 nmol	0.70 ± 0.07 (11)	0.37 ± 0.07 (7)	0.65 ± 0.30 (5)
PRL		Vehicle	30.39 ± 2.93 (11)	43.86 ± 5.62 (12)[c]	38.97 ± 5.39 (9)[b]
	24.53 ± 2.15 (23)	AMPA 0.5 nmol	14.25 ± 2.34 (13) [d]	38.11 ± 4.12 (11) [d]	32.66 ± 4.00 (9)
E_2		Vehicle	78.26 ± 8.10 (6)	98.61 ± 17.01 (9)[b]	66.21 ± 9.11 (7)
	62.51 ± 4.72 (16)	AMPA 0.5 nmol	80.96 ± 3.98 (5)	71.75 ± 8.32 (8)	87.21 ± 17.65 (5)

[a] Values are given as means $\pm$ SEM. Number of animals were indicated in brackets.
[b] $P < 0.05$ vs. levels on day 30.
[c] $P < 0.01$ vs. levels on day 30.
[d] $P < 0.01$ vs. corresponding vehicle injected group. (two-way ANOVA followed by Tukey's test).

types evidenced that NMDA activation results in precocious puberty [4,7], KA activation was ineffective [7] and AMPA activation delayed puberty onset. These data tend to indicate a complex interplay between endogenous excitatory aminoacids (EAAs) and their receptors and suggest that the final role of EAAs depends on the number and ligand-affinity of different receptor subtypes in hypothalamic areas involved in the onset of puberty. In this sense, during pubertal development an increase in the hypothalamic expression of NMDA receptor subunit NR1 and NR2b has been reported [12]. To our knowledge no study has addressed the evaluation of number, affinity or pattern of mRNA expression of AMPA receptors along pubertal development.

Different mechanisms may account for the reported AMPA-induced delay in puberty onset. Regulation of gonadotropin secretion by EAAs has been reeported [2]. Obviously, alteration of LH/FSH secretion after AMPA treatment may explain changes in timing of puberty. However, in line with our previous references, AMPA failed to alter gonadotropin release, but persistently inhibited PRL secretion.

Compelling evidenced points to a relevant role of PRL on puberty onset. During pubertal development serum PRL concentrations increases [13–16], showing peaks in the prepubertal period [17] and around the day of vaginal opening [15,18]. Hyperprolactinemia induced by hypothalamic lesions [19], dopaminergic antagonists [20,21], pituitary graft [22,23] or hormone administration [24,25] is associated with precocious puberty in the rat. Since AMPA did not change gonadotropin release neither after acute [9] or chronic administration (present results) the effects observed on estradiol secretion and vaginal opening may be derived from its action on PRL release. Present experiments confirmed the increase in serum PRL concentrations during pubertal development and the inhibitory effect induced by AMPA administration. Since PRL increased the number of ovarian LH receptors [21] and the responsiveness to gonadotropins [20], it is tempting to postulate that the decrease in PRL secretion after AMPA injection reduced ovarian LH receptors and decreased the effectiveness of LH leading to a reduced estradiol secretion which in turn delayed vaginal opening. In this sense, the effects of AMPA administration are consistent with the delay in puberty onset observed in other models of chronic suppresion of prolactin secretion [26].

In contrast to effects of AMPA on VO, treatment protocol failed to alter day of first estrous, which indicate that maturation of positive feedback between estradiol and LH was independent of the degree of activation of AMPA receptors.

In conclusion, present results confirmed that activation of AMPA receptors inhibits PRL secretion and suggests a link between a novel element of the neuroendocrine hypothalamic network (the AMPA receptors) and well-known regulators of puberty onset (prolactin and estrogens).

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