Sexual differences in the role of kainate receptors in controlling gonadotrophin secretion in prepubertal rats

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Glutamate stimulates LH secretion in adult female rats after activation of *N*-methyl-D-aspartic acid (NMDA), kainate, and 2-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid receptors. In contrast to the positive role of kainate receptors in the control of LH secretion in adult females, neither activation nor antagonization of kainate receptors in immature rats modified the onset of puberty. The present experiments were carried out to establish why, if kainate stimulates LH release in adult rats, it fails to advance puberty in immature rats, and to determine whether the role of kainate receptors is sexually dimorphic around puberty. In Expt 1, 4-, 8-, 12-, 16-, 20- and 30-day-old females were investigated 15 min after administration of vehicle or kainic acid, a kainate receptor agonist (2.5 mg kg^{-1}). In Expt 2, 30-day-old female rats were studied 2, 5 and 10 min after administration of vehicle, 2.5 mg kg^{-1} kainic acid or NMDA, an NMDA receptor agonist (15 mg kg^{-1}). In Expt 3, female and male rats were gonadectomized or sham-gonadectomized on day 23 and investigated on day 30 after injection of vehicle, kainic acid (2.5 mg kg^{-1} at -15 min) or 6,7 dinitroquinoxaline-2,3 dione (DNQX), a kainate antagonist (2 mg kg^{-1} in two injections at -120 and -60 min). Finally, 30-day-old female and male rats were investigated 15 min after injection of vehicle or NMDA (15 mg kg^{-1}) or 60 min after administration of different doses of 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), an NMDA antagonist (0.1 , 0.25 or 0.50 mg kg^{-1}). The results indicate that the role of kainate receptors in the control of gonadotrophin secretion is sexually dimorphic around puberty, since: (a) LH secretion was stimulated by kainic acid in male rats but inhibited in females; (b) FSH secretion was inhibited by kainic acid in ovariectomized females, but not in orchidectomized males; and (c) DNQX inhibited LH secretion in males but not in females. These differences were specific for kainate receptors since, in both sexes, NMDA stimulated and MK-801 inhibited LH secretion. It may be concluded that the secretion of gonadotrophins is modulated differently by kainate receptors in prepubertal male and female rats.

Introduction

Excitatory amino acids (EAAs) are the major activating neurotransmitters in the brain (van den Pol *et al.*, 1990) and play a crucial role in controlling neuroendocrine function (Brann and Mahesh, 1994). Glutamate or its agonists stimulate GnRH release from hypothalamic fragments (Donoso *et al.*, 1990; Brann and Mahesh, 1991; Arias *et al.*, 1993) and immortalized GnRH neurones (Mahachoklertwattana *et al.*, 1994), as well as LH release *in vivo* (Urbanski and Ojeda, 1987; Luderer *et al.*, 1993; Brann and Mahesh, 1994). Different glutamate receptor subtypes (Watkins, 1994) mediate glutamate effects on GnRH. Specifically, LH secretion is inhibited in adult female rats after administration of different antagonists of *N*-methyl-D-aspartic acid (NMDA) (Brann and Mahesh, 1991), kainate (Ping *et al.*,

1995) and 2-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid (AMPA) receptors (Ping *et al.*, 1997). LH secretion increases after activation of kainate receptors in adult cyclic female rats (Abbud and Smith, 1991, 1993) and in prepubertal males (Aguilar *et al.*, 1996). However, neither activation nor antagonization of kainate receptors affects onset of puberty in female rats (Brann *et al.*, 1993a), in contrast with the ability of NMDA treatment to induce precocious puberty (MacDonald and Wilkinson, 1990; Urbanski and Ojeda, 1990). Normal onset of puberty in female rats treated with kainate and 6,7 dinitroquinoxaline-2,3 dione (DNQX) (Brann *et al.*, 1993a) could be due to a lack of effect of these drugs on gonadotrophin secretion. The present study was undertaken to evaluate this hypothesis and to analyse whether the role of kainate receptors is sexually dimorphic around puberty by evaluating the effects of kainate, NMDA, DNQX and 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801) in prepubertal male and female rats.

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Materials and Methods

Animals and treatments

Prepubertal Wistar rats bred in our laboratory were used. The litter size was adjusted to eight pups per dam on the day of birth (day 1). Animals were weaned at 21 days of age and housed under controlled conditions of temperature (20°C) and light (12 h light:12 h darkness) with free access to pelleted food (Rodent Toxicology Diet; B&K Universal, Barcelona). Kainic acid and NMDA were obtained from Sigma, Barcelona. The kainate antagonist, DNQX, was obtained from Tocris (Langford, Bristol). The NMDA antagonist, MK-801, was purchased from Research Biochemicals International, Natick, MA. Kainic acid, NMDA and MK-801 were dissolved in saline immediately before use. DNQX was dissolved in 45% (w/v) 2-hydroxypropyl- β -cyclodextrin. Doses and timings were selected on the basis of previous studies (Ping *et al.*, 1995; Pinilla *et al.*, 1995, 1996a, b; Aguilar *et al.*, 1996, 1997).

Experimental design

In Expt 1, 4-, 8-, 12-, 16-, 20- and 30-day-old female rats were injected i.p. with vehicle or 2.5 mg kainic acid kg^{-1} and decapitated 15 min later. Since only a weak but significant decrease in serum LH concentrations was observed on day 30, and considering the rapid effect of kainic acid, in a second experiment (Expt 2) 30-day-old females were killed 2, 5 and 10 min after i.p. injection of vehicle, 2.5 mg kainic acid kg^{-1} or 15 mg NMDA kg^{-1} . NMDA was used as a positive control since it is known to stimulate LH release (Carbone *et al.*, 1992). In Expt 3, female and male rats were gonadectomized or sham-gonadectomized on day 23 and killed on day 30 after injection of vehicle or 2.5 mg kainic acid kg^{-1} at -15 min or administration of vehicle or 2 mg DNQX kg^{-1} in two injections at -120 and -60 min to evaluate sexual dimorphism in the role of kainate receptors in the control of gonadotrophin secretion. Finally, 30-day-old female and male rats were killed 15 min after injection of vehicle or 15 mg NMDA kg^{-1} or 60 min after administration of different doses of MK-801 (0.1, 0.25, 0.50 mg kg^{-1}) to analyse whether, as reported for kainate, the role of NMDA receptors in the control of gonadotrophin secretion is sexually dimorphic around puberty. In all experiments, trunk blood was collected. After centrifuging at 1650 g at 4°C for 20 min, serum was collected, frozen and stored at -20°C until use.

Hormone determination

LH and FSH concentrations were measured in duplicate using a double-antibody radioimmunoassay method and reagents supplied by NIH (Bethesda, MD). Rat-LH I-9 and rat FSH-I-8 were labelled with ^{125}I by the chloramine T method (Greenwood *et al.*, 1963). Hormone concentrations were expressed as ng serum ml^{-1} of the reference preparations LH-rat-RP-2 and FSH-rat-RP-3. The intra-assay coefficients of variation were 6 and 7% for LH and FSH, respectively.

Fig. 1. Serum LH and FSH concentrations at different ages in female prepubertal rats decapitated 15 min after injection of vehicle (□) or 2.5 mg kainic acid kg^{-1} (■). Values are given as mean $\pm$ SEM ($n = 10$ animals per group). * $P \leq 0.05$ versus vehicle-injected group (Student's t test). ND: not detectable.

Interassay variabilities were < 12%. Sensitivities were 0.075 ng ml^{-1} and 0.4 ng ml^{-1} for LH and FSH, respectively.

Statistics

Results are expressed as mean $\pm$ SEM. Since the criteria of homogeneity of variance were not fulfilled in all situations, statistical analyses were performed after logarithmic transformation of data. Differences between experimental groups were subjected to analysis of variance (ANOVA) followed by Tukey's test or by Student's t test.

Results

Effects of kainic acid on gonadotrophin secretion at different ages in female rats

Serum LH concentrations during the first week of life were below the limits of detection in most animals and increased later (Fig. 1). Administration of kainic acid did not change LH secretion at any age studied, except for a weak inhibition on day 30. Serum FSH concentrations decreased after day 16 and remained unchanged after kainic acid administration.

Fig. 2. Serum LH and FSH concentrations in 30-day-old female rats decapitated at different times after injection of vehicle (□), 2.5 mg kainic acid kg⁻¹ (■) or 15 mg N-methyl-D-aspartic acid kg⁻¹ (▨). Values are given as mean ± SEM (*n* = 10 animals per group). ***P* ≤ 0.01 versus vehicle-injected group (ANOVA followed by Tukey's test).

Time course of kainic acid and N-methyl-D-aspartic acid actions on gonadotrophin secretion in 30-day-old females

Serum LH concentrations increased significantly 5 and 10 min after administration of NMDA, while changes after treatment with kainic acid were not significant (Fig. 2). Serum FSH concentrations were not significantly affected by administration of NMDA or kainic acid.

Effects of kainic acid and 6,7 dinitroquinoxaline-2,3 dione on gonadotrophin secretion in intact and gonadectomized rats

Serum LH and FSH concentrations significantly increased 1 week after ovariectomy. Kainic acid inhibited LH secretion in intact and ovariectomized females and FSH secretion in ovariectomized females. In contrast to this inhibitory effect, kainic acid significantly stimulated LH release in intact male rats (Table 1).

In female rats, serum LH and FSH concentrations remained unchanged after administration of 2 mg DNQX kg⁻¹ in two doses at -120 and -60 min. In contrast, DNQX significantly decreased LH secretion in intact and orchidectomized males, while serum FSH concentrations remained unchanged.

Table 1. Serum concentrations of LH and FSH in intact and gonadectomized female and male rats decapitated after treatments with kainic acid or 6,7 dinitroquinoxaline-2,3 dione (DNQX)

Group	Treatment	LH (ng ml ⁻¹)	FSH (ng ml ⁻¹)
Intact females	Vehicle	0.76 ± 0.03	4.18 ± 0.44
	Kainic acid	0.44 ± 0.01*	3.98 ± 0.39
Ovariectomized females	Vehicle	5.22 ± 0.38	59.44 ± 3.02
	Kainic acid	2.60 ± 0.35*	47.40 ± 1.85*
Intact females	Vehicle	0.56 ± 0.03	3.98 ± 0.61
	DNQX	0.48 ± 0.01	4.44 ± 0.56
Ovariectomized females	Vehicle	6.08 ± 0.57	51.52 ± 1.73
	DNQX	7.96 ± 1.53	57.20 ± 3.43
Intact males	Vehicle	0.45 ± 0.15	15.86 ± 1.40
	Kainic acid	1.18 ± 0.08**	17.45 ± 1.01
Intact males	Vehicle	0.98 ± 0.34	19.50 ± 1.37
	DNQX	0.14 ± 0.09**	18.13 ± 1.12
Orchidectomized males	Vehicle	9.03 ± 1.28	48.57 ± 3.03
	DNQX	4.21 ± 0.82**	45.97 ± 1.90

Values are given as means ± SEM of ten animals per group.

Animals were injected with 2.5 mg kainate kg⁻¹ at -15 min and with 1 mg DNQX kg⁻¹ at -120 and -60 min.

P* ≤ 0.05, *P* ≤ 0.01 versus corresponding vehicle-injected group (ANOVA followed by Tukey's test).

Effects of N-methyl-D-aspartic acid and MK-801 on gonadotrophin secretion in intact rats

Secretion of LH increased significantly in males and females 15 min after administration of 15 mg NMDA kg⁻¹ and decreased 60 min after administration of different doses of MK-801 (Fig. 3).

Discussion

Puberty in female rats is a complex process initiated at the hypothalamus. Prepubertal GnRH neurones have low activity that is enhanced at the end of the juvenile period by an increase in stimulatory inputs (Ojeda and Urbanski, 1994). Some neurotransmitters, such as neuropeptide Y, noradrenaline and EAAs, have been implicated in the prepubertal activation of GnRH neurones (Ojeda and Urbanski, 1994). The actions of EAAs are mediated by activation of different postsynaptic receptor subtypes, such as NMDA, kainate and AMPA receptors. Activation of NMDA receptors advances the time of puberty in female rats (Urbanski and Ojeda, 1987; MacDonald and Wilkinson, 1990; Brann *et al.*, 1993a), while administration of antagonists of NMDA receptors, such as MK-801 or DL,2-amino-5-phosphono valeric acid (AP-5), delays vaginal opening and blocks the oestradiol-induced LH surge in prepubertal ovariectomized rats (Urbanski and Ojeda, 1990; Meijis Roelofs *et al.*, 1991). In contrast, the onset of puberty remained unaffected after activation or antagonization of kainate receptors with kainic acid or DNQX, respectively (Brann *et al.*, 1993a). The basis for this finding remains unexplained, since LH and FSH concentrations after treatment with kainic acid or

Fig. 3. Serum LH concentrations in 30-day-old (a) female and (b) male rats decapitated 15 min after injection of 15 mg *N*-methyl-D-aspartic acid (NMDA) kg⁻¹ (▨) or 60 min after injection of different doses (0.1, 0.25 and 0.50 mg kg⁻¹) of the NMDA antagonist MK-801 (■). Values from animals decapitated 15 or 60 min after vehicle injection were similar and were pooled for presentation (□). Values are given as mean ± SEM (*n* = 10 animals per group, except vehicle-injected group which included 20 animals). **P* ≤ 0.05, ***P* ≤ 0.01 versus vehicle-injected group (ANOVA followed by Tukey's test).

DNQX were not measured in these experiments (Brann *et al.*, 1993a).

In adult male and female rats, NMDA and kainic acid stimulate LH secretion (Price *et al.*, 1978; Schainker and Cicero, 1980; Abbud and Smith, 1991; Luderer *et al.*, 1993; Strobl *et al.*, 1993) and the antagonists of NMDA (AP-5, MK-801) and kainate receptors (DNQX) suppress pulsatile LH secretion in adult male rats (Ping *et al.*, 1995) and ovulatory LH surges in females (López *et al.*, 1990; Brann and Mahesh 1991, 1993b). NMDA increases LH secretion in prepubertal males (Aguilar *et al.*, 1996) and females (Carbone *et al.*, 1992; MacDonald and Wilkinson 1992). Aguilar *et al.* (1996) showed a clear increase in serum LH concentrations after administration of 2.5 mg kainic acid kg⁻¹ to prepubertal male rats. However, similar studies in prepubertal females have not yet been performed.

The present study was undertaken to analyse changes in LH and FSH secretion in prepubertal females after activation or antagonization of kainate receptors. Initially, a weak decrease in LH secretion was observed 15 min after administration of kainic acid in 30 day-old females. In the later experiments, this inhibitory action was observed in intact and ovariectomized

females. Furthermore, kainic acid was also able to reduce FSH secretion in ovariectomized female rats. Since the effects of EAAs appear to be mediated through changes in the release of GnRH (Gay and Plant, 1987; Bourguignon *et al.*, 1989; Donoso *et al.*, 1990; Strobl *et al.*, 1993), the findings of the present study point to an inhibition of GnRH secretion after activation of kainate receptors.

The effects of DNQX, a potent kainate receptor antagonist, on gonadotrophin secretion were analysed to evaluate whether kainate receptors are activated tonically in prepubertal female rats. Serum LH and FSH concentrations remained unchanged after administration of DNQX to intact and ovariectomized females, suggesting that gonadotrophin secretion, both in basal conditions and after ovariectomy, is not modulated by kainate receptors. On the contrary, the decrease in LH concentrations after administration of MK-801 points to a physiological role of NMDA receptors in the control of LH release in prepubertal female rats. Taken as a whole, the results of the present study suggest that the role of kainate receptors in the control of gonadotrophins during puberty in females is not as important as that of NMDA receptors. This finding may explain why the onset of puberty in female rats was not affected by administration of kainic acid or DNQX (Brann and Mahesh, 1993a).

At present, it is not clear why kainate does not stimulate LH in prepubertal female rats, while doing so in adult females. The ability of AMPA and NMDA receptor agonists to stimulate LH release was found to require a background of oestrogen (Brann and Mahesh, 1992; Ping *et al.*, 1997). It is possible that the kainate receptors involved in the regulation of LH release were upregulated by oestrogens and remained ineffective before puberty, when oestradiol concentrations were low. Alternatively, it is possible that, before puberty, kainate stimulates the secretion of an LH release-inhibiting factor of hypothalamic origin (Hwan and Freeman, 1987).

The inability of manipulations (activation or antagonization) of kainate receptors to modify gonadotrophin secretion in prepubertal females, where only a minor inhibitory effect of kainic acid on LH secretion was observed, is in marked contrast to data obtained for prepubertal males, in which kainic acid elicited LH release (Aguilar *et al.*, 1996). This finding suggests that the role of kainate receptors in the neuronal circuitry governing GnRH release is sexually dimorphic, at least before puberty. This dimorphism seems to be restricted to the involvement of kainate receptors in the control of GnRH secretion, since kainic acid stimulated GH release in both prepubertal male and female rats (Tena-Sempere *et al.*, 1995) and inhibited prolactin release in both sexes (Pinilla *et al.*, 1996a, b).

In conclusion, the results of the present study indicate that the role of kainate receptors in the control of gonadotrophin secretion in prepubertal rats is sexually dimorphic, being stimulatory only in males. Data obtained also suggest that, unlike NMDA receptors, kainate receptors play only a minor role in the control of gonadotrophin secretion in prepubertal female rats.

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