Regulation of prolactin secretion by α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors in male rats

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Abstract

The secretion of PRL is controlled by different hypothalamic signals. Depending on the experimental model, PRL secretion increases or decreases after activation of N-methyl-D-aspartic acid and kainate receptors. Recently we have described that activation of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors inhibits PRL secretion in prepubertal male rats. The aim of present study was to examine (1) the physiological relevance of this finding, (2) the possible age-related changes observed after activation or blockade of AMPA receptors, (3) the specificity of the AMPA effect, (4) the hypothalamic and/or pituitary localization of AMPA action, and (5) the mechanism(s) of action of AMPA agonists.

In a first set of experiments, neonatal males (5 and 10 days old) and prepubertal (23 days old) male rats were injected with AMPA (1, 2.5 or 5 mg/kg) or the antagonist of AMPA receptors 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo (f) quinoxaline-7-sulfonamide (NBQX; 0.25 or 0.50 mg/kg). Serum PRL concentrations decreased significantly 15 and 30 min after i.p. administration of AMPA in prepubertal male rats, while the inhibitory effect of AMPA was not observed in 5- and 10-day-old males. The effect of AMPA was abolished by NBQX but not by

MK-801 (a selective antagonist of NMDA receptors). NBQX alone (0.25 or 0.50 mg/kg) had no effect on PRL release. *In vitro*, AMPA slightly stimulated PRL secretion by hemipituitaries from prepubertal males, suggesting that the hypothalamus is likely the site of action for the reported inhibitory action of AMPA on PRL release. In this sense, the blockade of AMPA effects in animals pretreated with domperidone (a dopaminergic antagonist) or α -methyl-p-tyrosine (an inhibitor of dopamine synthesis) suggests that an increase in the release of hypothalamic dopamine is probably the mechanism involved in the effect of AMPA. In a second set of experiments, the effects of AMPA (2.5 mg/kg i.p.) and NBQX (0.5 mg/kg i.p. and 20 or 40 nmol i.c.v.) were tested in freely moving adult male rats sampled during periods of 2, 3 or 6 h. In contrast with data obtained in prepubertal rats, neither AMPA nor NBQX affected PRL secretion.

In conclusion, these data indicate that activation of AMPA receptors inhibits PRL secretion in prepubertal male rats. This effect probably involves the release of dopamine from the hypothalamus and disappears in adulthood.

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Introduction

Hypothalamic control of prolactin (PRL) secretion involves different PRL-releasing (PRF) and -inhibitory factors (for review see Neill & Nagy 1994). Excitatory amino acids (EAAs) are the major activating transmitters in the brain (van den Pol *et al.* 1990). In recent years, many authors have firmly established the role of EAA pathways in the control of neuroendocrine function (for review see Brann & Mahesh 1997). The actions of EAAs are mediated by different post-synaptic 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 (Collinbridge & Watkins 1994, Brann & Mahesh 1997). Depending on the experimental paradigm, NMDA and KA either stimulated or inhibited PRL secretion (Abbud & Smith 1991, Luderer *et al.* 1993, Carbone *et al.* 1994, Pinilla *et al.* 1996, 1998). It has been proposed that the effects of glutamate agonists on PRL secretion depend on the previous circulating concentrations of the hormone, with a conversion of the PRL response from stimulation when basal secretion is low to an inhibitory effect in hyperprolactinaemic situations (Abbud & Smith 1991). However, previous data from our laboratory indicated that, at least partially, the dual effect of NMDA on PRL

secretion is independent of prior PRL concentrations (Pinilla *et al.* 1998).

AMPA receptors are involved in the steroid-induced luteinizing hormone (LH) surge (Ping *et al.* 1997) and activation of AMPA receptors stimulates gonadotrophin-releasing hormone (GnRH) release from rat hypothalamic fragments and from immortalized GnRH neurones (Donoso *et al.* 1990, Spergel *et al.* 1994, Zuo *et al.* 1996). We reported very recently that activation of AMPA receptors stimulates growth hormone (GH) release and inhibits PRL secretion in prepubertal male and female rats (González *et al.* 1999a,b). The experiments presented herein were set out to obtain additional information about the control of PRL secretion by AMPA receptors. Specifically we examine (1) the physiological relevance of this finding, (2) the possible age-related changes after activation or blockade of AMPA receptors, (3) the specificity of the AMPA effect, (4) the hypothalamic and/or pituitary localization of AMPA action, and (5) the mechanism(s) of action of AMPA agonists.

Materials and Methods

Animals and drugs

Wistar rats born in our laboratory were kept under controlled conditions of light (12 h light:12 h darkness, lights on at 0700 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. ($\pm$)- α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA; an agonist of AMPA receptors), 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo (f) quinoxaline-7-sulfonamide (NBQX; an antagonist of AMPA receptors), dizolcipine maleate (MK-801; an antagonist of NMDA receptors) and domperidone (a dopaminergic antagonist) were purchased from Research Biochemicals International (Natick, MA, USA). Diethyl-dithiocarbamate (DDC; an inhibitor of dopamine- β -hydroxylase); α -methyl-p-tyrosine (α -MPT; an inhibitor of tyrosine hydroxylase) and 5-hydroxytryptophan methyl ester (5-HTP; a precursor of serotonin synthesis) were obtained from Sigma (Barcelona, Spain). Fluoxetine (an inhibitor of serotonin reuptake) was generously supplied by Dista (Barcelona, Spain). AMPA and NBQX were dissolved initially in a few drops of dimethylsulfoxide and domperidone in a few drops of methanol. Thereafter, the drugs were dissolved in saline up to the working concentration. The doses and route of administration of different drugs as well as the time-points for hormone measurements were selected based on the previously reported effectiveness (Negro-Vilar *et al.* 1979, Martin *et al.* 1995, Pinilla *et al.* 1998, 1999a, Gonzalez *et al.* 1999a,b) and the absence of behavioural changes. In addition, to analyse the effectiveness of AMPA to change PRL secretion after blockade of different receptor subtypes as well and in

hyper- and hypo-prolactinaemic situations, NBQX, MK-801, α -MPT, DDC, 5-HTP and fluoxetine were injected prior to AMPA administration.

Experimental designs

Experimental procedures were approved by the Córdoba University Ethical Committee for animal experimentation and were conducted in accordance with the European Union norms for care and use of experimental animals.

Neonatal and prepubertal males

In experiment 1, 23-day-old male rats ($n=10$ per group) were killed 15, 30 and 60 min after i.p. injection of AMPA (2.5 or 5 mg/kg) or vehicle and 60 and 120 min after i.p. injection of NBQX (0.25 and 0.50 mg/kg) or vehicle. Special precautions were taken to avoid any stressful influences (e.g. all animals were handled daily for a week before the experiment and killed by the same person, the different drugs being injected at random). Trunk blood was collected in polystyrene tubes and after centrifugation (1600 g at 4 °C for 20 min) serum was collected, frozen and stored at -20 °C until use. Pituitaries were removed, dissected and homogenized in 1 ml physiological saline containing urea (2.5 mol/l) by ultrasonic treatment (Haggi & Aoki 1981). Samples were centrifuged for 20 min at 2800 g and the supernatant was frozen at -20 °C until analysis for hormone content. In experiment 2, in order to detect the onset of the inhibitory effect of AMPA on PRL secretion, 5-, 10- and 23-day-old males ($n=10-12$ per group) were decapitated 15 min after administration of 1 or 2.5 mg/kg AMPA. In experiment 3, in order to determine whether the inhibitory effect of AMPA was mediated by its specific receptors, 23-day-old males ($n=10$ per group) were injected with NBQX (antagonist of AMPA receptors; 0.5 mg/kg) or MK-801 (antagonist of NMDA receptors; 0.25 mg/kg) at -60 min and with vehicle or AMPA (2.5 mg/kg) at -15 min. In experiment 4, in order to detect possible actions of AMPA at pituitary level in the control of PRL secretion, anterior hemipituitaries (10-12 per group) were obtained from 23-day-old male rats and placed in glass scintillation vials (one per vial) in a Dubnoff shaker at 38 °C in an atmosphere of 95% O₂-5% CO₂. Each vial contained 1 ml Dulbecco's Minimal Essential Medium (DMEM). After preincubation for 60 min, the medium was replaced by fresh medium containing AMPA (10^{-8} to 10^{-6} M). Samples of medium were obtained at 60 and 120 min of the incubation period. In experiment 5, in order to determine the possible involvement of dopamine on AMPA effects, 23-day-old males ($n=10$ per group) were injected with vehicle or domperidone (1 mg/kg at -60 min) and with vehicle or AMPA (2.5 mg/kg at -15 min). Other animals ($n=10$ per group) were injected with 250 mg/kg α -MPT (inhibitor of dopamine synthesis) or 500 mg/kg DDC

(inhibitor of dopamine- β -hydroxylase) 3 h before administration of vehicle or AMPA (2.5 mg/kg) and the animals were killed 15 min later. In experiment 6, in order to evaluate the ability of AMPA to inhibit serotonin-stimulated PRL secretion, 23-day-old males ($n=10$ per group) were injected s.c. with fluoxetine (10 mg/kg) and 5-HTP (100 mg/kg) 45 min before administration of vehicle or AMPA (2.5 mg/kg) and the animals were killed 15 min later.

Adult male rats

In this set of experiments, the role of AMPA receptors in the control of PRL secretion was studied in freely moving adult male rats. In experiment 7, the animals ($n=6-8$ per group) were implanted with intracardiac cannulae under sodium pentobarbital (50 mg/kg) anaesthesia. After surgery, the animals were placed directly in isolation test chambers for 5 days. In order to evaluate the role of AMPA and NBQX administered systemically, the animals were sampled for 2 h at 15-min intervals. At the end of the first hour of sampling, the animals were injected i.p. with vehicle, AMPA (2.5 mg/kg) or NBQX (0.5 mg/kg). In experiment 8, the animals were implanted with intracardiac and i.c.v. cannulae under sodium pentobarbital (50 mg/kg) anaesthesia. In order to evaluate the role of NBQX administered i.c.v., groups of male rats ($n=10$ per group) were sampled at 5-min intervals for periods of 3 h (1000–1300 h) as previously described (Carro *et al.* 1997). After the two initial samples, the animals received either vehicle or NBQX (20 nmol in 10 μ l) through the i.c.v. route. During the sampling period, the volume of blood withdrawn was replaced hourly by a suspension of blood cells in sterile saline warmed at 38 °C. Other animals ($n=5-6$ per group) were sampled at 15-min intervals for periods of 6 h (1000–1600 h).

PRL measurements

The PRL concentrations were measured in duplicate aliquots using a double-antibody method and radioimmunoassay (RIA) kits supplied by NIH (Bethesda, MD, USA). Rat-PRL-I-6 was labelled with iodine-125 by the chloramine T method (Greenwood *et al.* 1963) and rat-PRL-RP-3 was used as standard. Serum PRL concentrations were measured in 25–50 μ l aliquots and media from pituitary incubations were diluted 1/100 and 10 μ l of this solution used for PRL measurements. Intra- and interassay variations were 9% and 12% respectively, and assay sensitivity was 10 pg/tube.

Statistical analysis

Data are expressed as means $\pm$ S.E.M. Data were homogeneous and no transformation was carried out. Intergroup differences were determined by one- or two-

way analysis of variance (ANOVA) followed by Tukey's test. Specifically, we used two-way ANOVA to analyse interaction between different variables in experiment 1 (serum PRL concentrations at different times after different treatments), experiment 2 (serum PRL concentrations at different ages after different treatments), experiment 4 (PRL concentrations after different times of incubation in the presence of different doses of AMPA), and experiments 5 and 6 (effects of AMPA in animals previously treated with different drugs). Assessment of pulsatile PRL secretion was carried out using the ULTRA program (E Van Cauter, Department of Medicine, University of Chicago, Chicago, IL, USA).

Results

Effects of AMPA and NBQX on serum and pituitary PRL concentrations in 23-day-old male rats (experiment 1)

Administration of AMPA decreased PRL concentrations significantly ($P \leq 0.01$, two-way ANOVA). The analysis of interactions evidenced that the effect was significant 15 and 30 min after administration of 2.5 or 5 mg/kg AMPA and returned to basal levels 60 min after drug injection (Fig. 1). Serum concentrations of PRL remained unchanged in male rats 1 and 2 h after administration of NBQX (data not shown). Pituitary concentrations of PRL remained unchanged after AMPA or NBQX administration (data not shown).

Effects of AMPA on serum PRL concentrations in infantile rats (experiment 2)

Serum PRL concentrations were higher in 23-day-old animals than in neonatal ones ($P \leq 0.01$, two-way ANOVA). The analysis of interactions evidenced that the inhibitory effect of AMPA was not observed in 5- and 10-day-old males (Table 1).

Effects of pretreatment with NBQX and MK-801 on the inhibitory action of AMPA on PRL secretion (experiment 3)

Since it has been suggested that the same agonist might bind to more than one receptor subtype (Zanisi *et al.* 1994), we checked the effects of AMPA in the presence of NBQX (antagonist of AMPA receptors) and MK-801 (antagonist of NMDA receptors). In this experiment there was evidence that antagonization of NMDA receptors with MK-801 and activation of AMPA receptors significantly ($P \leq 0.01$) inhibited PRL secretion, the effects of MK-801 and AMPA being additive. NBQX blocked the AMPA action and the serum PRL concentrations were similar in animals treated with NBQX or NBQX+AMPA (Table 2).

Figure 1 Serum PRL concentrations in 23-day-old male rats 15, 30 and 60 min after i.p. administration of vehicle (open bars) or AMPA (hatched bars: 2.5 mg/kg; solid bars: 5 mg/kg). Values are given as means $\pm$ S.E.M. (10 animals per group). * $P \leq 0.05$, ** $P \leq 0.01$ vs vehicle-injected group (two-way ANOVA followed by Tukey's test).

Effects of AMPA on PRL secretion by hemipituitaries in vitro (experiment 4)

PRL concentrations in media were significantly higher at 120 min than at 60 min of incubation ($P \leq 0.01$, two-way ANOVA). Incubation of pituitary tissue in the presence of AMPA failed to inhibit PRL release. Furthermore, the analysis of interactions indicated a significant increase ($P \leq 0.01$) 120 min after incubation with the lowest dose tested (10^{-8} M) (Table 3).

Effects of changes in dopaminergic tone on the inhibitory action of AMPA on PRL secretion (experiment 5)

Domperidone (antagonist of dopaminergic D2 receptors) prompted a clearcut increase ($P \leq 0.01$, two-way ANOVA) in serum PRL concentrations. The analysis of interactions showed that the inhibitory effect of AMPA disappeared in domperidone-treated rats (Table 4).

α -MPT (a blocker of tyrosine hydroxylase that reduced dopamine synthesis) significantly increased serum PRL concentrations and abolished the inhibitory effect of AMPA (Table 4). In contrast, animals pretreated with DDC (inhibitor of dopamine- β -hydroxylase) showed a significant reduction in serum PRL concentrations. In these animals, a further decrease in PRL concentrations was induced by AMPA administration (Table 4).

Effects of AMPA on serotonin-stimulated PRL secretion (experiment 6)

Serum PRL concentrations increased significantly after administration of fluoxetine and 5-HTP ($P \leq 0.01$, two-way ANOVA). The analysis of interactions between treatments evidenced that the serotonin-stimulated PRL release was not inhibited by AMPA (Table 5).

Table 1 Serum PRL concentrations in 5-, 10- and 23-day-old male rats decapitated 15 min after AMPA administration. Values are given as means $\pm$ S.E.M. (10–12 animals per group)

Age (days)	Treatment	PRL (ng/ml)
5	Vehicle	2.08 $\pm$ 0.46
	AMPA (1 mg/kg)	1.42 $\pm$ 0.08
	AMPA (2.5 mg/kg)	1.89 $\pm$ 0.28
10	Vehicle	1.51 $\pm$ 0.22
	AMPA (1 mg/kg)	1.03 $\pm$ 0.10
	AMPA (2.5 mg/kg)	1.36 $\pm$ 0.20
23	Vehicle	9.37 $\pm$ 2.17
	AMPA (1 mg/kg)	3.63 $\pm$ 0.57**
	AMPA (2.5 mg/kg)	2.97 $\pm$ 0.44**

** $P \leq 0.01$ vs vehicle-injected group (two-way ANOVA followed by Tukey's test).

Table 2 Serum PRL concentrations in 23-day-old males injected with vehicle, NBQX (0.50 mg/kg at -60 min), MK-801 (0.25 mg/kg at -60 min), AMPA (2.5 mg/kg at -15 min), NBQX+AMPA or MK-801+AMPA. Values are given as means $\pm$ S.E.M. (10 animals per group)

Treatment	PRL (ng/ml)
Vehicle	5.27 $\pm$ 0.81 ^a
NBQX	9.57 $\pm$ 2.18 ^a
MK-801	2.32 $\pm$ 0.26 ^b
AMPA	3.11 $\pm$ 0.46 ^b
NBQX+AMPA	5.59 $\pm$ 1.17 ^a
MK-801+AMPA	1.41 $\pm$ 0.19 ^c

Groups with different superscript letters were statistically significant ($P \leq 0.05$, one-way ANOVA followed by Tukey's test).

Table 3 PRL secreted by hemipituitaries incubated in the presence of different concentrations of AMPA. Values are given as means $\pm$ S.E.M. (10–12 hemipituitaries per group)

	PRL (ng/ml)	
	60 min	120 min
DMEM	0.09 $\pm$ 0.01	0.18 $\pm$ 0.02
AMPA (10^{-6} M)	0.11 $\pm$ 0.01	0.16 $\pm$ 0.02
AMPA (10^{-7} M)	0.14 $\pm$ 0.02	0.30 $\pm$ 0.05
AMPA (10^{-8} M)	0.16 $\pm$ 0.02	0.43 $\pm$ 0.07**

** $P \leq 0.01$ vs DMEM (two-way ANOVA followed by Tukey's test).

Effects of systemic administration of AMPA and NBQX on PRL secretion in adult male rats (experiment 7)

In freely moving adult male rats, serum PRL concentrations showed a tendency to decrease during the sampling period. Neither AMPA nor NBQX administered systemically affected PRL secretion (data not shown).

Table 4 Effects of AMPA (2.5 mg/kg at -15 min) in 23-day-old male rats with or without pretreatment with domperidone (1 mg/kg at -60 min), α -MPT (250 mg/kg at -180 min) or DDC (500 mg/kg at -180 min). Values are given as means $\pm$ S.E.M. (10 animals per group)

Treatment		PRL (ng/ml)
-60 min	-15 min	
Vehicle	+vehicle	4.69 $\pm$ 2.24
Vehicle	+AMPA	1.78 $\pm$ 0.31**
Domperidone	+vehicle	56.53 $\pm$ 12.16 ^a
Domperidone	+AMPA	39.73 $\pm$ 5.81 ^a
-180 min	-15 min	
Vehicle	+vehicle	12.84 $\pm$ 2.13
Vehicle	+AMPA	4.34 $\pm$ 1.56**
α -MPT	+vehicle	27.49 $\pm$ 3.05 ^a
α -MPT	+AMPA	21.34 $\pm$ 2.88 ^a
DDC	+vehicle	2.08 $\pm$ 0.33 ^a
DDC	+AMPA	1.21 $\pm$ 0.11***

^a $P \leq 0.01$ vs corresponding vehicle-injected group; ** $P \leq 0.01$ vs corresponding group not injected with AMPA (two-way ANOVA followed by Tukey's test).

Table 5 Effects of AMPA (2.5 mg/kg at -15 min) in 23-day-old male rats with or without pretreatment with fluoxetine (Fx) and 5-HTP (10 and 100 mg/kg respectively, at -60 min). Values are given as means $\pm$ S.E.M. (10 animals per group)

Treatment	PRL (ng/ml)
Vehicle+vehicle	6.92 $\pm$ 1.68 ^a
Vehicle+AMPA	1.46 $\pm$ 0.20 ^b
Fx+5-HTP+vehicle	44.28 $\pm$ 6.13 ^c
Fx+5-HTP+AMPA	55.87 $\pm$ 9.94 ^c

Groups with different superscript letters were significantly different ($P \leq 0.01$) (two-way ANOVA followed by Tukey's test).

Effects of i.c.v. administration of NBQX on PRL secretion in adult male rats (experiment 8)

Vehicle-injected animals sampled for 3 or 6 h showed pulsatile PRL secretion (pulse frequency: 4.77 ± 0.4 pulses/6 h; pulse duration: 63 ± 4.7 min; pulse amplitude: 5.98 ± 3.8 ng/ml; mean PRL levels: 4.43 ± 1.12 ng/ml; through levels 1.38 ± 0.05 ng/ml; area under the curve: 864 ± 136), which remained unaffected after i.c.v. injection of NBQX (20 or 40 nmol) (data not shown).

Discussion

The present results clearly demonstrate that activation of AMPA receptors inhibits PRL secretion in prepubertal male rats, probably through a hypothalamic mechanism

involving the release of dopamine. Antagonization of AMPA receptors with NBQX did not change PRL secretion in males, in contrast with the stimulatory effect reported previously in females (González *et al.* 1999b), which suggests a possible sexually dimorphic role of AMPA receptors in the control of PRL secretion, a finding also described for GH (González *et al.* 1999b).

Activation of AMPA receptors had no effect in neonatal rats as significant inhibition of PRL secretion was not observed on days 5 and 10 of life. This finding is in striking contrast with the effects observed on GH, since AMPA significantly stimulated GH on day 5 (from 15.42 ± 2.56 ng/ml to 146 ± 37 ng/ml 15 min after administration of 1 mg/kg AMPA) (González *et al.* 1999a). These results suggest that the acquisition of AMPA receptor-mediated regulatory mechanisms for different pituitary hormones displays a different timing.

In the present study, pulsatile secretory profiles of PRL in adulthood were similar to those previously described (López *et al.* 1991). Our results indicate that the role of AMPA receptors in the control of PRL secretion disappeared in adulthood, since neither AMPA nor NBQX affected PRL secretion. Absence of effects were observed after systemic or i.c.v. administration of drugs, indicating that the ineffectiveness of these drugs was independent of their ability to cross the brain–blood barrier. Loss of the effects of AMPA on PRL secretion in adult rats is specific since, in the same experimental paradigm, AMPA administration increased GH secretion (González *et al.* 1999a). Our results in adult male rats contradict those of Wagner *et al.* (1994a,b) showing that systemic administration of NBQX decreased serum PRL concentrations. Differences may be due to the very high doses of NBQX (20 and 60 mg/kg) required to detect this phenomenon.

The precise mechanisms involved in the inhibition of PRL secretion after activation of AMPA receptors have yet to be determined. In theory, an inhibitory effect at pituitary level, an increase in dopamine release or a decrease in the secretion of hypothalamic PRFs might be responsible for the inhibitory effect of AMPA. Despite the presence of AMPA receptors in pituitary gland (Kiyama *et al.* 1993, Meeker *et al.* 1994, Bhat *et al.* 1995, Villalobos *et al.* 1996), the only pituitary effect observed was a weak stimulatory action after incubation of hemipituitaries with 10^{-8} M AMPA. The results strongly suggest that the activation of AMPA receptors does not affect the secretion of PRL via a pituitary mechanism.

In order to analyse the involvement of dopamine in the inhibitory actions of AMPA, dopaminergic receptors were blocked with domperidone and dopamine synthesis with α -MPT. In these experimental paradigms, AMPA failed to decrease PRL secretion, suggesting that an increase in dopamine release might be involved in the action of AMPA. Further experiments are necessary in order to determine whether activation of AMPA receptors also decreased the secretion of PRFs, such as vasointestinal

peptide, thyrotrophin-releasing hormone or the newly characterized PRL-releasing peptide (Kato *et al.* 1978, Fagin & Neill 1981, Hinuma *et al.* 1998).

AMPA also failed to reduce PRL secretion in animals pretreated with fluoxetine and 5-HTP. This finding is consistent with our hypothesis that the mechanism behind the action of AMPA involves the release of hypothalamic dopamine since, when dopamine was infused intravenously, elevating its concentration in portal vessels, 5-hydroxytryptamine-induced PRL release was not prevented (Pilote & Porter 1981).

The physiological relevance of the inhibitory effect of AMPA on PRL secretion in rats remains to be elucidated. However, the fact that this action is only observed in prepubertal animals opens the possibility that pubertal development might be dependent on the degree of activation of NMDA and AMPA receptors. Activation of NMDA receptors advanced puberty onset through an increase in LH-releasing hormone release (Urbanski & Ojeda 1987). In contrast, repeated i.c.v. injections of AMPA to immature female rats diminished PRL and oestradiol secretion and delayed puberty (Pinilla *et al.* 1999b). Since, in these experiments, LH secretion remained unchanged after systemic or i.c.v. administration of AMPA, it is tempting to postulate that the delay of puberty after AMPA treatment is mediated by the decrease in PRL secretion, since it is well known that PRL increased the number of ovarian LH receptors (Advis *et al.* 1981), the responsiveness to gonadotrophins (Advis & Ojeda 1978) and advanced the onset of puberty in female and male rats (Clemens *et al.* 1969, Aguilar *et al.* 1988). Experiments are in progress to determine whether activation of AMPA receptors modifies pubertal development in male rats.

In conclusion, these results demonstrate that activation of AMPA receptors inhibits PRL release in prepubertal male rats, probably through a hypothalamic mechanism involving the release of dopamine.

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References

- Abbud R & Smith MS 1991 Differences in the luteinizing hormone and prolactin responses to multiple injections of kainate, as compared with N-methyl-D,L-aspartate, in cycling rats. *Endocrinology* **129** 3254–3258.
- Advis JP & Ojeda SR 1978 Hyperprolactinemia-induced precocious puberty in the female rat: ovarian site of action. *Endocrinology* **103** 924–935.

- Advis JP, Richards JS & Ojeda SR 1981 Hyperprolactinemia-induced precocious puberty. I. Studies on the mechanisms by which prolactin (PRL) enhances ovarian progesterone responsiveness to gonadotropins in prepubertal rats. *Endocrinology* **108** 1333–1342.
- Aguilar R, Bellido C, Sánchez-Criado JE & Aguilar E 1988 Mechanisms of precocious puberty induced in male rats by pituitary grafts. *Journal of Reproduction and Fertility* **83** 879–883.
- Bhat GK, Mahesh VB, Chu ZW, Chorich LP, Zamorano PL & Brann DW 1995 Localization of the N-methyl-D-aspartate R₁ receptor subunit in specific anterior pituitary hormone cell types of the female rat. *Neuroendocrinology* **62** 178–186.
- Brann DW & Mahesh VB 1997 Excitatory amino acids: evidence for a role in the control of reproduction and anterior pituitary secretion. *Endocrine Reviews* **18** 678–700.
- Carbone S, Szwarcfarb B, Rondina D, Scacchi P & Moguilevski J 1994 Effect of ovarian hormones on the prolactin response to excitatory amino acid system during sexual maturation. *Neuroendocrinology Letters* **16** 247–255.
- Carro E, Pinilla L, Seoane ML, Considine RV, Aguilar E, Casanueva FF & Diéguez C 1997 Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. *Neuroendocrinology* **66** 375–377.
- Clemens JA, Minagushi H, Storey R, Voogt JL & Meites J 1969 Induction of precocious puberty in female rats by prolactin. *Neuroendocrinology* **4** 150–156.
- Collinbridge GL & Watkins JC 1994 *The NMDA Receptor*. Oxford: Oxford University Press.
- Donoso A, López F & Negro-Vilar A 1990 Glutamate receptors of the non-N-methyl-D-aspartic acid type mediate the increase in luteinizing hormone-releasing hormone release by excitatory amino acids. *Endocrinology* **126** 414–420.
- Fagin KD & Neill JD 1981 The effect of dopamine on thyrotropin hormone-induced prolactin secretion *in vitro*. *Endocrinology* **109** 1835–1840.
- González LC, Pinilla L, Tena-Sempere M & Aguilar E 1999a Regulation of growth hormone secretion by AMPA receptors in infantile, prepubertal and adult rats. *Endocrinology* **140** 1279–1284.
- González LC, Pinilla L, Tena-Sempere M & Aguilar E 1999b Role of AMPA receptors in the control of anterior pituitary secretion in prepubertal female rats. *Journal of Endocrinology* **162** 417–422.
- Greenwood FC, Hunter WM & Glover JS 1963 The preparation of 131-I-labelled human growth hormone of high specific radioactivity. *Biochemical Journal* **89** 114–123.
- Haggi E & Aoki A 1981 Prolactin content in rat pituitary gland. RIA of prolactin after different extraction procedures. *Acta Endocrinologica* **97** 338–342.
- Hinuma S, Habata Y, Fujii R, Kawamata Y, Hosoya M, Fukusumi S, Kitada C, Masuo Y, Asano T, Matsumoto H, Sekiguchi M, Kurokawa T, Nishimura O, Onda H & Fujimo M 1998 A prolactin-releasing peptide in the brain. *Nature* **393** 272–276.
- Kato Y, Iwasaki Y, Iwasaki J, Abe H, Yanaihara N & Imura H 1978 Prolactin release by vasoactive intestinal peptide in rats. *Endocrinology* **103** 554–558.
- Kiyama H, Sato K & Tohyama M 1993 Characteristic localization of non-NMDA type glutamate receptor subunits in the rat pituitary gland. *Molecular Brain Research* **19** 262–268.
- López FJ, Sánchez-Criado JE & Negro-Vilar A 1991 A physiological role for sporadic interruptions of the dopaminergic tone in the genesis of big mass prolactin pulses. *Endocrinology* **129** 1471–1480.
- Luderer U, Strobl FJ, Levine JE & Schwartz NB 1993 Differential gonadotropin responses to N-methyl-D,L-aspartate in metestrous, proestrous, and ovariectomized rats. *Biology of Reproduction* **48** 857–866.
- Martin AI, Fernandez-Ruiz J & Lopez Calderon A 1995 Effects of catecholamine synthesis inhibitors and adrenergic receptor antagonists on restraint-induced LH release. *Journal of Endocrinology* **144** 511–515.
- Meeker RB, Greenwood RS & Hayward JN 1994 Glutamate receptors in the rat hypothalamus and pituitary. *Endocrinology* **134** 621–629.
- Negro-Vilar A, Ojeda SR, Advis JP & McCann SM 1979 Evidence for noradrenergic involvement in episodic prolactin and growth hormone release in ovariectomized rats. *Endocrinology* **105** 86–91.
- Neill JD & Nagy GM 1994 Prolactin secretion and its control. In *The Physiology of Reproduction*, pp 1833–1860. Eds E Knobil & JD Neill. New York: Raven Press.
- Pilotte NS & Porter JC 1981 Dopamine in hypophysial portal plasma and prolactin in systemic plasma of rats treated with 5-hydroxytryptamine. *Endocrinology* **108** 2137–2141.
- Ping L, Mahesh VB, Bhat GK & Brann DW 1997 Regulation of gonadotropin-releasing hormone and luteinizing hormone secretion by AMPA receptors. *Neuroendocrinology* **66** 246–253.
- Pinilla L, Tena-Sempere M & Aguilar E 1995 The role of excitatory amino acid pathways in the control of pituitary function in neonatally oestrogenized male rats. *Journal of Endocrinology* **147** 51–57.
- Pinilla L, González D, Tena-Sempere M, Aguilar R & Aguilar E 1996 Effects of N-methyl-D-aspartate and kainic acid on prolactin secretion in prepubertal female rats. *European Journal of Endocrinology* **135** 464–468.
- Pinilla L, Tena-Sempere M, Aguilar R & Aguilar E 1998 Effects of N-methyl-D-aspartic acid and kainic acid on prolactin secretion in hyper- and hypoprolactinaemic conditions. *European Journal of Endocrinology* **138** 460–466.
- Pinilla L, Gonzalez L, Tena-Sempere M, Dieguez C & Aguilar E 1999a Gonadal and age-related influences on NMDA-induced growth hormone secretion in male rats. *Neuroendocrinology* **69** 11–19.
- Pinilla L, Gonzalez L, Tena-Sempere M & Aguilar E 1999b Efectos de la administración intracerebroventricular (i.c.v.) de AMPA durante el periodo prepuberal a ratas hembras. XXX Meeting Sociedad Española de Ciencias Fisiológicas. *Journal of Physiology and Biochemistry* **55** (Abstract).
- van den Pol AN, Waurin J & Dudek F 1990 Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. *Science* **250** 1276–1278.
- Spergel DJ, Krsmanovic L, Stojilkovic S & Catt K 1994 Glutamate modulates calcium and gonadotropin-releasing hormone secretion in immortalized hypothalamic GT1-7 neurons. *Neuroendocrinology* **59** 309–317.
- Urbanski HF & Ojeda SR 1987 Activation of luteinizing hormone-releasing hormone release advances the onset of female puberty. *Endocrinology* **46** 273–276.
- Villalobos C, Nuñez L & García-Sancho J 1996 Functional glutamate receptors in a subpopulation of anterior pituitary cells. *FASEB Journal* **10** 654–660.
- Wagner EJ, Moore KE & Lookingland KJ 1994a Neurochemical evidence that AMPA receptor-mediated tonic inhibition of hypothalamic dopaminergic neurons occurs via activation of inhibitory neurons. *Brain Research* **660** 319–322.
- Wagner EJ, Moore KE & Lookingland KJ 1994b Non-NMDA receptor-mediated regulation of hypothalamic dopaminergic neurons in the rat. *European Journal of Pharmacology* **254** 105–112.
- Zanisi M, Galbiati M, Messi E & Martini L 1994 The anterior pituitary gland as a possible site of action of kainic acid. *Proceedings of the Society for Experimental Biology and Medicine* **206** 431–437.
- Zuo Z, Mahesh VB, Zamorano PL & Brann DW 1996 Decreased gonadotropin-releasing hormone neurosecretory response to glutamate agonists in middle-aged female rats on proestrus afternoon: a possible role in reproductive aging? *Endocrinology* **137** 2334–2338.

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