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Interactions between GABAergic and aminoacidergic pathways in the control of gonadotropin and GH secretion in pre-pubertal female rats

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ABSTRACT. Present experiments were carried out in 23-day-old female rats to analyze the interaction between excitatory amino acids (EAAs) and gamma-aminobutyric acid (GABA) in the control of gonadotropin and GH secretion. For this purpose, serum concentrations of LH, FSH and GH were measured after injection of different agonists of EAA receptor subtypes [N-methyl-D-aspartate (NMDA); kainic acid (KA), ±-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)], antagonists of GABA receptors (bicuculline, phaclofen) or the combined administration of both types of drugs. The results obtained indicated that: 1) GABA has a minor physiological role in the control of LH and GH secretion, since neither LH nor GH serum concentrations changed after administration of bicuculline (antagonist of $GABA_A$ receptors) or phaclofen (antagonist of $GABA_B$ receptors); 2) GABA has a sex-specific physiological role in the control of FSH secretion in female rats, in which FSH secretion increases after phaclofen administration; 3) GH secretion was enhanced after administration of NMDA, KA and AMPA, while LH increased only after activation of NMDA receptors; 4) the stimulatory effect of NMDA on LH secretion was counteracted by administration of phaclofen; and 5) bicuculline and phaclofen reduced the ability of NMDA and AMPA to stimulate GH secretion. In conclusion, present experiments evidenced a physiological role of GABA, mediated by $GABA_B$ receptors, in the control of FSH secretion and a cross-talk between excitatory and inhibitory amino acids in the control of anterior pituitary secretion.

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INTRODUCTION

The hypothalamic control of anterior pituitary hormone secretion includes complex networks in which many neurotransmitters are involved (1). Excitatory amino acids (EAAs) play a crucial role in the control of hypothalamic-pituitary function acting through their interaction with different receptor subtypes such as N-methyl-D-aspartate (NMDA); kainic acid (KA), ±-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and metabotropic receptors (2). Activation of AMPA, NMDA and KA receptors stimulates GH secretion (3, 4). NMDA stimulated LH secretion in male and female rats, whereas KA was only stimulatory in males (5), and AMPA was ineffective in both sexes (6-7). Activation of NMDA and KA receptors stimulated or inhibited PRL secretion depending on the experimental model used (8-12), while activation of AMPA receptors inhibited PRL secretion (6, 7). Gamma aminobutyric acid (GABA) is also involved in the control of anterior pituitary hormone secretion, and stimulatory or inhibitory actions of GABA on GH, gonadotropins and PRL secretion have been described (13).

Physiological interactions between different neurotransmitters involved in the control of hypothalamic or pituitary hormone secretion have been described. For example, bicuculline (antagonist of $GABA_A$ receptors) and phaclofen (antagonist of $GABA_B$ receptors) blocked glutamic acid-evoked LHRH secretion by arcuate nucleus-median eminence (AC-ME) preparations (14). Similarly, the stimulatory effect of muscimol (agonist of $GABA_A$ receptors) on LH secretion was blocked by MK-801 (antagonist of NMDA receptors) (15). In this sense, we recently described the cross-talk between excitatory and inhibitory amino acids in the regulation of GH secretion in neonatal rats, since the stimulatory effect of GABA on GH secretion was abolished by pretreatment with MK-801 (an antagonist of NMDA receptors) (16).

Present experiments were carried out to analyze the

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interactions between GABA and EAAs pathways in the control of gonadotropin and GH secretion in prepubertal female rats. Specifically, we tried to analyze whether the stimulatory effect of EAAs receptor agonists on GH and LH secretion was modified by pretreatment with antagonists of GABA receptors.

MATERIAL AND METHODS

Animals

Female Wistar 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. The pups were separated from their mothers immediately before starting treatments and they were kept warm by a heating source next to their cage.

Drugs

The following drugs were used:
EAA receptors: agonist of NMDA receptors, of KA receptors, of AMPA receptors (Research Biochemical Inc., RBI, M.A., U.S.A.). The drugs were dissolved in saline (NMDA and KA) or dimethylsulfoxide (DMSO)-saline (AMPA) and injected intraperitoneally (ip) at a dose of 15 mg/kg (NMDA) or 2.5 mg/kg (KA and AMPA) 15 min prior to sacrifice.
Antagonists of GABA receptors: bicuculline (antagonist of $GABA_A$ receptors, RBI); phaclofen (antagonist of $GABA_B$ receptors, RBI). The drugs were dissolved in saline and injected ip at a dose of 5 mg/kg (bicuculline) and 1 mg/kg (phaclofen), 30 min prior to sacrifice.

Experimental design

On day 23 of age, female rats were randomly divided in groups of 8-10 animals and injected (30 and 15 min prior to sacrifice) with 1) vehicle+vehicle; 2) vehicle+NMDA; 3) vehicle+KA; 4) vehicle+ AMPA; 5) bicuculline+vehicle; 6), phaclofen+vehicle; 7) bicuculline+NMDA; 8) bicuculline+KA; 9) bicuculline+AMPA; 10) phaclofen+NMDA; 11) phaclofen+KA; 12) phaclofen+AMPA. Animals were killed by decapitation and trunk blood was collected. Doses and timing for drug administration were selected on the basis of previous studies (5-13). Since we observed an unexpected increase in FSH levels after phaclofen administration, the effects of bicuculline and phaclofen were also tested in 23-day-old male rats.
The experiment design was approved by the Córdoba University Ethics Committee for animal experimentation and was conducted in accordance with the European Union normative for care and use of experimental animals. At the doses used no behavioral effects were observed.

Hormonal determinations

After centrifugation (1600 g at 4 C for 20 min), serum was collected, frozen and stored at -20 C until use. The concentrations of LH, FSH and GH were measured in 10-25 µl by means of a double antibody RIA method using RIA kits supplied by the National Institutes of Health (NIH, Bethesda, MD, U.S.A.). Rat-LH-I-9, Rat-FSH-I-8 and Rat-GH-I-6 were labeled with ^{125}I by the chloramine T method (17) and hormone concentrations were expressed using reference preparation (RP) LH-RP-3, FSH-RP-2 and GH-RP-2 as standards. Intra-assay coefficients of variation were below 8%. The sensitivity of the assays were 3.75, 50 and 20 pg/tube for LH, FSH and GH, respectively. For each hormone, all samples were measured in the same assay.

Statistics

Values are expressed as means±SE. Differences between groups were analyzed using one-or two-way ANOVA followed by Tukey's test.

RESULTS

Blockade of $GABA_A$ receptors with bicuculline did not affect serum concentrations of LH, FSH and GH. Administration of phaclofen (an antagonist of $GABA_B$ receptors) selectively increased FSH secretion in females. This effect was sex-dependent and was not observed in male rats (Table 1).

Table 1 - *Effects of bicuculline and phaclofen on LH, FSH, and GH secretion (ng/ml) in male and female rats.*

Treatment	Females			Males		
	LH	FSH	GH	LH	FSH	GH
Vehicle (10)	0.50±0.09	8.43±1.43	1.59±0.38	0.14±0.02	5.63±0.82	3.21±0.42
Bicuculline (10)	0.17±0.05	8.06±1.26	0.50±0.05	0.16±0.02	3.76±0.56	3.03±0.36
Phaclofen (10)	0.45±0.16	15.14±1.30*	2.81±0.43	0.14±0.03	6.74±1.26	2.73±0.34

Values are expressed as mean±SE, number of animals in brackets. *$p \leq 0.01$ *vs* vehicle-injected group (ANOVA followed by Tukey's test).

LH secretion was increased by NMDA but not by KA or AMPA (Fig. 1). Serum LH concentrations were similar in animals treated with any of the agonists of EAA receptors alone or combined with bicuculline. In contrast, the stimulatory effect of NMDA on LH secretion was partially blocked by pretreatment with phaclofen, which also induced a reduction on LH secretion in female rats treated with KA (Fig. 1).

FSH secretion remained unchanged after activation of AMPA, NMDA and KA receptors (Fig. 2). In bicuculline-pre-treated rats KA stimulated significantly ($p<0.01$) FSH secretion. Co-administration of NMDA+phaclofen or KA+bicuculline increased FSH secretion when compared with control animals (Fig. 2).

GH secretion was stimulated by NMDA, KA and AMPA. The stimulatory effect of AMPA was significantly reduced by co-administration of phaclofen and bicuculline. The reduction of NMDA-stimulated GH secretion by bicuculline or phaclofen was in the limits of significance. In contrast, phaclofen and bicuculline potentiated the releasing effect of KA on GH secretion (Fig. 3).

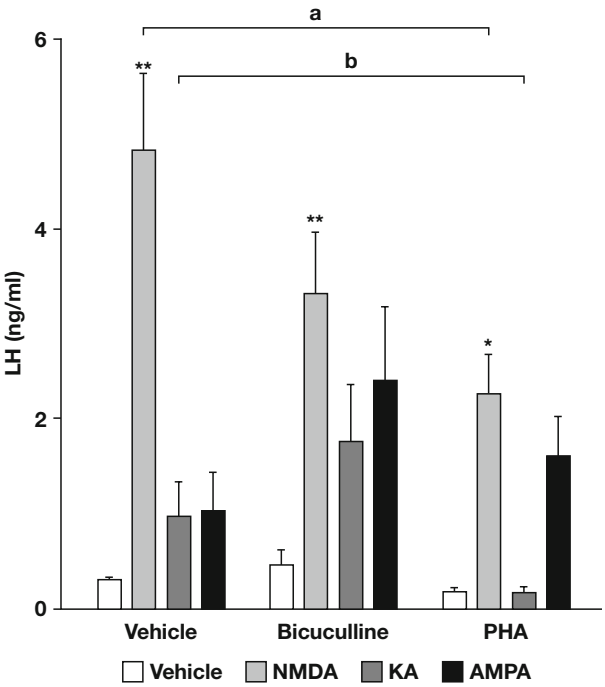

Fig. 1 - *Serum concentrations of LH in pre-pubertal female rats injected with N-methyl-D-aspartate (NMDA), kainic acid (KA) and ±-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) alone or in combination with bicuculline or phaclofen. *p≤0.05 and **p≤0.01, respectively, vs corresponding vehicle-injected group. a=p≤0.01; b=p≤0.05 (ANOVA followed by Tukey's test).*

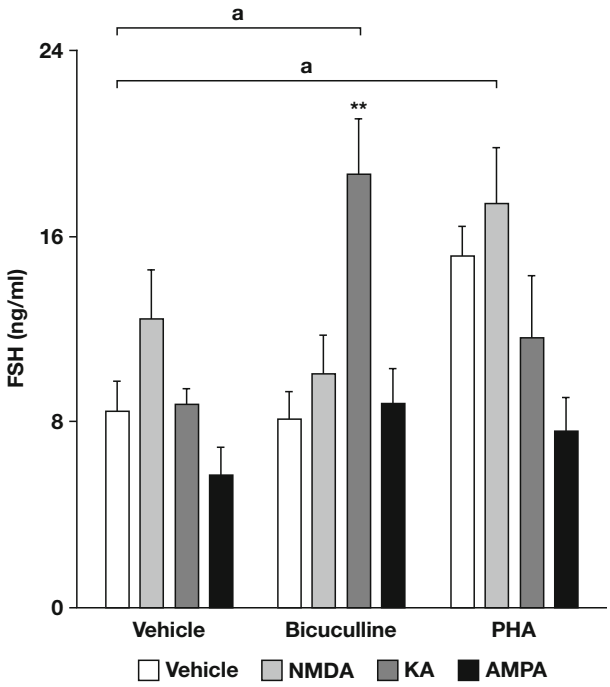

Fig. 2 - *Serum concentrations of FSH in pre-pubertal female rats injected with N-methyl-D-aspartate (NMDA), kainic acid (KA), and ±-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) alone or in combination with bicuculline or phaclofen. **p≤0.01 respectively vs vehicle-injected group. a=p≤0.05 (ANOVA followed by Tukey's test).*

DISCUSSION

Present results indicate that in pre-pubertal female rats: 1) GABA has a sex-specific physiological role in the control of FSH secretion which increases after phaclofen administration; 2) GH secretion is activated by all subtypes of receptors for EAAs; 3) some interactions between GABA and EAA pathways in the control of gonadotropin secretion occur, since phaclofen reduced the stimulatory effect of NMDA on LH release and coadministration of NMDA and phaclofen or KA and bicuculline stimulated FSH release; 4) the stimulatory effect of AMPA on GH secretion is counteracted by bicuculline and phaclofen which potentiated the KA-stimulated GH release.

The role of GABA in the control of gonadotropin secretion is complex and depends on sex and age studied. In gonadectomized adult female rats, administration of bicuculline and phaclofen (antagonists of $GABA_A$ and $GABA_B$ receptors) increases LH secretion, suggesting a GABAergic inhibition of the release of LHRH (18), $GABA_B$ receptor antagonists being more effective than the $GABA_A$ receptor antagonists (19). Baclofen (an agonist of $GABA_B$ receptors) inhibited *in vitro* the effects of LHRH on LH release (20). In 16-day-

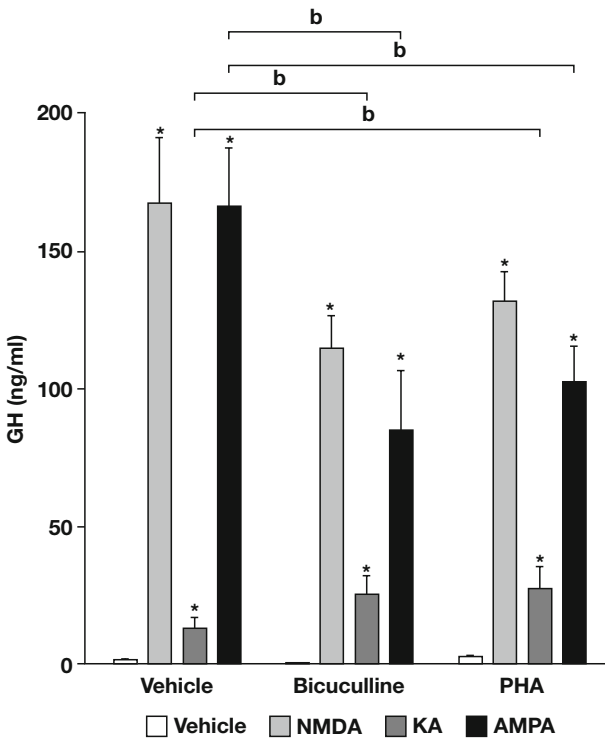

Fig. 3 - *Serum concentrations of GH in pre-pubertal female rats injected with N-methyl-D-aspartate (NMDA), kainic acid (KA), and ±-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) alone or in combination with bicuculline or phaclofen. *p≤0.01 vs corresponding vehicle-injected group. b=p≤0.05 (ANOVA followed by Tukey's test).*

old female rats, LH secretion is stimulated by muscimol (a $GABA_A$ agonist) and inhibited by baclofen (a $GABA_B$ agonist) (15). The role of $GABA_A$ receptors in the control of LH changes during pubertal development, and $GABA_A$ receptor agonists stimulated LH secretion in pre-pubertal rats (16-day-old) and inhibited it in peripubertal ones (30-day-old) (21). Our results indicate that LH secretion in 23-day-old females is independent of activation of $GABA_A$ or $GABA_B$ receptors, since neither bicuculline nor phaclofen changed serum LH concentrations. The selective increase in FSH secretion in female rats after phaclofen administration was unexpected. However, our results resemble those obtained after administration of 5-hydroxytryptophan (5-HTP: precursor of serotonin synthesis) in pre-pubertal rats. Administration of 5-HTP increases FSH secretion in male but not in female rats (22) and the increase in FSH levels was not accompanied by concomitant increases in LH secretion (23). The possibility that phaclofen increased LHRH secretion is difficult to reconcile with the absence of changes in LH secretion. Alternatively, different studies have repeatedly proposed the existence of a selective FSH-releasing factor (24, 25), which could mediate the effects of phaclofen on FSH. It should also be considered that the selective release of both gonadotropins is related to the frequency and amplitude of LHRH pulses. Pituitary stimulation with a high frequency of LHRH pulses increases LH secretion, while a stimulation with low frequency of LHRH pulses increases selectively FSH secretion (26, 27), it being possible that phaclofen stimulated selectively FSH secretion through a change in the frequency of LHRH pulses.

Present experiments confirmed and extended previous results from our laboratory indicating that GH secretion is activated by NMDA, KA and AMPA receptors, whereas LH increased only after administration of NMDA and FSH was unaffected (3, 5). These findings evidenced a distinct role of EAA receptor subtypes in the control of different pituitary hormones.

Interactions between excitatory and inhibitory amino acids in the control of gonadotropin secretion are evident from present experiments. The reduction of NMDA-stimulated LH secretion after phaclofen treatment indicates that $GABA_B$ receptors play a permissive role in the effects of NMDA on LH secretion. It has previously been described that NMDA stimulates LH via LHRH (28) and phaclofen decreased the stimulatory effect of NMDA on LHRH release by median eminence-arcuate nucleus (14). The experiments reported herein indicated that the interactions between NMDA and $GABA_B$ receptors in the control of LHRH observed *in vitro* (14) are also operative *in vivo*.

In recent experiments (16), we demonstrated that the stimulatory effect of GABA on GH secretion was counteracted by MK-801 (an antagonist of NMDA receptors). Present results indicated that, inversely, NMDA and AMPA effects were partially counteracted by phaclofen (an antagonist of $GABA_B$ receptors), whereas KA effect was potentiated by bicuculline. As a whole, these interactions suggest that GABA receptors differently modulate the functional role of different subtypes of EAA receptors in the control of GH secretion.

In conclusion, our results suggest that $GABA_B$ receptors play a selective physiological role in the control of FSH secretion in pre-pubertal female rats and that a cross-talk between excitatory and inhibitory amino acids operates in the control of gonadotropin and GH secretion in pre-pubertal female rats. In contrast with recent results demonstrating that the stimulatory effect of GABA on GH secretion required the activation of NMDA receptors (16), present experiments suggest that the full stimulatory effect of AMPA receptors on GH secretion requires the activation of $GABA_A$ and $GABA_B$ receptors. As a whole, the results obtained suggest that the stimulatory effect of EAAs in the control of GH are modulated by GABA receptors.

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