ENDOCRINE RESEARCH, 26(3), 399–410 (2000)

INTERRELATIONSHIPS OF GABAERGIC, SEROTONINERGIC AND EXCITATORY AMINO ACID SYSTEMS IN ITS REGULATORY EFFECT ON PROLACTIN SECRETION IN PREPUBERTAL RATS

Jaime A. Moguilevsky, Berta Szwarcfarb, Silvia Carbone, Dora Rondina, Osvaldo Ponzo and Pablo Scacchi
Departamento de Fisiologia, Facultad de Medicina, Universidad de Buenos Aires
Paraguay 2155 Piso 7 1121 Buenos Aires, Argentina

ABSTRACT

GABAergic, serotoninergic and excitatory amino acid systems (EAAs) regulate the prolactin (PROL) secretion in prepubertal female rats. The aim of the present paper was to determine the interrelationships of these systems on the control of this pituitary hormone. It was carried out through the following scheme: 1. The participation of the EAAs and serotonin in the effect of GABAergic system on PROL release, determined by evaluating the GABA A and GABA B receptor agonists. It was carried out on animals that were previously treated with AAEs receptor antagonist or p-chlorophenylamphetamine (PCA), this one depleting serotonin in the hypothalamus. 2. The participation of GABAergic system in the effect of serotonin and EAAs systems, determined by the evaluation of the effects of EAAs receptor agonists and of 5-HTP, a serotonin precursor. With this purpose the rats were previously treated with GABA A and GABA B receptor antagonists. 3. The interrelationships between the EAAs and the serotoninergic systems in the control of PROL secretion, determined (a) by using EAAs agonists (in rats depleted of serotonin by PCA) and (b) using EAAs antagonists (in rats treated with 5-HTP, a serotonin precursor). The administration of GABAergic agonists significantly increased PROL secretion in prepubertal female rats. Neither EAAs antagonists nor the depletion of serotonin in the brain, modified the stimulatory effects of the GABAergic system on PROL levels. This is a clear indication that the activity of the GABAergic system is independent of the serotoninergic and of the EAAs system effects on the pituitary hormone. The EAAs neurotransmitter system agonists significantly increase PROL levels. This

effect was blocked by the GABAergic system antagonists but was not modified by serotonin depletion. Taking into account these facts it may be considered that the GABAergic system is involved in the stimulatory effect of EAAs on PROL secretion, this effect being independent of the serotoninergic system. 5-HTP significantly increased PROL plasma levels, and this effect was modified neither by the GABAergic nor by the EAAs receptor antagonists. These results indicate that the stimulatory effect of serotonin on PROL release is independent of the GABAergic and EAAs systems. In conclusion it may be considered that in prepubertal female rats, the GABAergic and serotoninergic systems stimulate PROL secretion by independent mechanisms that do not include EAAs. On the other hand, the effects of EAAs neurotransmission are exerted via the GABAergic system.

INTRODUCTION

The participation of neurotransmitters and neuromodulators are relevant mechanisms in the different neuroendocrine processes involved in the hypothalamic control of prolactin (PROL) (1) which results in one integrated response on PROL secretion. Prepubertal control of PROL seems to be quantitative and qualitative different than that operated in adult female rats. We had previously demonstrated that GABAergic system have a different qualitative effect on PROL secretion in prepubertal than in adult female rats, since while in prepubertal rats has a stimulatory effect, in adult rats, inhibits this pituitary hormone secretion. This change takes place in the peripubertal period (2). On the other hand, qualitative differences in the neurotransmitter effect on PROL secretion in prepubertal versus adults rats had been demonstrated on the excitatory amino acids (EAAs) (3) and serotonin systems (4). It was carried out through the systemic administration of NMDA, its agonists (5,6) or else of 5-hydroxytryptophan (5-HTP) (that increases the hypothalamic content of 5-HT). In all cases the secretion of PROL was stimulated, both in prepubertal and adult rats. The effect was higher in adult than in prepubertal animals.

It is interesting to note that the effect of neurotransmitters on LH and FSH secretion is also qualitative and quantitative different in prepubertal and in adult

rats. These differences are bounded with the various interrelationships among the systems engaged in the hypothalamic control of both gonadotrophins.

It has been proposed that the quantitative and qualitative differences in the prepubertal, peripubertal and adult hypothalamic control of PROL and gonadotrophin secretions in female rats are in some way connected with the neuroendocrine events involved in the onset of puberty and sexual maturation (7-12).

Many of the interactions between the neurotransmitter system control on PRO and gonadotrophin secretion had been established on adult rats (1,13,14). Recently we determined some of these events occurring in prepubertal animals. Important differences were shown in these neuroendocrine processes during sexual maturation (14-16)

The aim of the present paper was to determine the interrelationships among the stimulatory effects of GABAergic, EAAs and serotonin systems on PROL secretion in prepubertal female rats. With this purpose, three EAAs antagonists, MK-801 (antagonist of NMDA receptors), CNQX (antagonist of no-NMDA receptors) (5, 17) and PCA (para-chloro amphetamine) (for the depletion of serotonin in the hypothalamus) (18) were used to evaluate in the animals the interrelationships of GABAergic system and PROL secretion. On the other hand, the participation of the GABAergic system on the stimulatory effects of serotonin and EAAs were also determined: two exogenous agonists of EAAs receptor, NMDA and kainate, and a serotonin precursor, 5-HTP (7), were used in rats treated with bicuculline and phaclofen (antagonists of GABA A and GABA B receptors respectively).

MATERIALS AND METHODS

Animals used:

Sixteen days old (at the time of sacrifice) prepubertal female rats from the Department of Physiology of the Faculty of Medicine, University of Buenos Aires, were used. They had been kept in a light and temperature controlled environment (light on: 06.00 to 20.00hr, 22 °C).

Drugs:

The following drugs were used: Muscimol* (5-aminomethyl-3-hydroxyisoxazole), Baclofen* . (4-amino-3-[4-chlorophenyl]-butanoic acid), Bicuculline* (bicuculline-methiodide), Phaclofen** (3-amino-2-(4-chlorophenyl) propylphosphonic acid), NMDA* (N-methyl-D-aspartic acid), Kainic acid* (2-carboxy-3-carboxymethyl-4-isopropenylpyrrolidine), MK 801**, CNQX** (6-cyano-7-nitroquinoxaline-2,3-dione), 5-HTP *(5-hydroxytryptophan), PCA (p-chloroamphetamine), Regis Chemical, Morton Grove, Ill, USA [* Sigma Chemical Co., St. Louis, Mo., USA; ** RBI, Ma., USA]. All substances were previously dissolved in saline.

Doses and treatment:

The minimal doses and schedule of administration of agonists inducing a maximal response on PROL and the minimal doses of antagonists that completely blocked the effect of the corresponding antagonists on PROL level were determined in previous experiments. Muscimol, 1mg/kg, IP, 90 min before sacrifice; Baclofen 5mg/kg IP, 120 min before sacrifice; Bicuculline 1mg/kg IP 120 and 240 min before sacrifice; Phaclofen 1mg/kg IP 120 and 240min before sacrifice; NMDA 30mg/kg SC (pH 5.5) 10 min before sacrifice (in selected experiments was also injected 20min before sacrifice to discard a delayed response); kainate 2.5mg/kg IP 15 and 30min before sacrifice; MK 801 0.1mg/kg SC 60min before sacrifice; CNQX 0.3mg/kg 60min before sacrifice; 5-HTP 75mg/kg IP 1hour before sacrifice; PCA 5mg/kg IP at 12.00hr three consecutive days, starting 76hours before sacrifice.

Experimental Design

The following series of animals (8 to 10 each) were studied. Control groups of each series were injected with the corresponding vehicle.

Each of the stated drugs were used because of the following effects: Muscimol: (GABA A agonist); baclofen (GABA B agonist); bicuculline (GABA A antagonist); phaclofen (GABA B antagonist; NMDA (NMDA neurotransmission agonist); kainate (non NMDA receptor agonist); MK 801 (NMDA neurotransmission antagonist); CNQX (kainate-quisqualate non NMDA

competitive receptor antagonist); 5-HTP (serotonin precursor); PCA (serotoninergic neuron toxin, i.e. serotonin depletor)

Series 1: Interaction of the GABAergic effects on prolactin secretion with EAAs and serotoninergic systems: A: control; B: muscimol; C: baclofen; D: muscimol + MK 801; E: baclofen + MK 801; F: muscimol + CNQX; G. Baclofen +CNQX; H: muscimol + PCA; I: baclofen + PCA.

Series 2: Interactions of the EAAs effects on PROL secretion with GABAergic and serotoninergic systems: A: control; B: NMDA; C: kainate; D: NMDA + bicuculline; E: NMDA+phaclofen; F: kainate + bicuculline; G: acido kainico +phaclofen; H: NMDA + PCA; I: kainate + PCA.

Series 3: Relationships of serotoninergic effects on PROL secretion with GABAergic and EAAs systems: A: control; B: 5-HTP; C: 5-HTP + PCA; D: 5-HTP + bicuculline; E: 5-HTP + phaclofen; F: 5-HTP + MK 801; G: 5-HTP + CNQX.

Blood collection: The animals were killed by decapitation at 16.00 to 17.00hr. Blood was collected from the trunk and allowed to clot at 4o.C. The samples were centrifuged for 10min at 2500 rpm and the sera separated and stored frozen until PROL assays were carried out.

Hormone Determinations:

Serum PROL was determined in duplicate, using a double antibody radioimmunoassay technique. The reagents for it were kindly provided by the NIAMDD rat pituitary program. Intra and interassay coefficients of variation were 8% and 10%, respectively.

Statistical Analysis:

The results were subjected to analysis of variances (ANOVA) and Tukey's multiple range test (19). When comparing only two treatments the Student's t test was applied. All results showing a $p < 0.05$ were considered statistically significant. Values are expressed as mean±SEM.

RESULTS

Series 1: Interaction of the GABAergic effects on prolactin secretion with EAAs

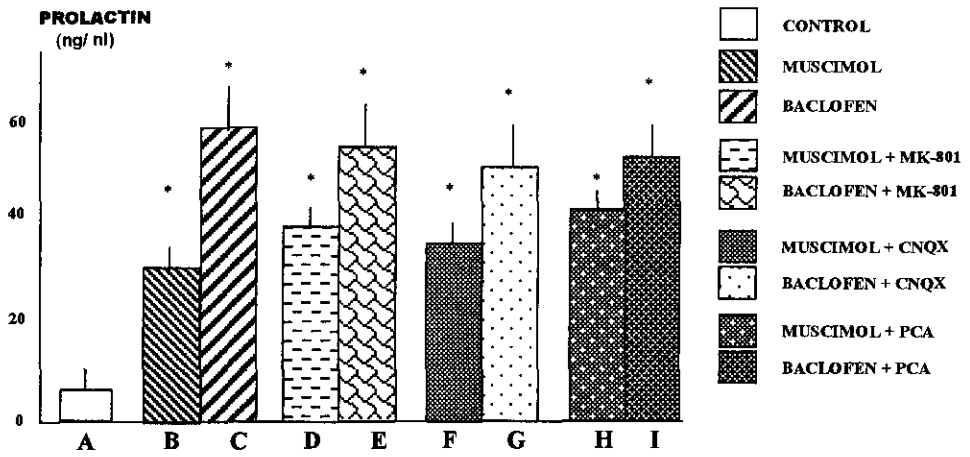

FIGURE 1.
Effect of GABA A (muscimol) and GABA B (baclofen) agonists on PROL levels in prepubertal rats treated with NMDA (MK-801) and non NMDA (CNQX) receptor antagonists and with depletion of brain serotonin levels (PCA) *p<0.01 vs control

and serotoninergic systems: As shown in figure 1, the administration of muscimol and baclofen (GABA A and GABA B agonists) significantly increases PROL secretion. Neither MK 801, CNQX (NMDA and non-NMDA receptor antagonists, respectively) nor PCA (serotonin depletor) modified the stimulatory effect of muscimol and baclofen.

A: 4.8 ± 0.3; B: 30.3 ± 1.8; C: 57.8 ± 4.3; D: 38.3 ± 3.8; E: 54.0 ± 6.1; F: 36.2 ± 4.1; G: 50.3 ± 6.3; H: 42.3 ± 4.1; I: 53.2 ± 7.1 (ng/ml) Control vs. Experimental groups: $p < 0.01$.

Series 2: Interactions of the EAAs effects on PROL secretion with GABAergic and serotoninergic systems: Figure 2 shows that NMDA and kainate (agonists of NMDA and non NMDA neurotransmission, respectively) significantly increased PROL levels ($p < 0.01$). This effect was blocked by MK 801and CNQX (NMDA and non-NMDA receptor antagonists, respectively), by bicuculline and by phaclofen (GABA A and GABA B receptor antagonists, respectively). PCA

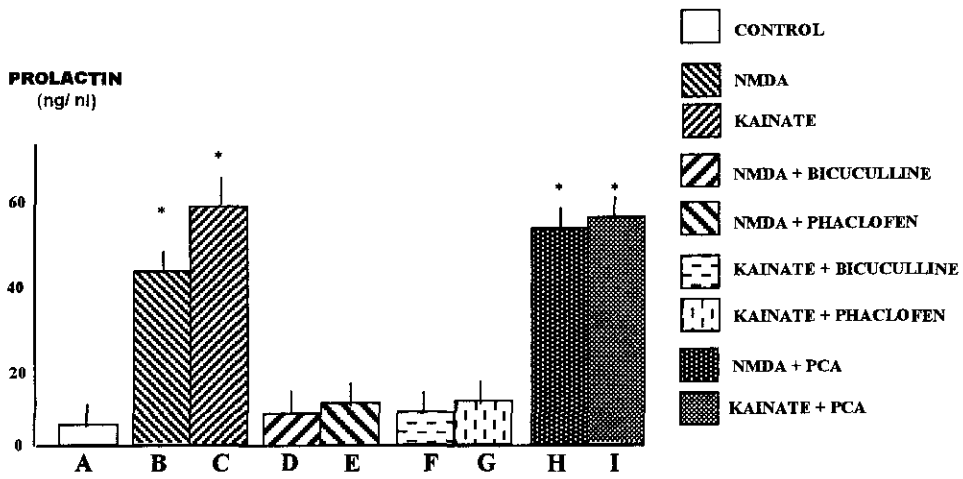

FIGURE 2
Effect of NMDA (NMDA) and non NMDA (kainate) agonists on PROL secretion in prepubertal rats treated with GABA A (bicuculline) and GABA B (phaclofen) antagonists and with serotoninergic neurotoxin (PCA) p*<0.01 vs control.

(serotonin depletor) did not modify the PROL release effect of NMDA and kainate.

A: 5.4 ± 0.6; B: 42.2 ± 5.3; C: 58.2 ± 6.3; D: 6.2 ± 0.7; E: 9.1 ± 1.2; F: 6.3 ± 0.7; G: 9.1 ± 1.0; H: 48.9 ± 5.6; I: 60.2 ± 7.3. (ng/ml) Control versus B, C, H and I: $p < 0.01$. Control vs. D, E, F, and G: not significant.

Series 3: Relationships of serotoninergic effects on PROL secretion with GABAergic and EAAs systems: The figure 3 shows that 5-HTP (serotonin precursor) significantly increased the plasmatic level of PROL ($p < 0.01$). This effect was blocked by PCA (serotonin depletor). On the other hand neither bicuculline nor phaclofen (antagonists of GABAergic receptors), nor MK 801 and CNQX (NMDA and non-NMDA receptor antagonists) modified the stimulatory effect of 5-HTP on PROL secretion.

A: 6.3 ± 0.7; B: 59.3 ± 7.8; C: 6.3 ± 0.42; D: 50.0 ± 5.8 E: 58.3 ± 8.1; F: 57.0 ± 5.3; G: 56.3 ± 8.1.(ng/ml) Control versus B, D, E, F and G: $p < 0.01$. Control vs. C: not significant.

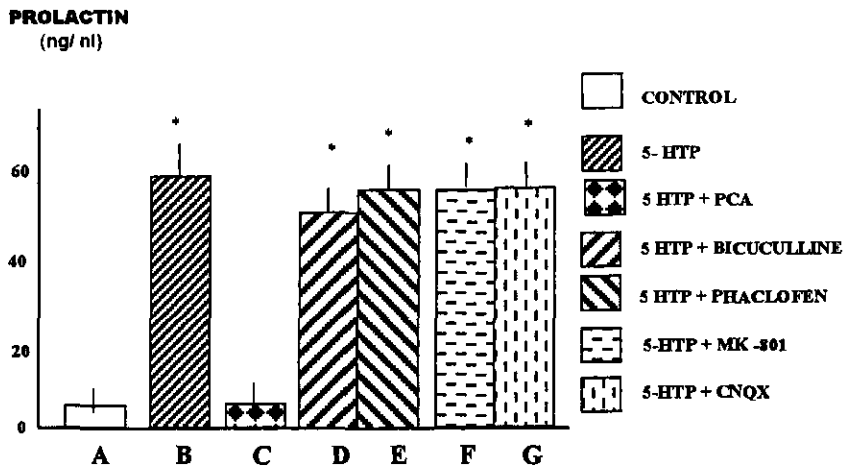

FIGURE 3
Effect of serotoninergic precursor (5-HTP) on PROL secretion in prepubertal rats treated with GABA A (muscimol), GABA B (phaclofen), NMDA (MK-801) and non NMDA (CNQX)(receptor antagonists *p>0.01 vs control.

DISCUSSION

In previous studies we have demonstrated that during prepubertal state, GABAergic, serotoninergic and excitatory amino acid systems have a stimulatory effect on PROL secretion in rats. We also showed that the prepubertal stimulatory effect of GABA is mediated by GABA A and GABA B receptors (2-4). This stimulatory effect of the GABAergic system changes to an inhibitory action in adult rats. On the other hand the serotoninergic and excitatory amino acid systems stimulate PROL secretion both in prepubertal and in adult rats, being higher in adults than in prepubertal. The present work is an attempt to determine the interactions of these neurotransmitter systems on the stimulatory prepubertal control of PROL secretion, by using different GABAergic, serotoninergic and EAAs agonists and antagonists. Its results confirm those of our previous papers (2-4) indicating that GABAergic, serotoninergic and EAAs neurotransmission stimulate PROL secretion in prepubertal rats and that the GABAergic effect is exerted through the GABA A and GABA B receptors.

On the bases of the present results neither the serotoninergic nor the EAAs systems participate in the stimulatory effect of GABA on PROL release, since the administration of the antagonists of these systems (PCA, MK 801 and CNQX) did not modify the stimulatory effect of the administration of both GABA A and GABA B agonists.

On the other hand, the administration of NMDA and kainate (agonists of NMDA and non-NMDA neurotransmission) significantly increases PROL secretion. Moreover, these effects were completely blocked by bicuculline and baclofen (GABA A and GABA B antagonists). On this bases it could be considered that the stimulatory effects of NMDA and non-NMDA neurotransmission are mediated through the GABAergic system. No modifications in the stimulatory effects of EAAs neurotransmission on PROL secretion were observed in animals treated with PCA (antagonist of the serotoninergic system). According to that, it could be concluded that the serotoninergic system is not involved in the increase of PROL secretion induced by NMDA and non-NMDA neurotransmission.

The stimulatory effect of the serotoninergic system on PROL secretion was only blocked by PCA (the antagonist of this system), remaining unmodified after the administration of GABA A, GABA B and EAAs antagonists. These results appear to indicate that the stimulatory effect of serotonin on PROL secretion is not connected with the stimulatory effects of GABAergic and EAAs neurotransmission.

In conclusion it may be considered that in the female prepubertal rat, while the GABAergic and serotoninergic systems stimulate PROL secretion by independent mechanisms that not include EAAs, the effects of EAAs neurotransmission are exerted via GABAergic systems.

It is interesting to note that, on PROL secretion, the GABAergic system changes from the stimulatory prepubertal action to an inhibitory action in adult rats. On the other hand, a similar stimulatory effect on the hormone were observed with serotoninergic and EAAs in immature and mature animals (2-4). Since the EAAs stimulatory effect in prepubertal rats is exerted through the

GABAergic system, it is clear that, in adult rats in which GABA inhibits PROL secretion, the functional relationships change, and this change takes place during sexual maturation. More experimental evidences are needed to clarify this point. Nevertheless, additional evidences of changes in the interrelationships of the different hypothalamic neurotransmitter systems during the onset of puberty have been previously mentioned. These changes appear to be connected with a neuroendocrine process involved with sexual maturation (14).

ACKNOWLEDGEMENTS

Supported by the Agencia Nacional de Promocion Cientifica, Consejo Nacional de Investigaciones Cientificas y Tecnicas and Universidad de Buenos Aires. * JAM and PS are established investigator from National Research Council. Address correspondence to Prof. Dr. Jaime A. Moguilevsgy. Departamento de Fisiologia, Fac. de Medicina. UBA. Paraguay 2155.CP 1121 Buenos Aires Argentina Telefax: 0054 1 4963 6286, Email jmoquile@fmed.uba.ar

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