Neuroendocrinology Letters Nos.1/2, Feb-Apr, Vol.24, 2003
 ISSN 0172–780X **www.nel.edu**

ORIGINAL ARTICLE

Effects of gabaergic and serotoninergic systems on hypothalamic content of catecholamines during sexual development in female rats

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Submitted: *October 10, 2002*
Accepted: *November 11, 2002*

Key words: **puberty; serotonin; GABA; catecholamines**

Neuroendocrinology Letters 2003; ***24****(1/2):46–49 pii: NEL241203A06* ***www.nel.edu***

Abstract

OBJECTIVES: The aim of the present investigation was to determine whether the catecholaminergic system is involved in gabaergic and serotoninergic effects on gonadotrophin secretion during sexual development. To this end, we studied the effect of GABAergic and serotoninergic systems on hypothalamic catecholamine content at different stages of sexual development.

METHODS: The effect of GABA A and GABA B agonists and 5-hydroxy-L-tryptophan on hypothalamic noradrenaline and dopamine content were determined in prepubertal (16 days old) and peripubertal (30 days old) rats.

RESULTS: At 16 days of age_GABA agonists did not modify hypothalamic noradrenaline content, whereas a significant decrease in catecholamine concentration was observed in peripubertal rats at 30 days of age. Similar changes were observed with GABA agonists administration on dopamine hypothalamic levels, i.e no effects at 15 days of age and a significant decrease at 30 days. The administration of 5-hydroxy-l-tryptophan (5-HTP) induced a decrease of hypothalamic concentration of noradrenaline and dopamine at both ages.

CONCLUSION: Results indicate that the GABAergic system modifies the hypothalamic catecholamine content in peripubertal but not in prepubertal rats while serotonin has an inhibitory effect at both stages of sexual maturation. Even though both systems induce similar ontogenic modifications on the gonadotrophin axis (stimulatory effect in prepubertal and inhibitory action in peripubertal and adult rats) the present results appear to indicate that GABAergic and serotoninergic systems regulate gonadotrophin secretion by different hypothalamic mechanisms.

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Introduction

It is well known that hypothalamic neurotransmitter systems are directly involved in the control of gonadotrophin [1,2]. For example, evidence has accumulated [3] indicating an important role of γ-aminobutyric acid (GABA) and catecholamines (CA) [4,5] in the control of gonadotrophin secretion in adult female rats. The infusion of GABA or muscimol in regions containing GnRH neurons inhibits the pulsatile release of LH in ovariectomized rats treated with aminooxoacetic acid (a blocker of GABA degradation, that increases the hypothalamic concentration of GABA and also inhibits the pulsatile secretion of LH). On the other hand, the inhibition of noradrenaline (NE) synthesis by blockade of α adrenergic receptors or destruction of the ventral noradrenergic bundle with 6-hydroxydopamine results in the supression of LH release in ovariectomized rats. Clearly, CA, and specifically noradrenergic and adrenergic neurons are involved in LH release [4].

In previous studies we have demonstrated that the effect of GABA and serotoninergic system on GnRH-gonadotrophin axis in prepubertal female rats are qualitatively different from those observed in peripubertal and adult rats [6, 7, 8, 9, 10]. Moreover these changes are connected with modifications in the interactions among the different neurotransmitters systems [10] involved in the maturative processes of the hypothalamic-pituitary-gonadal axis.

CA are directly involved in the hypothalamic control of GnRH [1, 4, 5] and evidences have been presented indicating that the different neurotransmitter systems could act on gonadotrophin secretion trough the hypothalamic release of CA. On this basis, the aim of the present investigation was to study the effect of GABAergic and serotoninergic systems on the hypothalamic CA content at different stages of sexual development. For these purposes, the effects of GABA A and GABA B agonists and 5-hydroxytriptophan (5-HTP) (a precursor of serotonin that increases hypothalamic serotonin) on the hypothalamic content of NE and dopamine (D) were determined in prepubertal (16 days old) and peripubertal (30 days old) female rats.

Material and Methods

Animals: Prepubertal (16 days old) and peripubertal (30 days old) Wistar female rats from the Department of Physiology, School of Medicine, University of Buenos Aires, were used. They were kept in a light and temperature controlled environment (lights on from 6 AM to 8 PM, at 22° C).

Drugs: *Muscimol* (5-aminomethyl-3-hydroxy-isoxazole), Sigma Chemical Co St Louis, Mo, USA; *Baclofen* (4-amino-3-(4- chlorophenyl) – butanoic acid) Sigma Chemical Co. St Louis, Mo, USA; *5-HTP* (5-hydroxytryptophan) Calbiochem Chemical La Jolla Cal. USA.

Muscimol, a GABA-A agonist, was administered at 1 and 2 mg/ kg i.p., 90 min before sacrifice; Baclofen, a GABA-B agonist, was administered at 5 and 10 mg/kg i.p., 120 min before sacrifice. [6,7]. 5-HTP, an immediate serotonin precursor that increases serotonin levels in the hypothalamus, was administered i.p. at 75 mg/kg, 60 min before sacrifice [8]. Previous results demonstrated that these are the minimal doses that induce maximal modifications in the GnRH-gonadotrophin axis .

Tissue Processing. After decapitation the brains were rapidly removed and the hypothalami dissected out with a single razor blade. Hypothalamic samples containing the anterior preoptic and medial basal areas (APOA-MBH) were dissected with the help of a stereomicroscope. The hypothalamic samples were bordered laterally by the hypothalamic sulci; rostrally, 3 mm anterior to the optic chiasma, and caudally, by the mammillary bodies; the depth at which the samples were acquired was 3–4 mm. The hypothalamic samples were weighed and homogenized in 1ml 0.3 M perchloric acid, using a Vibra Cell refrigerated with ice. No significant differences were found among the weights of different samples. The homogenate was centrifuged for 2 minutes at 13000 rpm. The supernatant plus Tris-HCl buffer, 2M (pH 8.6) plus 200 mg dehydrated alumina, di-hydroxy benzyl amine was added to the supernatant as internal standard. After shaking for 15 min the samples were centrifuged at 13000 rpm and the pellets washed twice. The samples were re-suspended in 100 μl of acetic-phosphoric acid solution (80:20). After shaking for 15 min, the samples were centrifuged. Twenty μl of the supernatant was injected in a HPLC analytical column (LKB) (Spherisorb C-18, ODS, 2,5μ. 4 x 100mm) to separate CA, which were detected by means of an electrochemical detector (LKB) connected to an integrator.

Standard curves were processed simultaneously. The mobile phase was prepared with sodium phosphate monohydrate (13.78 g), octane-sulfonic acid (35 mg), EDTA (100 mg/l) and acetonitrile (15 ml) pH 3.0.

Statistical Analysis: Results were examined by means of the analysis of variance (ANOVA) followed by Tukey's multiple range test [11]. When comparing only two treatments a Student's *t-test* was used. Statistically significant differences were considered at $p < 0.05$. Values were expressed as mean ± SEM.

Results

Effect of Muscimol and Baclofen administration on hypothalamic NE and D content

Figure 1 shows the effect of muscimol and baclofen (GABA A and B agonists respectivelly) on NE hypothalamic content in 16 days old prepubertal and 30 days old peripubertal female rats. At 16 days of age, GABA agonists did not modify the hypothalamic NE content while a significant decrease in CA concentration was observed in peripubertal rats at 30 days of age. Similar changes were observed (Figure 2) with GABA agonists administration on D hypothalamic levels, i.e. no effects at 16 days of age and a significant decrease at 30 days of age. (**Prepubertal** (16 days old): Noradrenaline : Control: 1380.2 ± 105.3; Muscimol: 1230.4 ± 130.3; Baclofen 1140.5 ± 114.6 Dopamine: Control: 230.4 ± 10.5, Mus-

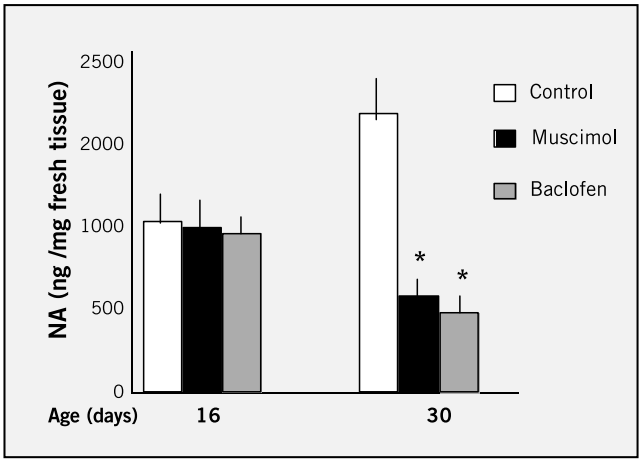

Fig. 1: Effect of Muscimol and Baclofen on hypothalamic noradrenaline content. Mean ± standard error of 8–10 determinations * < 0.01 vs control.

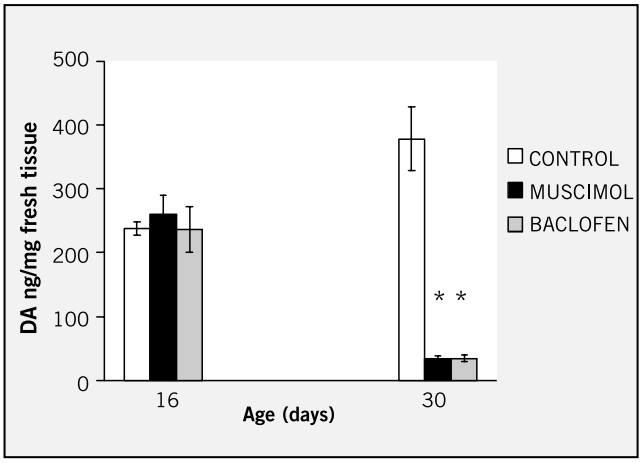

Fig. 2: Effect of Muscimol and Baclofen on hypothalamic dopamine content. Mean ± standard error of 8–10 determinations * < 0.01 vs control.

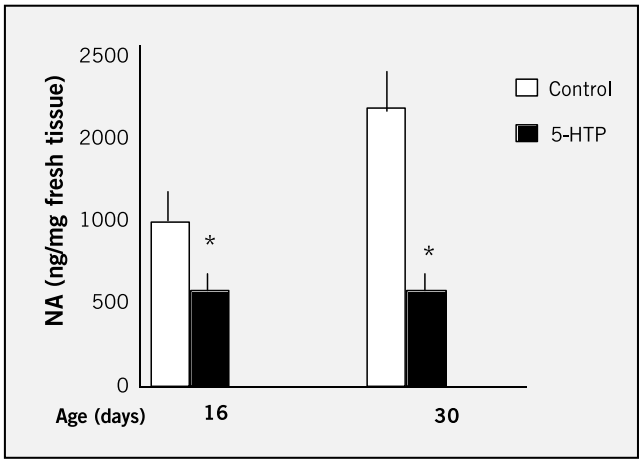

Fig. 3: Effect of 5 HTP on hypothalamic noradrenaline content. Mean ± standard error of 8–10 determinations * < 0.01 vs control.

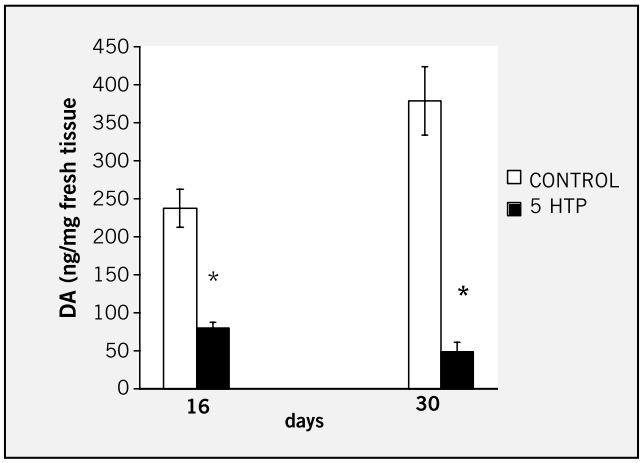

Fig. 4: Effect of 5-HTP on hypothalamic dopamine content. Mean ± standard error of 8-10 determinations * < 0.01 vs control.

cimol 260.3 ± 60.2; Baclofen 245.5 ± 50.6. **Peripubertal** (30 days old) Noradrenaline: Control: 2110.3 ± 315.7; Muscimol: 754.4 ± 65.2; Baclofen 543.4 ± 51.3 (p <0.01 vs control) Dopamine: Control: 370.3 ± 70.8, Muscimol 25.2 ± 3.2; Baclofen 27.8 ± 3.4. ng /mg fresh tissue respectivelly (p <0.01 vs control))

Effect of 5 HTP administration on hypothalamic NE and D content

Figures 3 and 4 show the effect of 5-HTP on the hypothalamic concentration of NE and D. It can be seen the effect was similar at both ages since the 5-HT precursor significantly decreased NE and D at both studied ages. (**Prepubertal**: Noradrenaline: Control: 1250.2 ± 145.3; HTP: 750.4 ± 69.6 Dopamine: Control: 240.4 ± 25.5, HTP: 75.2 ± 16.3**. Peripubertal**: Noradrenaline: Control: 1949.2 ± 145.3; HTP: 753.4 ± 69.6 (p <0.01 vs control) Dopamine: Control: 379.4 ± 35.5, HTP: 55.2 ± 6.3. ng /mg fresh tissue respectivelly (p <0.01 vs control)).

Discussion

In previous studies we have demonstrated that sexual maturation and the onset of puberty in female rats implicate complex neuroendocrine changes. They include qualitative and quantitative modifications in the effect of the neurotransmitter systems on the GnRH-gonadotrophin axis as well as changes in the interactions between the neurotransmitters involved in the hypothalamic control of gonadotrophin release [6,7,8,9,10]. For example, the GABAergic and serotoninergic systems have a stimulatory effect on gonadotrophin secretion in prepubertal rats and an inhibitory one in peripubertal rats.

It is clear that during sexual maturation new neuronal contacts as well as receptors development take place. These processes involve changes in the interrelationships between the different neurotransmitter systems as well as in the effect of these systems on hypothalamic gonadotrophin control .

The results of the present study indicate that while the GABAergic system has no effect on NE and D hypo-

thalamic content in prepubertal rats, it decreases CA content in peripubertal rats of 30 days of age. Conversely, serotonin has an inhibitory effect on hypothalamic NE and D at both ages of sexual maturation. This clearly indicates a different effect of GABAergic and serotoninergic systems on the hypothalamic catecholaminergic system during sexual maturation.

Previous results [8] indicate similar modifications of the gonadotrophin axis by the GABAergic and serotoninergic systems during sexual maturation, i.e. a change from an stimulatory effect in prepubertal to an inhibitory one in peripubertal and adult rats. GABA did not modify the hypothalamic CA content in prepubertal rats but decreased both NE and D in peripubertal rats. These findings appear to indicate that the stimulatory effect of GABA on the gonadotrophin axis in prepubertal rats and the inhibitory one in peripubertal animals are mediated by different types of interrrelationships between GABA and the catecholaminergic system. On the other hand serotonin decreases hypothalamic CA at both ages. These results could indicate that the relationship between both system of neurotransmission does not change during sexual maturation, and that the change in the effect of serotonin on the hypothalamic-gonadal axis during sexual maturation implicates additional mechanisms that remain to be studied.

In summary the results of the present paper indicates that in spite of GABAergic and serotonin system induces similar ontogenic modifications in theirs effects on gonadotrophin axis these modifications are induced by different neuroendocrine mechanisms.

Acknowldgments

This work was supported by Grants from Agencia Nacional de Promoción Científica y Técnica (BID 1201. PICT 6240). The fellowship Carrillo-Oñativia, (Ministerio de Salud de la Nación) and the University of Buenos Aires.

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