

Changes in the Effect of Testosterone on Hypothalamic Nitric Oxide Synthetasa During Sexual Maturation. Its Relationship with GnRH Release

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Abstract

OBJECTIVES: To determine the effect of testosterone administration to prepubertal (15 days old) and peripubertal rats (30 days old) on hypothalamic nitric oxide synthetase (NOS), and GnRH release. **METHODS:** Hypothalamic samples containing the anterior preoptic and medial basal areas (APO-MBH) were incubated for 30 minutes in 500 l of Earle's medium with glucose (1 mg/ml) and bacitracin (20 mM). GnRH was determined by RIA in the medium and NOS activity was determined in APO-MBH after 10 min of incubation by the conversion of 14 C arginine to 14 C citrulline. **RESULTS:** Treatment with testosterone propionate, significantly decreased NOS hypothalamic activity in prepubertal male rats. (Control: 58.41 ± 0.85 ; Testosterone: 25.61 ± 1.40 , $p < 0.001$) and had no effect in peripubertal male rats (Control 49.28 ± 1.50 ; Testosterone 51.48 ± 5.2 pmoles NO/10 min/hypothalamus). On the other hand, in prepubertal rats the treatment decreased Gn-RH release (Control: 3.62 ± 0.23 ; Testosterone: 1.38 ± 0.11 (pg/ ml medium, $p < 0.001$) and had no effect on Gn-RH release in 30 days old rats (Control: 3.65 ± 0.33 ; Testosterone: 4.15 ± 0.36 pg/ ml. medium). **CONCLUSION:** These results clearly demonstrated that testosterone has an inhibitory effect on hypothalamic NOS activity in prepubertal rats while it did not affect the concentration of this neurotransmitter system in peripubertal rats. This pattern is similar to that observed with GnRH hypothalamic release since testosterone has an inhibitory effect in prepubertal rats and did not modify the GnRH release in peripubertal rats. Taking into account the well known stimulatory effect of NO on GnRH and the decrease in the sensitivity of GnRH-gonadotrophin axis to the inhibitory feedback effect of testosterone during sexual maturation and the onset of puberty, it is proposed that the changes here described are connected with maturational modifications in the sexual hormones on-GnRH axis connected with the onset of puberty.

Introduction

The involvement of nitric oxide (NO), as an intracellular and extracellular gaseous neurotransmitter synthesized from L-arginine by nitric oxide synthetase (NOS), [1] in the hypothalamic control of gonadotrophin secretion has been demonstrated [2,3,4,5,6,7]. NO as a diffusible signaling gas has the ability to control and synchronize the activity of the neighboring cells. GnRH neurons are under the regulatory effect of NO. At the median eminence NO activates GnRH terminals which, at the same time increases the release of the hypothalamic hormone [3]. NO secretion in the median eminence was also involved in the modulation of GnRH release during the estrous cycle and in the genesis of pulsatile GnRH secretion [3,4,6].

There are clear evidences that GnRH is the primary messenger involved in the activation of pituitary gonadotrophins during sexual maturation and in the onset of puberty [8]. GnRH hypothalamic neurons are under the regulatory activity of different neurotransmitter and neuromodulator systems which change its effects during sexual maturation and at the time of the onset of puberty [9,10,11]. The decrease in the inhibitory effect of testosterone on gonadotrophin secretion appears to be one of the neuroendocrine mechanisms involved in the increases of these pituitary hormones during the onset of puberty [8]. Apparently these changes are related with changes in sensitivity of neurotransmitters-GnRH neurons to sexual hormones during sexual maturation. [10]. On these bases the possibility arises that the sexual hormones could modify NOS hypothalamic activity during sexual maturation as one of the neuroendocrine mechanisms implicated in the changes in the sensitivity of hypothalamic-pituitary axis to the negative feedback effect of testosterone that take place during the onset of puberty.

The aim of the present paper was to determine the effect of testosterone on hypothalamic NOS activity in prepubertal and in peripubertal male. On the other hand the effect of testosterone on hypothalamic GnRH release was also determined at both ages.

Material and Methods.

Animals: Prepuberal (15 days of age) and peripubertal (30 days of age) male rats from 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 (lights on for 06.00 to 20.00 h, 22 °C).

Drugs: the following drugs were used: Testosterone propionate (Sigma Chemical Co., Saint Louis Mo), Dowex, AG50 W x8 resin. Biorad Laboratories, CA USA, L(U-¹⁴C) arginine (Amersham-Buckinghamshire, UK) with specific activity 11,26 GBq/mmol.

The animals were injected with testosterone propionate (Sigma Chemical, Co) 20 µg/kg subcutaneously, 72 hrs before sacrifice (Previous experiments demonstrated that this is the minimal dosage that induced

changes at any of the studied ages). Controls were administered with the solvent.

Hypothalamic Incubation : After decapitation the brains were rapidly removed and the hypothalami dissected out with a single razor blade as described previously [12]. Hypothalamic samples containing the anterior preoptic and medial basal areas (APO-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, caudally, by the mamillary bodies, the depth was 3-4 mm. After dissection, similar samples of the APO-MBH were put into plastic chambers containing 500 ul of Earle's medium with glucose (1 mg/ml) and bacitracin (20 mM). The pH was adjusted to 7.4. Each chamber was incubated in a Dubnoff shaker at 37 °C with constant shaking (60 cycles/min) under an atmosphere 95% O₂, 5% CO₂. After a 30 min preincubation period the medium was discarded and replaced with fresh medium. The samples were incubated for 30 min. The medium was removed and stored at -20 °C for subsequent GnRH assays.

Gn-RH determination GnRH concentration was measured in duplicated samples by radioimmunoassay (RIA) The intra and interassays coefficient of variation was lower than 9% and 10% respectively and the sensitivity was 0.2 pg/tube. Results were expressed as pg/ml medium.

NOS activity: Was determined in similar animal groups (15 and 30 days of age male rats controls and treated with testosterone). After dissecting the APO and HMB as previously described NOS was determined by conversion of ¹⁴C arginine to ¹⁴C citrulline using a modification of the method of Bredt and Snyder. [13]. As NOS converts arginine in equimolar quantities of citrulline and NO, the results were expressed as pmoles NO/10 min of incubation/hypothalamus

Results

Effect of the administration of testosterone on hypothalamic NOS activity

As can be seen in Figure 1, the treatment with testosterone propionate, significantly decreases NOS activity in prepubertal male rats as compared with controls and expressed as pmol NO/10 min of incubation/hypothalamus (Control: 58.41±0.85; Testosterone: 25.61±1.4, p<0.001) and has no effect in peripubertal male rats. (Control: 49.28±1.50; Testosterone: 51.48±5.2. pmol./hypothalamus).

Effect of the administration of testosterone on hypothalamic Gn-RH release

The administration of testosterone propionate (Fig. 2) to prepubertal rats decreases Gn-RH release compared with controls. (Control: 3.62±0.23; Testosterone: 1.38±0.11 (pg/ml medium, p<0.001) and has no effect on Gn-RH release in 30 days old rats (Control: 3.65±0.33; Testosterone: 4.15±0.36 pg/ ml. medium).

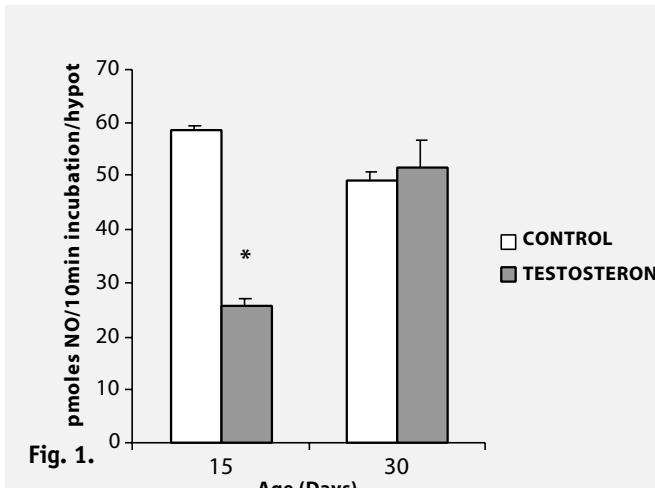


Fig. 1.

Fig. 1. Hypothalamic concentration of NOS after 10 min incubation. Means \pm standard error of 8–10 determinations * $p < 0.001$

Fig. 2. Effect of Testosterone on GnRH by hypothalamic tissue (APOA MBH). Means \pm standard error of 8–10 determinations * $p < 0.001$.

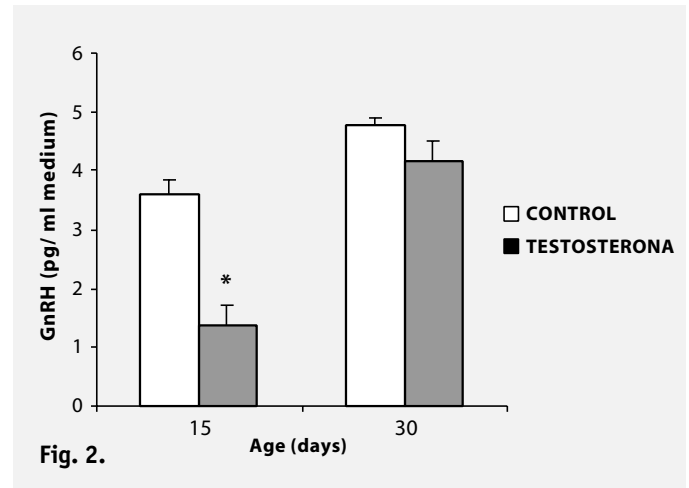


Fig. 2.

Statistical Analysis

The results are expressed as the means \pm S.E.M. Significance was assessed by analysis of variance (ANOVA) and Tukey's multiple range test [14]. Where appropriate, Student's *t* test was used when comparing only two treatments; $P < 0.01$ was considered significant.

Discussion

The sexual maturation and the onset of puberty implicates qualitative and quantitative modifications in the effect of neurotransmitter system on GnRH control. We propose that these modifications are connected with the neuroendocrine processes related to neural maturational mechanisms as well as the development of new interneuronal connections that characterize the onset of puberty [10]. These modifications also implicates changes in the effect of sexual hormones not only on the activity of these neurotransmitter systems but also on the synthesis and release of GnRH. For instance, during sexual maturation the negative feedback effect of testosterone on gonadotrophin release decreases and this appears to be one of the mechanism involved in the increase of gonadotrophin secretion that characterized the onset of puberty. This change appears to be connected with modifications in the sex hormone-neurotransmitter-GnRH axis relationships [8].

The results of the present paper indicate that in prepubertal rats the administration of testosterone not only decreases GnRH release but also the hypothalamic NOS activity. On the other hand in peripubertal rats the sexual hormone neither modified the hypothalamic release of GnRH nor NOS activity. The present results appear to indicate that NOS is in some way involved in the neuroendocrine mechanisms by which there is a decrease in the sensitivity of GnRH-gonadotrophins axis to the negative feedback of testosterone.

There are several experimental evidences that sexual hormones modified the NOS activity in the hypothalamus that are correlated with changes in GnRH secretion. Recent studies suggested that the vascular endothelium of the median eminence was involved, via NO secretion, in the modulation of GnRH release during estrus cycle and that E_2 is able to modulate endothelial NOS [6].

It has been demonstrated that androgens may modulate NOS activity. It has been recently showed the presence of androgens receptors in the NOergic neuron of the major pelvic ganglion in the male rat; it provides anatomical evidence for the direct association of androgens with NOS activity and synthesis [15].

Taking into account the experimental evidences of the regulatory influence of sexual hormones on NO activity as well as the participation of this neurotransmitter system in the control of GnRH, it is clear that testosterone may probable exerts its negative feedback effect on gonadotrophin axis by acting on hypothalamic NO activity and on other neurotransmitter and neuromodulator systems. The effects of gonadal steroids upon Gn-RH neuronal system are thought to be mediated by interactions with central neurotransmitter systems. The amino acids neurotransmitters system appears to be relevant in these neuroendocrine processes. [16,17,18,19,20]. For instance the positive and negative feedback effects of estrogens on gonadotrophin secretion are connected with modifications in the hypothalamic release of excitatory and inhibitory amino acid neurotransmitters (glutamate, aspartate and GABA) [17]. On the other hand it has been demonstrated the interrelationships of NO with hypothalamic amino acids neurotransmission [7]. On these basis the possibility arises that the amino acids neurotransmitter system could be involved in the changes in the sensitivity of NOS to testosterone during the sexual maturation here described. It is clear that more experimental evidence is needed in this respect.

In conclusion the results of the present paper demonstrated that the inhibitory effect of testosterone on hypothalamic NOS activity observed in prepubertal rats disappears in peripubertal rats. Taking into account the stimulatory effect of NOS on GnRH the possibility arises that during the onset of puberty the increase in the hypothalamic release of GnRH could be connected with the lack in the inhibitory effect of the sexual hormone on hypothalamic NO activity. This in turn would modify the inhibitory feed back effect on GnRH. It is clear that this could be an important neu-

roendocrine maturational mechanism involved in the onset of puberty.

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