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Androgen receptors are required for full masculinization of nitric oxide synthase system in rat limbic–hypothalamic region

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ABSTRACT

The neuronal nitric oxide synthase (nNOS) is involved in the control of male and female sexual behavior and its distribution in several regions of the limbic–hypothalamic system, as well as its coexistence with gonadal hormones' receptors, suggests that these hormones may play a significant role in controlling its expression. However, data illustrating the role of gonadal hormones in controlling the nNOS expression are, at present, contradictory, even if they strongly suggest an involvement of testosterone (T) in the regulation of nNOS. The action of T may be mediated through androgen (AR) or, after aromatization to estradiol (E_2), through estrogen receptors.

To elucidate the role of AR on nNOS expression, we compared male and female rats with a non-functional mutation of AR (*Tfm*, testicular feminization mutation) to their control littermates. We investigated some hypothalamic and limbic nuclei involved in the control of sexual behavior [medial preoptic area (MPA), paraventricular (PVN), arcuate (ARC), ventromedial (VMH) and stria terminalis (BST) nuclei]. In BST (posterior subdivision), VMH (ventral subdivision), and MPA we detected a significant sexual dimorphism in control animals and a decrease of nNOS positive elements in *Tfm* males compared to their littermate. In addition, we observed a significant increase of nNOS positive elements in BST (posterior) of *Tfm* females. No significant changes were observed in the other nuclei. These data indicate that, contrary to current opinions, androgens, through the action of AR may have a relevant role in the organization and modulation of the nNOS hypothalamic system.

Introduction

Nitric oxide (NO) is a gaseous regulatory molecule that acts both as a second messenger and as a neurotransmitter. It is synthesized from arginine by the enzyme nitric oxide synthase (NOS) and it has been implicated in the regulation of several physiological and behavioral functions (for reviews see Prast and Philippu, 1992; Stuehr, 1997; Hull et al., 1999; Mungrue et al., 2003).

Molecular cloning and the study of immunological properties suggested that there are at least three isoforms of NOS that have been purified and characterized from nervous tissue, macrophages, and endothelial cells (Alderton et al., 2001). All these isoforms are present within the brain in different cellular compartments, but the neuronal isoform (nNOS) is largely predominant (Bredt et al., 1990).

Several reports demonstrated the presence of nNOS in structures belonging to neural circuits implicated in the control of reproductive behavior, i.e. the preoptic nucleus, the bed nucleus of the stria terminalis (BST), the ventromedial nucleus (VMH), the bed nucleus of the accessory olfactory tract and the medial amygdaloid nucleus, structures that belong to the vomeronasal system (VNS) (McDonald et al., 1993; Hadeishi and Wood, 1996; Collado et al., 2003; Gotti et al., 2005; Carrillo et al., 2007) which has been demonstrated to be sexually dimorphic (Segovia and Guillamon, 1993, 1996). In addition, competitive inhibitors of nNOS reduce mount rates (Sato et al., 1998), and prevent ejaculations (Benelli et al., 1995) in male rats. Moreover, disruption of nNOS gene in knockout mice results in inappropriate sexual behavior in male mice (Nelson et al., 1995). Conversely, treatment of rats with L-arginine, a natural nNOS substrate, facilitates sexual behavior in male rats (Benelli et al., 1995; Sato et al., 1998), whereas treatment with an inhibitor of nNOS (Mani et al., 1994) or with an inhibitor of NO-stimulated cyclic GMP production (Chu and Etgen, 1997) inhibits lordosis behavior in female rats.

The distribution of nNOS in several brain regions of mammals overlaps that of gonadal hormones' receptors. Regions like the BST, the amygdala, the preoptic region, the mediobasal hypothalamus, or the magnocellular nuclei are characterized by the presence of two types of estrogen receptors (ERα and ERβ) (Shughrue and Merchenthaler, 2001; Merchenthaler et al., 2004), of androgen receptors (AR) (Simerly et al.,

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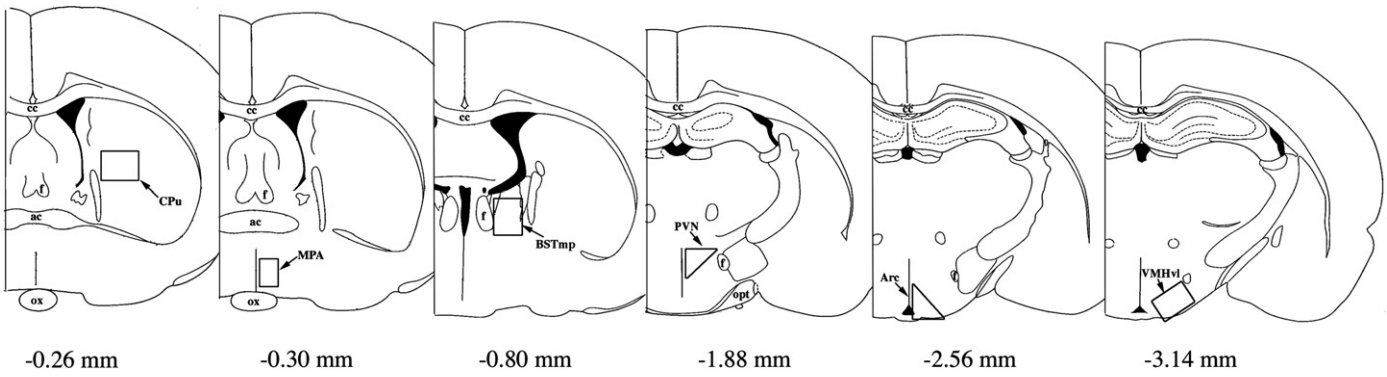

Fig. 1. Drawings of rat brain sections showing the nuclei sampled in this study. Boxed areas represent the regions in which cells were counted and immunoreactive staining was quantified. The stereotaxic coordinates listed are from rat brain atlas (Paxinos and Watson, 1986) based on the sections' relation to bregma. Arc, arcuate nucleus; BSTmp, bed nucleus of the stria terminalis, medial posterior subdivision; CPu, caudate putamen; MPA, medial preoptic area; PVN, paraventricular nucleus; VMHvl, ventromedial nucleus, ventrolateral subdivision.

1990), and of progesterone receptors (Lauber et al., 1991). Moreover, several studies investigated the coexistence of these receptors with nNOS-immunoreactive (ir) elements as well as the role played by gonadal hormones in the regulation of nitrinergic system in adult (for a review see Panzica et al., 2006). NO action on reproductive behavior is probably mediated by interactions with other neurotransmitter system such as dopamine (DA) in males (for reviews see: Hull et al., 1997, 1999, 2002) and noradrenaline in females (Chu and Etgen, 1996, 1999). In male rat, testosterone (T) acts by increasing nNOS immunoreactivity (Du and Hull, 1999), NO, in turn, stimulates the release of DA in the medial preoptic area (MPA) (Lorrain and Hull, 1993; Lorrain et al., 1996; Dominguez et al., 2004). The increased DA release enhances responsiveness to stimuli from an estrus female and increases the probability, rate, and efficiency of copulation (Lorrain et al., 1996). However, it was not known which metabolite(s) of T regulate(s) DA and/or nNOS in the MPA of male rats. The results of a recent study indicate that estradiol upregulates nNOS expression in the MPA and it maintains tissue content of DA at levels similar to those in T-treated rats. Dihydrotestosterone (DHT) did not influence nNOS immunoreactivity, while attenuating the effect of castration on tissue DA content (Putnam et al., 2005).

Rats with the testicular feminization mutation (*Tfm*) are partially insensitive to androgens. Testicular development and testosterone secretion appear to be normal in *Tfm* males and they do not display lordosis despite their external feminine phenotype (Olsen, 1979), so they are defeminized. They also show infrequent and incomplete male copulatory responses to receptive females (Beach and Buehler, 1977), but that could be due to either incomplete masculinization of brain circuitries or to the absence of normal male genitalia.

Tfm rats have significantly lower aromatase activity and significantly higher circulating T, DHT, and estradiol levels than their control male littermates (Roselli et al., 1987), but because they have a mutation in the AR gene involving a single nucleotide substitution (Yarbrough et al., 1990), only 10–15% of the AR protein binds androgen (Naess et al., 1976). As *Tfm* rats have decreased AR binding but apparently normal ER binding (Attardi et al., 1976), these animals offer a good model to understand the role played by the AR on nNOS

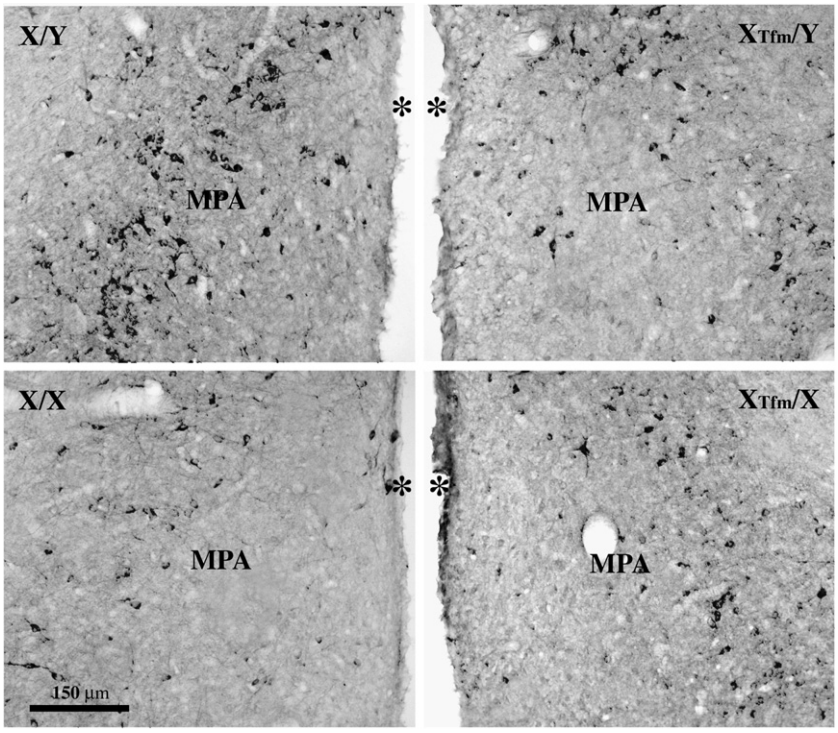

Fig. 2. Photographs of medial preoptic area (MPA) showing differences in nNOS-ir cell number in X/Y, X/X, X^{Tfm}/Y, and X^{Tfm}/X rats. Asterisk = third ventricle.