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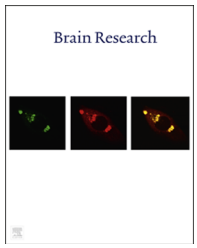

Research Report

Effects of sex steroids and estrogen receptor agonists on the expression of estrogen receptor alpha in the principal division of the bed nucleus of the *stria terminalis* of female rats

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ABSTRACT

Estrogen actions on neurons of the principal division of the bed nucleus of the *stria terminalis* (BNSTpr) are essential for the regulation of female sexual behavior. However, little is known about the effects of estradiol and progesterone (P) on estrogen receptor alpha (ERα) expression in this nucleus. To study this subject, we used stereological methods to estimate the total number of ERα-immunoreactive (ERα-ir) neurons in the BNSTpr of female rats at each stage of the estrous cycle and of ovariectomized rats after administration of estradiol benzoate (EB) and/or P. To ascertain the percentage of ERα-positive neurons in the BNSTpr, the total number of neurons in this nucleus was also estimated. In order to identify the specific role played by the selective activation of each ER in the expression of ERα, ovariectomized rats were injected with the ERα agonist, propyl-pyrazole triol (PPT), or the ERβ agonist, diaryl-propionitrile (DPN). Data show that ERα is expressed in 40–60% of the BNSTpr neurons and that the number of ERα-ir neurons is lowest at proestrus. This value is paralleled by the administration of EB. The number of ERα-ir neurons was not modified by P. PPT induced no changes in the number of ERα-ir neurons. Contrariwise, DPN induced a decrease in the total number of ERα-ir neurons to values similar to those of EB-treated rats. These results show that P has no effect in the modulation of ERα expression and demonstrate that estradiol regulation of ERα in BNSTpr neurons is mediated by activation of ERβ.

Abbreviations: ANOVA, analysis of variance; BNST, bed nucleus of the stria terminalis; BNSTpr, principal division of the bed nucleus of the stria terminalis; DPN, diaryl-propionitrile; EB, estradiol benzoate; ER, estrogen receptor; ERα, estrogen receptor alpha; ERα-ir, estrogen receptor alpha-immunoreactive; ERβ, estrogen receptor beta; PBS, phosphate-buffered saline; P, progesterone; PR, progesterone receptor; PPT, propyl-pyrazole triol.

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1. Introduction

The principal nucleus of the bed nucleus of the stria terminalis (BNSTpr), also known as the encapsulated part (Young, 1936) or the posterior medial subnucleus of the BNST (De Olmos et al., 1985; Moga et al., 1989), is a small-sized, densely packed group of round- to oval-shaped neurons located in the medial part of the posterior division of the BNST (Ju and Swanson, 1989; Moga et al., 1989). Rostrally, it is a distinct round cluster of neurons partially encapsulated by a cell-poor area and located above the anterior commissure, whereas caudally it forms a large ribbon of cells oriented ventromedially, lateral to the fornix and stria medullaris (Ju and Swanson, 1989; Moga et al., 1989). Its neurons express high levels of receptors for sex steroid hormones, namely, the estrogen receptor α (ERα) and the ERβ (Laflamme et al., 1998; Shughrue et al., 1997, 1998), androgen receptors (Simerly et al., 1990) and progesterone receptors (Parsons et al., 1982). The BNSTpr, together with the medial nucleus of the amygdala with whom it shares strong bidirectional connections, is an important component of the accessory olfactory pathway that conveys pheromonal information from the vomeronasal organ to the hypothalamus (Gu et al., 2003; Segovia and Guillamón, 1993) and, therefore, it plays a significant role in the regulation of neuroendocrine and autonomic responses, and of social behaviors (Dong and Swanson, 2004; Gu et al., 2003).

The neuroendocrine control of female social behaviors, like reproduction and aggressiveness, seems to be mediated by ERα-dependent mechanisms (Nelson and Chiavegatto, 2001; Ogawa et al., 1998; Rissman et al., 1997). Sex steroid hormone receptors are ligand-activated nuclear transcription factors that regulate the expression of target genes, including those of their own receptors. The observation that estradiol administration reduces [^{3}H]estradiol binding and the levels of ERα mRNA and protein in the preoptic area and hypothalamus (Brown et al., 1996; DonCarlos et al., 1995; Li et al., 1993; Osterlund et al., 1998) led to the general belief that estrogens downregulate ERα expression (reviewed in Blaustein and Erskine, 2002). However, a careful review of the literature revealed that this effect is not apparent from estimates of ERα-positive neuron numbers (Chakraborty et al., 2003). In particular, in the BNST it was shown that estradiol administration decreases the amount of ERα protein evaluated by measurement of immunocytochemical staining intensity (DonCarlos et al., 1995; Li et al., 1993), but does not alter the number of neurons immunoreactive for ERα estimated from level-matched sections (Gréco et al., 2001). Also, and in dissonance with the effects of exogenous estradiol, the increase of endogenous estrogen and progesterone (P) plasma levels during the proestrus stage of the estrous cycle has been reported either not to change or, alternatively, to increase or decrease ERα mRNA and protein expression in several preoptic and hypothalamic nuclei (Shughrue et al., 1992; Zhou et al., 1995).

Despite the recognized role of the BNSTpr in the neural circuits that regulate neuroendocrine responses and sexually dimorphic social behaviors that are mediated by ERα activation, very little is known about the effects of estradiol and P on the expression of ERα by its neurons. In addition, to our knowledge no studies have so far addressed the role played by each ER subtype in the modulation of ERα expression by BNSTpr neurons. Here, we address these questions by estimating, using stereological techniques, the total number of ERα-immunoreactive (ERα-ir) neurons in intact female rats at each phase of the estrous cycle, and in ovariectomized rats injected with estradiol benzoate (EB) and P, alone or in sequence, and with the specific agonists of the ERα, propyl-pyrazole triol (PPT) and ERβ, diaryl-propionitrile (DPN). In order to assess the percentage of neurons that express ERα in the BNSTpr, we have also estimated the total number of neurons in the BNSTpr using the same quantitative methods.

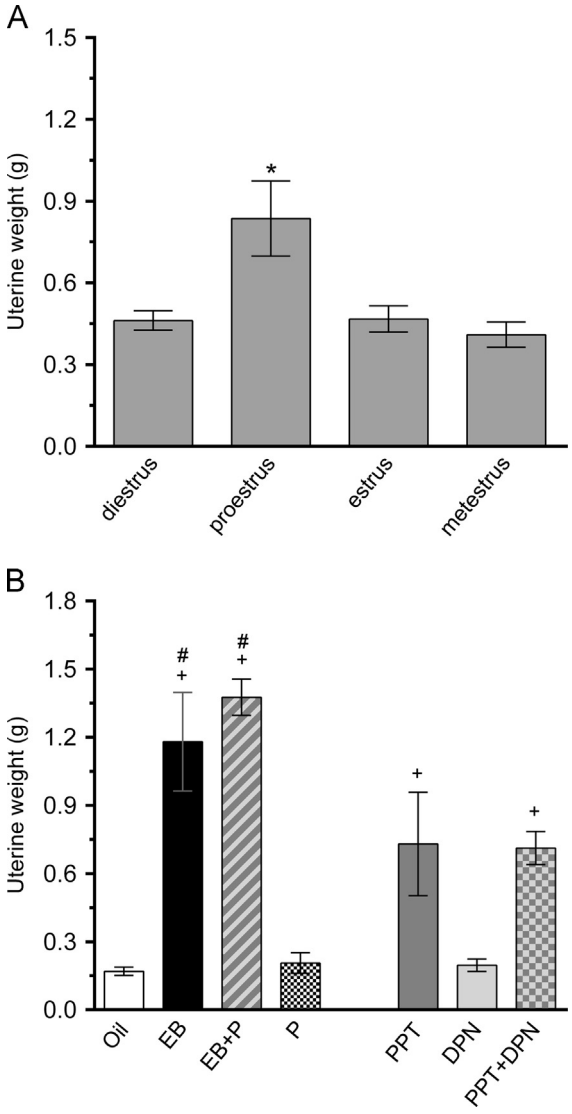

Fig. 1 – Graphic representation of uterine weights of cycling and ovariectomized rats. (A) Uterine weights of rats at each stage of the estrous cycle. (B) Uterine weights of rats injected with oil, EB, EB+P, P, PPT, DPN and a combination of PPT and DPN (PPT+DPN). Columns represent means and vertical bars 1 SD. Tukey's post-hoc tests: $^{*}P<0.0005$, compared with diestrus, estrus and metestrus; $^{+}P<0.0005$, compared with oil, P and DPN groups; $^{\#}P<0.0005$, compared with PPT and PPT+DPN groups.