FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2013/2014

Cláudia Alexandra da Rocha Nunes Leite

Effects of sex steroids on the expression of estrogen receptors in the principal division of the female bed nucleus of the *stria terminalis*

março, 2014

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Mestrado Integrado em Medicina

Área: Anatomia

Trabalho efetuado sob a Orientação de:
Doutora Susana Isabel Ferreira da Silva de Sá

Trabalho organizado de acordo com as normas da revista:
Brain Research

março, 2014

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Effects of sex steroids on the expression of estrogen receptors in the principal division of the female bed nucleus of the *stria terminalis*

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

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. Nevertheless, little is known about the effects of estradiol and progesterone (P) in the expression of estrogen receptor alpha (ERα) in this nucleus and the role played by the activation of each nuclear ER. To study this subject, we used unbiased stereological methods to determine the total number of ERα-immunoreactive (ERα-ir) neurons in the BNSTpr of young female rats across the four stages of the estrus cycle and of young ovariectomized rats after exogenous administration of estradiol benzoate (EB) and/or P. In order to identify the specific role played by the selective activation of each ER, young ovariectomized rats were injected with the ERα agonist, propyl-pyrazole triol (PPT) or the ERβ agonist, diaryl-propionitrile (DPN). The results show that the number of ERα-ir neurons is lowest at proestrus, when hormone levels are higher, and that the value seen at proestrus may be mimicked by the administration of EB but not of P. Administration of PPT induced no changes in the number of ERα-ir neurons. Contrariwise, the administration of DPN induced a decrease in the total number of ERα-ir neurons to values similar to the ones obtained after EB administration. These results suggest that P has no effect in the modulation of ERα expression and that estradiol regulation of ERα in BNSTpr neurons is mediated by activation of ERβ.

Keywords: Estrogen receptors; Estrogen receptor agonists; estrus cycle; Bed nucleus of the *stria terminalis*; Immunohistochemistry; Stereology

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 medularis (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 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 down regulate ERα (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 measured by intensity of immunocytochemical staining (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 estrus cycle has been reported either not to interfere 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 circuit that regulates 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 BNST 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 (OVX) 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).

2. Results

2.1. Uterine weights

Uterine weight was used as an index of peripheral response to ovarian hormone levels. We found significant overall effects of stage of the estrous cycle ($F(3,16) = 31.15$, $P < 0.0005$) and treatment ($F(6,28) = 74.74$, $P < 0.0005$) on uterine weights. Uteri were 80% heavier in proestrus than in any other stage of the estrous cycle (Fig. 1A). The uterine weights of rats injected with EB, EB+P, PPT and PPT+DPN were higher (4-7 times) than in oil-, P- and DPN-injected rats. The uteri weights of rats injected with EB or EB+P were also higher (about 1.5 times) than in rats injected with PPT or PPT+DPN (Fig. 1B).

2.2. Influence of stage of the estrous cycle on the expression of ERα

The stage of the estrus cycle influenced the total number of ERα-ir neurons in the BNSTpr ($F(3,16) = 12.38$, $P < 0.0005$; Fig. 2). The number was lower in rats at proestrus and estrus (25% and 20%, respectively) than in rats at metestrus or diestrus. In addition, the total number of neurons did not differ between proestrus and estrus rats as well as between metestrus and diestrus rats.

2.3. Effect of EB, P, and ERα and ERβ agonists on the expression of ERα

The total number of ERα-ir neurons in the BNSTpr of OVX rats was influenced by the administration of EB, P and ER agonists ($F(6,28) = 14.87$, $P < 0.0005$; Fig. 3). The total

number of neurons was 25% lower in rats treated with EB and with EB+P than in oil-injected rats. In DPN-treated rats, the total number of neurons was similar to that of EB-treated rats and smaller (23%) than in oil-injected rats. The total number of ERα-ir neurons did not differ between rats treated with P alone, PPT, PPT+DPN and oil.

3. Discussion

Data from the present study show that the total number of ERα-ir neurons in the BNSTpr is significantly reduced in rats at the proestrus and estrus stages of the estrous cycle relative to the numbers estimated in rats at metestrus and diestrus stages. Our findings are in agreement with data from an earlier study centered in the whole preoptic area in which a decrease of ER protein expression, measured by western blot, from metestrus to proestrus was noticed (Zhou et al., 1995). The increase in the circulating concentrations of estradiol after the ovarian surge of estradiol at proestrus is followed by a surge in P levels a few hours later, making this stage of the estrus cycle the one where both hormones are at their highest levels. The levels of these hormones start to decline almost immediately to reach basal concentrations at the estrus stage. There is a second smaller surge of P at metestrus that returns to basal levels at diestrus (Butcher et al, 1974; Smith et al., 1975). Therefore, the decrease in the number ERα-ir neurons at the proestrus and estrus stages may be mediated by the surge of estradiol at proestrus and/or of P at proestrus or metestrus. In fact, previous studies have shown that both hormones can induce the decrease in the concentration of ERs in the hypothalamus-preoptic area (reviewed in Blaustein and Erskine, 2002). In addition, in a study carried out in the medial preoptic nucleus it was shown that ER mRNA levels are highest at

estrus and metestrus, and thenceforth start to decline to reach the lowest value at proestrus (Shughrue et al., 1992). Assuming a similar mechanism for the regulation of ERα mRNA expression in the BNSTpr, it is conceivable that P might reduce the expression of ERα protein after its surge at proestrus or the levels of ERα mRNA at metestrus, which would precede a decrease in protein expression at proestrus. Also, the decrease of ERα mRNA levels at proestrus stage might prevent the ERα protein replenishment at the estrus stage.

To evaluate the influence of estrogens and P in the number of ERα-ir neurons we injected OVX rats with EB, P and EB followed by P. Our results show that the administration of EB leads to a marked decrease in the total number of ERα-ir neurons in the BNSTpr. This finding is at odds with data from an earlier study (Gréco et al., 2001) showing that EB- and oil-injected rats do not differ with respect to the number of ERα-ir neurons estimated from level-matched sections of the BNST. It is possible that the inconsistency between these and our own data might rely on differences on the parameters estimated (number of neurons in level-matched sections vs. total neurons numbers) and/or on the dose of EB administered (20 µg in two pulses 24 hours apart vs. 10 µg in one single pulse). Actually, previous studies have shown a dose-dependent effect in the estradiol-induced down regulation of ERα mRNA and protein expression (Brown et al., 1996; Osterlund et al., 1998). There is evidence from studies in several preoptic and hypothalamic nuclei, that P has the capability of modulating the expression of the ERα (Blaustein and Brown, 1984; Brown and MacLusky, 1994; Malikov and Madeira, 2013). Only a relatively small number of neurons in the BNSTpr contain progesterone receptors, but the expression of these receptors is significantly up-regulated by estradiol (Gréco et al., 2001; Parsons et al., 1982). However, our results demonstrate that the number of ERα-ir neurons in the BNSTpr is not influenced by P

levels. In fact, the injection of P in association with EB, which simulates the proestrus surge, did not interfere with the EB-induced decrease in the total number of ERα-ir neurons. In the same vein, the administration of P to OVX rats, which mimics the metestrus surge of P, did not modify the total number of ERα-ir neurons relative to oil-injected rats. These results suggest that, in the BNSTpr, P has no effect in the modulation of the number of neurons that express ERα.

Present results also show that the estradiol-dependent decrease in the number of BNSTpr neurons that express ERα is mediated by the activation of the ERβ, which suggests that this receptor is able to induce the repression of ERα in neurons that co-express both types of ER (Shughrue et al., 1998). Actually, the administration of DPN to OVX rats provoked a reduction in the total number of ERα-ir neurons similar to that induced by EB. Conversely, the administration of PPT alone or in association with DPN (PPT+DPN group) did not induce any change in the total number of ERα-ir neurons relative to those estimated in oil-injected rats. Taking into account that the BNST contains high levels of ERβ and ERα mRNA and protein, and that 95% of the neurons containing ERα co-express ERβ (Laflamme et al., 1998; Shughrue et al., 1997, 1998), the observation that the simultaneous administration of PPT and DPN did not induce any change in the total number of ERα-ir neurons was somehow surprising in view of the reduction of 25% observed in EB-injected rats. Previous studies have shown that the selective activation of ERα or ERβ may result in similar or divergent effects and that ERβ has a role in the modulation of ERα gene transcription (Frasor et al., 2003; Hall and McDonnell, 1999; Lindberg et al., 2003; Sá et al., 2009, 2013). PPT is a potent ERα agonist that binds to ERα with 400-fold preference and demonstrates almost no binding to ERβ (Stauffer et al, 2000). In contrast, DPN is a potency-selective agonist for ERβ with more than 70-fold higher binding affinity for ERβ than ERα (Meyers et al., 2001).

Because DPN has a lower binding affinity for ERβ than PPT has for ERα, it is possible that this discrepancy between the results obtained in PPT+DPN and EB-injected rats might result from the doses of PPT and DPN used in the experiments.

In summary, our results show that the decrease in the number of ERα-ir neurons that occurs at the proestrus stage of the estrous cycle is a consequence of the increase in circulating levels of estrogens. They also reveal the down regulation induced by estrogens in the number of ERα-ir neurons is mediated by the activation of the ERβ. The BNSTpr plays an important role in the transmission of olfactory information to the hypothalamic nuclei involved in the control of neuroendocrine responses and social behaviors that are modulated by ERα activity. The ERβ activation-induced decrease in the number of neurons that express ERα in the BNSTpr may be a way for estradiol to activate a mechanism of negative feedback to ERα action, and, in this way, regulate behavioral answers.

4. Experimental procedures

4.1. Animals

Female Wistar rats were obtained from the Institute for Molecular and Cell Biology (Porto, Portugal), and maintained under standard housing conditions (lights on between 07:00 and 19:00 h), and ambient temperature of 23 ºC. Food and water were freely available. Starting at 2 months of age, estrous cycles were monitored by daily vaginal smear cytology; only females exhibiting at least two consecutive 4 to 5-day estrous cycles were used. Vaginal smears were stained with Giemsa, and the phase of the

estrous cycle was determined by the proportion of leukocytes, nucleated epithelial cells, and cornified epithelial cells identified in the smears (Pompili et al., 2010). At 10 weeks of age, 35 normal cycling rats were bilaterally ovariectomized under deep anesthesia induced by sequential injections of promethazine (10 mg/kg b.w., subcutaneous), and a solution of xylazine (2.6 mg/kg b.w., intramuscular) and ketamine (50 mg/kg b.w., intramuscular). They were allowed to recover for 12 days before being randomly assigned to different treatment groups (n=5), as described below. In order to determine changes in the expression of ERα in BNSTpr neurons of intact cycling rats, 20 untreated rats were sacrificed at 12 weeks of age, in each stage of the estrous cycle (n=5/stage). All animal experimentation was conducted in agreement with accepted standards of humane animal care and in accordance with the European Communities Council Directives of 22 September 2010 (2010/63/EU) and Portuguese Act no 129/92.

4.2. Treatments

Sesame oil, EB and P were purchased from Sigma–Aldrich Company Ltd. (Madrid, Spain) and PPT and DPN from Tocris BioScience (Bristol, UK). Solutions were all prepared in 0.1 ml of sesame oil and injected subcutaneously. After recovery, ovariectomized rats were randomly allocated to seven groups and injected twice, 24 h apart, with the following: (1) 0.1 ml oil (oil group); (2) 10 µg EB (EB group); (3) 10 µg EB followed by one injection of 500 µg P, 4 h before sacrifice (EB+P group); (4) 500 µg P (P group); (5) 500 µg PPT (PPT group); (6) 500 µg DPN (DPN group); (6) 500 µg PPT+500 µg DPN (PPT+DPN group). The hormone and PPT doses used in this study are recognized as the optimal priming doses for the induction of sexual receptivity in ovariectomized rats and PR expression in several regions of the brain, and for

facilitating P-regulated sexual behavior (Mazzucco et al., 2008; McEwen and Alves, 1999; Sá et al., 2013). Although DPN does not induce sexual behavior or PR expression, whatever the dose employed, the dose used in this study was able to avert the action of ERα in the induction of PRs (Mazzucco et al., 2008; Sá et al., 2013).

4.3. Tissue preparation

Treated rats were sacrificed 48 h after the last injection, except in the EB+P group in which rats were sacrificed 4 h after the P injection. We chose this time interval because there is evidence that this is the optimal interval for the induction of P-mediated female sexual behavior (Boling and Blandau, 1939; Pfaff and Sakuma, 1979; Pfaus et al., 2006). Intact cycling rats were sacrificed at 3 months of age, between 14:00 and 16:00 h. Rats were anesthetized with 2 ml/kg b.w. of a solution containing sodium pentobarbital (10 mg/ml) given intraperitoneally, and sacrificed by intracardiac perfusion of a fixative solution containing 4% paraformaldehyde in phosphate buffer, pH 7.6. The brains were removed from the skulls, weighed, immersed in the same fixative solution for 1 h at 4 ºC, and then transferred to a solution of 10% sucrose in phosphate buffer at 4 ºC, where they were maintained overnight.

To confirm the stage of the estrous cycle in intact rats and the efficacy of the treatments in ovariectomized rats, the uteri were excised, freed from connective tissue and fat, and weighed.

4.4. Immunocytochemical staining of BNST neurons

Blocks of brain tissue containing the preoptic area and hypothalamus were mounted on a Vibratome with the rostral surface up and serially sectioned in the coronal plane at 40 µm. Sections containing the BNST were sampled at regular intervals of 120 µm (one out of 3), collected in phosphate-buffered saline (PBS) and, then, transferred to a cryoprotectant solution where they were maintained until processing for immunocytochemical detection of ERα-positive neurons. Because of the high number of animals and sections, one animal from each group was processed at a time for immunohistochemistry in order to minimize variation across groups.

Sections were thoroughly washed in PBS to remove the cryoprotectant, treated with 3% H_2O_2 for 10 min to inactivate endogenous peroxidase, washed again in PBS and blocked with 10% normal goat serum for 45 min. Sections were then incubated for 74 h, at 4º C, in rabbit polyclonal ERα antibody (MC-20, sc-542, Santa Cruz Biotechnology, Germany), at a 1:1000 dilution, followed by incubation, for 1 h, at room temperature with biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA, USA) at a 1:400 dilution. Sections were then incubated in avidin–biotin peroxidase complex (Vectastain Elite ABC Kit; Vector Laboratories), diluted 1:800, for 1 h, at room temperature. After that, they were incubated for 90 s in 0.05% diaminobenzidine (DAB; Sigma–Aldrich) to which 0.01% H_2O_2 was added, and rinsed with PBS to wash out all DAB. Stained sections were mounted on gelatin-coated slides. After air-drying overnight at room temperature, sections were counterstained with Giemsa solution for the examination of cytoarchitectural details (Fig. 4), dehydrated in a series of ethanol solutions (50%, 70%, 90% and 100%), cleared in xylol, and coverslipped using Histomount (National Diagnostics, Atlanta, GA, USA).

4.5. Estimation of the total number of ERα-immunoreactive neurons

The total number of ERα-ir neurons was estimated by applying the optical fractionator method (Madeira et al., 1997; West et al., 1991). For this purpose, sections were analyzed using a modified Olympus BH-2 microscope interfaced with a color video camera and equipped with a Heidenhain ND 281 microcator (Traunreut, Germany), a computerized stage, and an object rotator (Olympus, Albertslund, Denmark). A computer fitted with a framegrabber (Screen Machine II, FAST Multimedia, Germany) was connected to the monitor. The boundaries of the BNSTpr were consistently defined based on cytoarchitectonic criteria (Fig. 4B; Ju and Swanson, 1989). By using the C.A.S.T. – Grid system software (Olympus), the fields of view were sampled in each section at regular intervals of 80 µm along the x and y axes. The disector used had a counting frame area of 991 μm^2 at the tissue level and a fixed depth of 10 µm. The estimations were performed using a 100× oil immersion lens with a numerical aperture of 1.40. A cell profile was considered immuno-positive for ERα if dark brown reaction product was present within the cell nucleus (Fig. 4C). The mean coefficient of error (Gundersen et al., 1999) of the estimates was 0.06. All counts were made by an observer who was blind to the group assignment.

4.6. Statistical analyses

The effect of the stage of the estrus cycle and of the treatment regimen used was assessed by one-way analysis of variance (ANOVA) followed by post-hoc Tukey's HSD test. A probability value of 0.05 was used to determine statistical significance.

Acknowledgments

The authors wish to thank Professor José Paulo Andrade for helping with BNST photomicrographs. This work was supported by National Funds through FCT - Fundação para a Ciência e a Tecnologia within the scope of the Strategic Project UI 121 – 2014 (PEst-OE/SAU/UI0121/2014).

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Figure Legends

Fig. 1 - Graphic representation of uterine weights of normal cycling and ovariectomized rats. (A) Uterine weights of rats over 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.

Fig. 2 - Graphic representation of the effect of the stage of the estrous cycle on the total number of ERα-ir neurons in the BNSTpr. Columns represent means and vertical bars 1 SD. Tukey's post hoc tests: *$P < 0.05$, **$P < 0.005$, compared with diestrus and metestrus group.

Fig. 3 - Graphic representation of the effect of hormones and ERα and ERβ agonists on the total number of ERα-ir neurons in the BNSTpr 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: *$P < 0.05$, **$P < 0.005$, compared with oil, P and PPT groups; $^{+}P < 0.05$, compared with PPT+DPN group.

Fig. 4 - Coronal section of the female rat brain immunostained for ERα and counterstained with Giemsa solution. (A) Photomicrograph of a coronal section through the BNSTpr at bregma level approx. -0.80 mm. 3V, third ventricle; LV, lateral ventricle, oc, optic chiasm. Scale bar = 2 mm. (B) Higher magnification of the shaded area represented in A. The dashed line marks the BNSTpr limits. f, fornix; sm, stria

medularis; st, stria terminalis. Scale bar = 500 μm. (C) Higher magnification of the area delineated by the box in B. ERα-ir neurons have a dark brown immunostained nucleus and a relatively unstained cytoplasm. Scale bar = 20 μm.

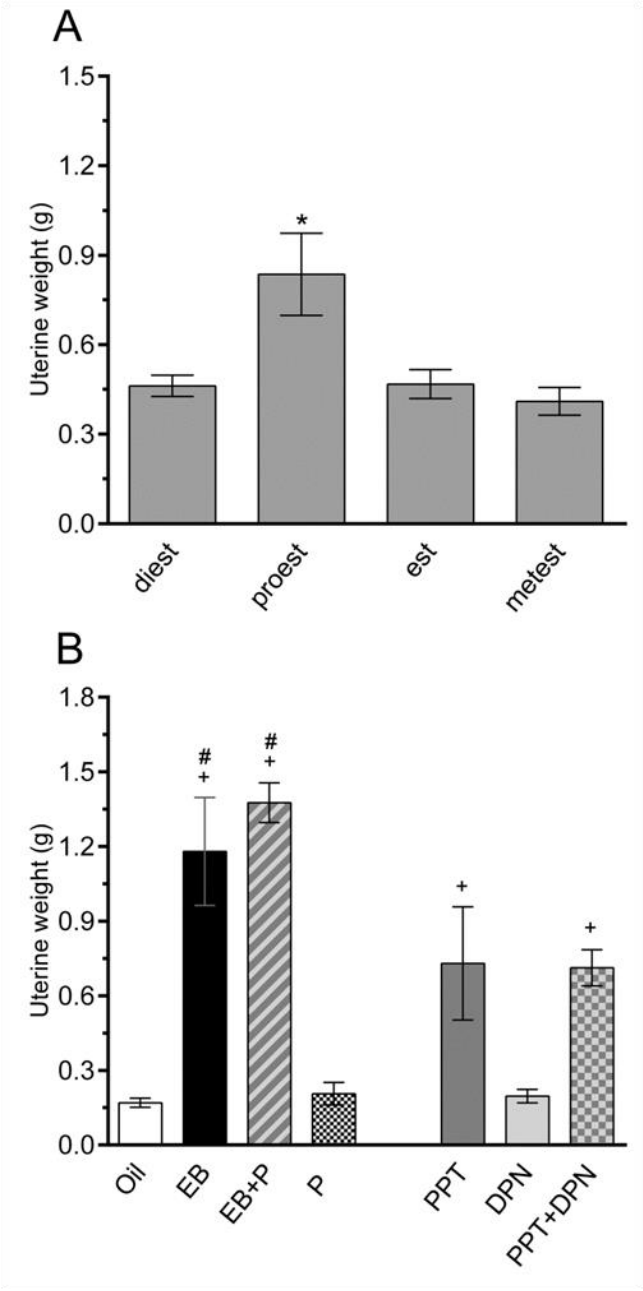
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Fig. 1

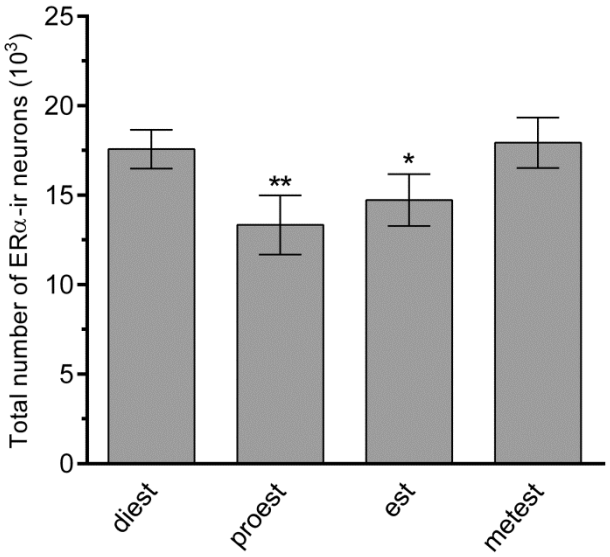
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Fig. 2

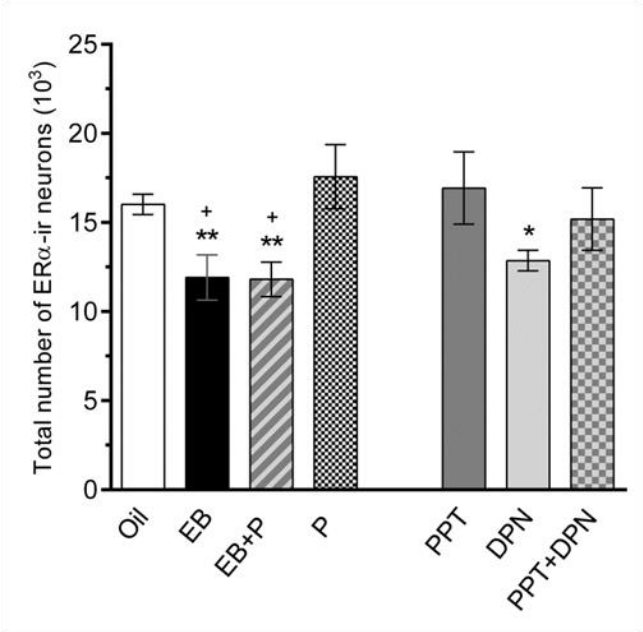
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Fig. 3

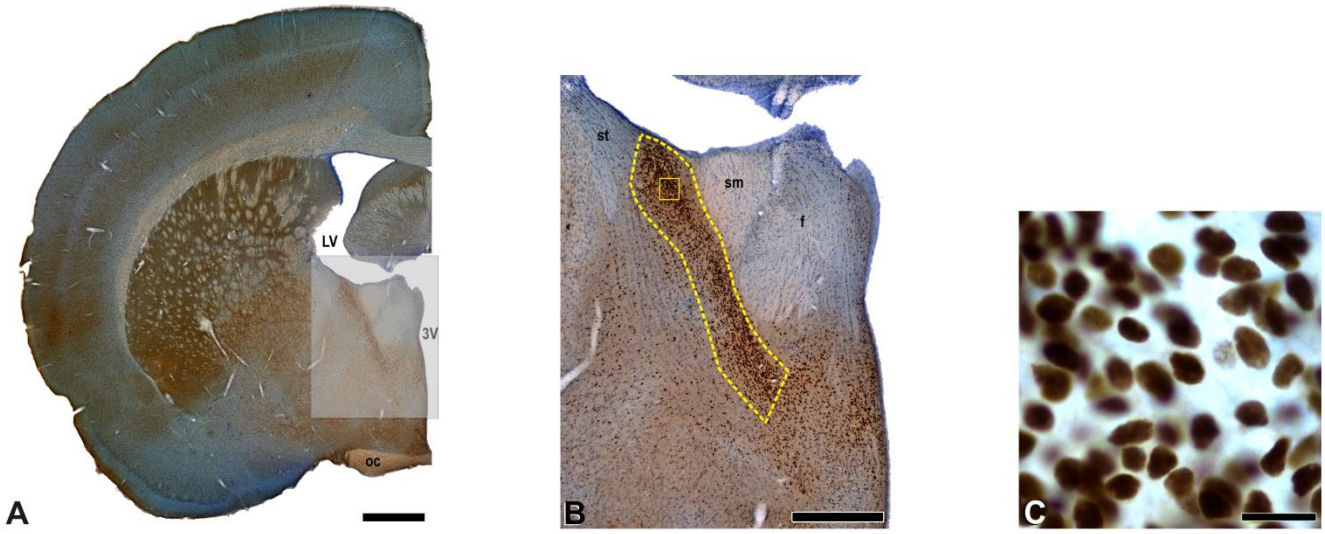

Fig. 4

Highlights

Higher hormone levels at proestrus and estrus lower the number of ERα neurons in BNST

Progesterone has no effect in the total number of ERα positive BNSTpr neurons

Estradiol decreases the total number of ERα positive BNSTpr neurons by activating ERβ

ERα expression in BNSTpr is dependent on estradiol-dependent ERβ activation

Agradecimentos

Gostaria de agradecer a todos aqueles que colaboraram na composição da presente tese.

À Professora Doutora Maria Dulce Cordeiro Madeira, Directora do Departamento de Anatomia da Faculdade de Medicina da Universidade do Porto, agradeço profundamente por me ter aceitado neste local de aprendizagem e investigação, assim como pelas suas insubstituíveis orientações e laboriosas revisões do meu trabalho.

À minha Orientadora de Tese de Mestrado, a Professora Doutora Susana Isabel Sá, agradeço carinhosamente por me ter facultado a excelente oportunidade de aprender consigo, tal como pelas suas contínuas instruções e dinâmicas correcções do meu trabalho. Sem esta formidável Orientadora, a concretização desta tese teria sido impossível.

Aos Professores Doutores José Paulo Alves Vieira de Andrade e Armando Cardoso, agradeço a pacientíssima colaboração na estruturação iconográfica do meu trabalho.

ANEXO

Guia de Publicação de Artigos para a Revista **Brain Research**

PS104

Total number of ERα-immunoreactive neurons of the principal division of the bed nucleus of the stria terminalis in female rat brain during the estrous cycle

C. Leite (1), M.D. Madeira (1) and S.I. S'ç (1)

(1) Department of Anatomy, Faculty of Medicine, University of Porto, Portugal

AIM

To evaluate the total number of ERα-ir neurons in the BNSTpr of three-month-old female Wistar rats during the estrous cycle.

INTRODUCTION

The BNST is a relay nucleus of the rostral forebrain closely related to the amygdala. It is extensively connected to some preoptic and hypothalamic nuclei involved in the neuroendocrine regulation of the reproductive behavior [1]. The effects of estrogens on the BNSTpr are essential for its role in the regulation of sexual and defensive behaviors. The BNST is known to express abundantly both types of nuclear estrogen receptors (ER) and their expression is modulated by hormone levels [2,3]. Estrogen receptor content significantly changes over the estrous cycle, with brain levels being highest during metestrus, attenuated on diestrus, and lowest during proestrus and estrus [4].

METHODS

The estrous cycle of 3-mo-old rats was monitored daily by vaginal smear cytology. Prior to perfusion, blood samples were taken directly from the heart and estradiol and progesterone serum levels were assayed. The animals were perfused, the brains were sectioned and the uteri were surgically isolated and weighed. The BNST-containing sections were processed for immunohistochemistry using an anti-rabbit ERÔ± antibody. The estimates of the total number of neurons were obtained by using stereological methods. The results were statistically analyzed using a one-way ANOVA.

RESULTS

The total number of ERα-ir neurons in the BNSTpr significantly altered over the estrous cycle, being about 35% lower during proestrus and estrus when compared with metestrus and diestrus.

CONCLUSION

These results suggest that gradual combinations of ovarian hormone levels have the ability to modulate the expression of ERα in the neurons of the BNSTpr. On the one hand, the prominent surge in progesterone levels which is observed in proestrus is correlated with a decrease in ERα expression. On the other hand, slight estradiol levels which are observed in metestrus and diestrus are correlated with an increase in ERα expression. This may be a way to the hormone control of the relay mechanism of the BNSTpr for the olfactory information to the hypothalamic nuclei involved in the control of sexual behavior.

This work was supported by National Funds through FCT.

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TOTAL NUMBER OF ERË±-IMMUNOREACTIVE NEURONS OF THE PRINCIPAL DIVISION OF THE BED NUCLEUS OF THE STRIA TERMINALIS IN FEMALE RAT BRAIN DURING THE ESTROUS CYCLE

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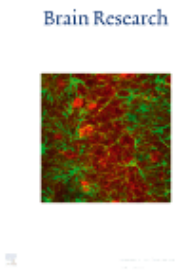

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