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ROLE OF ESTROGEN RECEPTOR α AND β IN THE INDUCTION OF PROGESTERONE RECEPTORS IN HYPOTHALAMIC VENTROMEDIAL NEURONS

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Abstract—The estrogen induction of progesterone receptors (PRs) in the ventrolateral division of the hypothalamic ventromedial nucleus (VMNvl) is critical for the regulation of female sexual behavior. VMNvl neurons express PRs and both types of estrogen receptors (ERα and ERβ), and their sequential activation initiates the molecular mechanisms underlying sexual behavior. To assess the relative importance of each ER subtype in the induction of PRs, we have estimated the total number of PR-immunoreactive neurons and quantified the total amount of PR protein in the VMNvl of adult ovariectomized rats that were injected with either estradiol benzoate (EB) or the specific agonists of the ERα, propyl-pyrazole triol (PPT), and of the ERβ, diaryl-propionitrile (DPN), in different doses and schedules. The administration of EB and of PPT alone, but not of DPN alone, increased the total number of PR-immunoreactive neurons and PR protein levels. When the specific agonists were administered sequentially, the total number of PR-immunoreactive neurons also increased, particularly when PPT was administered before DPN. Conversely, the concomitant administration of PPT and DPN did not increase the number of PR-immunoreactive neurons. The observation that PPT increases the number of PR-immunoreactive neurons and the levels of PR protein far less than EB shows that the estradiol induction of PRs in the VMNvl does not involve solely the activation of the ERα and suggests that it might also implicate the activation of membrane receptors. The present results also show that ERβ activation averts the action of ERα in the induction of PRs.

Key words: estrogen receptors, Western blot, estrogen receptor agonists, progesterone receptor, ventromedial nucleus, stereology.

INTRODUCTION

In rodents, the ventrolateral division of the hypothalamic ventromedial nucleus (VMNvl) is well known for its role in the modulation of goal-oriented behaviors that are vital for the survival of the species (Simerly, 1995), namely the display of lordosis, the receptive component of female sexual behavior (Pfaff and Sakuma, 1979; Blaustein and Erskine, 2002). Although in ovariectomized rodents the administration of estradiol followed by progesterone, or of high estradiol levels alone, can elicit this behavior, in intact female rodents, the sequential release of estradiol and progesterone is required for the synchronization of sexual behavior and ovulation (Wallen and Thornton, 1979). Progesterone facilitates the display of the paracopulatory (proceptive) and copulatory (receptive) phases of female sexual behavior (Whalen, 1974; Tennent et al., 1980; Blaustein and Erskine, 2002), and this action is mediated through specific intracellular receptors (for a review, see Frye, 2011). According to their response to estradiol, there are two distinct classes of progesterone receptors (PRs) in the brain of rodents: receptors not induced by estradiol, which were identified in the cortex and cerebellum (MacLusky and McEwen, 1978, 1980), and those that markedly increase after estradiol priming, the estradiol-induced PRs. These are distributed in the hypothalamus, preoptic area, bed nucleus of the stria terminalis, amygdala and pituitary (Parsons et al., 1982; Gréco et al., 2001). The VMNvl contains estrogen-regulated PRs and, in the adult female rat, their levels vary in parallel with the plasma concentrations of estradiol during the estrous cycle (Blaustein and Erskine, 2002; Guerra-Araiza et al., 2003), and their mRNA and protein levels increase with the administration of estradiol to ovariectomized rats (Lauber et al., 1991; Sá et al., 2010).

There is the general belief that nuclear estrogen receptors (ERs) mediate most of the actions of estrogens in the VMNvl. Neurons in the VMNvl express ERs of both subtypes (α and β) and PRs (Warembourg et al., 1989). Although the ERα largely predominates, some of the VMNvl neurons express the ERβ or co-express both receptor subtypes (Shughrue et al., 1998; Shughrue and Merchenthaler, 2001). The idea that the estrogen induction of female sexual behavior and of PR expression in the VMNvl is mediated by activation of the ERα emerged from investigations conducted in knockout mice models. These studies have shown that

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Abbreviations: ANOVA, analysis of variance; DAB, diaminobenzidine; DPN, diaryl-propionitrile; EB, estradiol benzoate; ER, estrogen receptor; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; PBS, phosphate-buffered saline; PPT, propyl-pyrazole triol; PR, progesterone receptor; PR-ir, progesterone receptor-immunoreactive; VMN, hypothalamic ventromedial nucleus; VMNvl, ventrolateral division of the hypothalamic ventromedial nucleus.

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the presence of a functional ERα is essential for the display of lordosis behavior (Ogawa et al., 1998; Kudwa and Rissman, 2003; Musatov et al., 2006) and for the full expression of PRs in response to estradiol (Moffatt et al., 1998). Subsequent studies in female rats have confirmed these observations in what concerns both the induction of sexual behavior (Rhodes and Frye, 2006; Mazzucco et al., 2008; Spiteri et al., 2010) and the expression of PRs in the brain (Harris et al., 2002), and revealed that the activation of the ERβ does not elicit lordosis and proceptive behavior (Mazzucco et al., 2008). However, it was also shown that in the ERαKO female and in double knockout female mice for ERα and ERβ estradiol maintains the capacity of inducing the expression of PRs in the caudal part of the VMNvl (Moffatt et al., 1998; Kudwa and Rissman, 2003). This observation suggests that, unlike sexual behavior, the induction of PRs by estradiol involves mechanisms that transcend the activation of nuclear ERs.

The use of knockout mice has limitations associated with development failures, altered hormonal profiles and production of ER splice variants that have complicated the interpretation of findings in what concerns the mechanisms through which estrogens act upon VMNvl neurons to induce the expression of PRs (Couse et al., 1995; Harris et al., 2002). Moreover, there is evidence that the two ERs exert different roles in the sexual differentiation of the brain (Kudwa et al., 2006; Gonzales et al., 2008) and, consequently, the lack of functional ERα or ERβ during development might compromise the differentiation of the hypothalamic ventromedial nucleus (VMN) and change the expression of estrogen-dependent physiological and behavioral processes in the adult rat. Therefore, to elucidate further the relative importance of each ER subtype in the estrogen induction of PRs in the VMNvl and the idea that estradiol-induction of PR-expression may not depend solely on nuclear ERs activation, we have used ER subtype-selective ligands as an alternative approach. Adult ovariectomized rats were injected subcutaneously with the specific agonists of the ERα, propyl-pyrazole triol (PPT), and of the ERβ, diaryl-propionitrile (DPN), alone, in combination or sequentially, and at dose levels that are known to induce changes in the connectivity of VMNvl neurons similar to those elicited by physiological doses of estradiol (Sá et al., 2009). To reveal the effects of these treatments on the expression of PRs, we have estimated the total number of PR-immunoreactive neurons and the amount of PR-protein expressed in the VMNvl and compared these data with those obtained in ovariectomized rats injected with estradiol benzoate (EB) or vehicle.

EXPERIMENTAL PROCEDURES

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 7:00 and 19:00 h) and ambient temperature of 23 °C. Food and water were freely available. Starting at 2 months of age, estrous cycles were monitored daily by vaginal smear cytology; only females exhibiting at least two consecutive 4- to 5-day estrous cycles were used. At 10 weeks of age, rats were bilaterally ovariectomized under deep anesthesia induced by sequential injections of promethazine (10 mg/kg b.w., s.c.), and a solution of xylazine (2.6 mg/kg b.w., i.m.) and ketamine (50 mg/kg b.w., i.m.). They were allowed to recover for 12 days before being randomly assigned to different treatment groups, as described below. 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 n°129/92.

Treatments

Estradiol benzoate was 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 (Sigma–Aldrich Company Ltd.) and injected subcutaneously. Starting 12 days after ovariectomy, rats were separated in thirteen groups and allotted to one of the following treatments: (1) 0.1 ml oil (oil group); (2) two pulses of 10 μg EB 24 h apart (EB group); (3) two pulses of 500 μg PPT 24 h apart (PPT group); (4) two pulses of 1 mg PPT 24 h apart (2PPT group); (5) two pulses of 3 mg PPT 24 h apart (3PPT group); (6) one pulse of 500 μg PPT followed, 24 h later, by 0.1 ml sesame oil (PPT–O group); (7) 0.1 ml sesame oil followed, 24 h later, by one pulse of 500 μg PPT (O–PPT group); (8) two pulses of 500 μg DPN 24 h apart (DPN group); (9) one pulse of 500 μg DPN followed, 24 h later, by 0.1 ml sesame oil (DPN–O group); (10) 0.1 ml sesame oil followed, 24 h later, by one pulse of 500 μg DPN (O–DPN group); (11) two pulses of a solution containing 500 μg PPT + 500 μg DPN, 24 h apart (PPT + DPN group); (12) one pulse of 500 μg PPT followed, 24 h later, by one pulse of 500 μg DPN (PPT–DPN group); and (13) one pulse of 500 μg DPN followed, 24 h later, by one pulse of 500 μg PPT (DPN–PPT group).

The dose of EB used in this study is widely recognized as the optimal priming dose for the induction of sexual receptivity in ovariectomized rats (Pfaff and Lewis, 1974; McEwen et al., 1999; Flanagan-Cato et al., 2006; Pfaus et al., 2006). The three doses of PPT used have been shown to induce PR expression in several regions of the brain and to facilitate progesterone-regulated sexual behavior in a dose-dependent way (Harris et al., 2002; Mazzucco et al., 2008). Because DPN does not induce sexual behavior or PR expression, whatever the dose employed, we have used one single dose that has been recognized as effective in promoting other behavioral responses (Lund et al., 2005; Mazzucco et al., 2008).

Experiments

Effect of EB, and ERα and ERβ agonists on the induction of PRs. The total number of PR-immunoreactive neurons and the amount of PR protein expressed in the VMNvl of rats of the EB, 2PPT, DPN and oil groups were comparatively analyzed. The 2PPT group was used in this experiment because the dose of PPT administered to this group evoked the highest increase in the number of PR-immunoreactive neurons in the VMNvl (see Results section).

Influence of dose and schedule of PPT and DPN administration in the total number of PR-immunoreactive neurons. The effect of different doses of PPT (PPT, 2PPT, 3PPT, PPT–O, and O–PPT groups) and DPN (DPN, DPN–O and O–DPN groups) on the total number of PR-immunoreactive neurons in the VMNvl was analyzed.

Influence of chronology of PPT and DPN administration in the total number of PR-immunoreactive neurons. The effect of simultaneous (PPT + DPN group), and isolated (PPT–O, DPN–O, O–PPT and O–DPN groups) or sequential (PPT–DPN,