Hormones and Behavior 75 (2015) 11–17

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Effects of postnatal estrogen manipulations on juvenile alloparental behavior

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ARTICLE INFO

Article history:
Received 6 January 2015
Revised 21 July 2015
Accepted 23 July 2015
Available online 26 July 2015

Keywords:
Estradiol
PPT
DPN
Alloparental behavior
Prairie vole
Estrogen receptor alpha

ABSTRACT

Sex- and species-specific patterns of estrogen receptor (ER)-α expression are established early in development, which may contribute to sexual differentiation of behavior and determine male social organization. The current study investigated the effects of ERα and ERβ activation during the second postnatal week on subsequent alloparental behavior and ERα expression in juvenile prairie voles. Male and female pups were treated daily with 17β-estradiol (E2, ERα/ERβ agonist), PPT (selective ERα agonist), DPN (selective ERβ agonist), or the oil vehicle on postnatal days (PD) 8–14. Alloparental behavior and ERα expression were examined at PD21. PPT treatment inhibited prosocial motivation in males and increased pup-directed aggression in both sexes. E2 and DPN had no apparent effect on behavior in either sex. PPT-treated males had increased ERα expression in the medial preoptic area (MPN), medial amygdala (MEApd) and bed nucleus of the stria terminalis (BSTpr). DPN treatment also increased ERα expression in males, but only in the BSTpr. Female ERα expression was unaffected by treatment. These results support the hypothesis that ERα activation in early life is associated with less prosocial patterns of central ERα expression and alloparental behavior in males. The lack of an effect of E2 on behavior suggests that ERβ may antagonize the effects of ERα on alloparental behavior. The results in DPN-treated males suggest that ERα in the MEApd, and not the BSTpr, may be a primary determinant of alloparental behavior in males.

Introduction

Prosocial behaviors consist of "positive" social interactions that benefit other individuals (Penner et al., 2005). Reproductive strategies often involve a trade-off between mating potential and prosocial behavior. Thus, highly prosocial strategies are characterized by delayed maturation, the formation of long-term social bonds and higher levels of caring for young, whereas less prosocial strategies involve rapid maturation, a focus on short-term mating opportunities and reduced care for young. Aggression and prosocial behavior, while not mutually exclusive, are typically considered to be opposite ends of social behavior, with high levels of aggression being considered to limit the expression of prosocial behavior — especially caring for young (Trivers, 1972; Wingfield et al., 1990).

Steroid hormones have been associated with both prosocial behavior and aggression (Del Giudice, 2009; Fernandez-Duque et al., 2009; Rilling and Young, 2014; Soma et al., 2008; Trainor et al., 2006; Yildirim and Derksen, 2012). However, studies on the role of estrogen in regulating these behaviors have produced mixed findings, which may reflect a number of factors including timing of treatment, sex, species, and/or the study design. Adding to this complexity, the two primary estrogen receptors (ERα and ERβ) can have opposing, synergistic or sequentially coordinated influences over behavior (Rissman, 2008). In general, ERα is associated with increased aggression, anxiety and emotionality — traits that should inhibit prosocial behavior — whereas ERβ is associated with reduced aggression and anxiety and enhanced cognition — traits that should facilitate prosocial behavior (Nomura et al., 2002; Ogawa et al., 1998; Oyola et al., 2012; Scordalakes and Rissman, 2004; Walf et al., 2009; Walf and Frye, 2005). Therefore, we hypothesized that ERα activation would reduce prosocial behavior in naïve males and females, whereas ERβ activation would enhance prosocial behavior.

Alloparental care in the prairie vole (*Microtus ochrogaster*) provides an excellent opportunity to study the role of estrogen receptors in regulating prosocial behavior and aggression in naïve males and females. As juveniles, both sexes are highly alloparental and rarely attack pups (Bales et al., 2004; Lonstein and De Vries, 2001). Reproductively-naïve adult males remain highly alloparental, whereas naïve adult females are more likely to show pup-directed aggression (Bales et al., 2004; Lonstein and De Vries, 1999, 2000a). Thus, adolescence involves the reduction in prosocial behavior in females only, unlike most other rodent species in which both sexes show a developmental decline in

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http://dx.doi.org/10.1016/j.yhbeh.2015.07.019
0018-506X/

alloparental behavior (Lonstein and De Vries, 2000b). The majority of adult female prairie voles will only revert to displaying high levels of alloparental behavior once they have given birth to pups (Hayes and De Vries, 2007). Estrogen and ER are thought to contribute to the reorganization of female prosocial behavior during motherhood (Olazábal et al., 2013), the mechanisms underlying its reorganization in naïve individuals during adolescence are less clear.

In part because social monogamy is distinguished by increased prosocial behavior by males, we have a greater understanding of the mechanisms regulating male prosocial behavior. While many factors contribute to male prosocial behavior, low levels of ERα expression in the medial amygdala (MEApd) and bed nucleus of the stria terminalis (BSTpm) appear to be a critical determinant (Cushing et al., 2008; Cushing and Wynne-Edwards, 2006; Lei et al., 2010). ERα expression in the MEApd and BSTpm is relatively limited during the first postnatal week and increases dramatically between the second and third postnatal weeks in both sexes, but with an attenuated rise in males that produces a significant sex difference (Yamamoto et al., 2006). Males show a further reduction in ERα expression in the MEApd and BSTpm between weaning and adulthood (Cushing et al., 2004; Kramer et al., 2006; Yamamoto et al., 2006), which renders these brain regions less sensitive to ERα activation. Several studies have shown that overriding the reduced ERα expression in these regions with viral vectors containing ERα cDNA (Cushing et al., 2008; Lei et al., 2010) or neonatal castration (Cushing and Kramer, 2005; Lonstein et al., 2002) reduces male prosocial behavior.

Therefore, to test the hypothesis that ERα activation reduces prosocial behavior in naïve males and females, we treated voles with estradiol (E2) or ER-selective agonists during the second postnatal week and examined their alloparental behavior one week later at weaning. We predicted that selective ERα activation would increase pup-directed aggression and reduce prosocial motivation in both sexes, and increase ERα expression in the MEApd and BSTpm of males only (i.e., reorganize the brain into a less prosocial configuration). We predicted that ERβ activation would increase prosocial behavior, decrease aggression and reduce ERα expression in the MEApd and BSTpm of males; however, as control juveniles were expected to be highly prosocial, these behavioral effects might be obscured by an apparent "ceiling effect".

Materials and methods

Husbandry

Prairie voles were maintained on a 14:10 h light:dark cycle (lights on at 06:00) and provided with high fiber rabbit chow and water ad libitum. On the day of birth, animals were sexed and marked for identification with a single toe clip — a standard and approved technique for Microtines, as they lack extensive pinnae and there is no other way to reliably mark individuals for later identification across treatment and testing phases. Subjects remained with the dam, sire, and litter mates until testing at postnatal day (PD) 21, the typical age for weaning. In no case were subjects exposed to their mother's subsequent litter. Thus, the alloparental test was the first experience with pups for all subjects. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were preapproved by the University of Illinois Committee on the Use and Care of Animals.

Treatments

Animals were randomly assigned within each litter to receive one of four daily treatments between PD8–14: 5 μg of 17-β-estradiol (E2; Sigma; (Kuiper et al., 1997)), 5 μg of the ERα-selective agonist 4,4′,4″-(4-propyl-[1*H*]-pyrazole-1,3,5-triyl)*tris*phenol (PPT; Tocris Bioscience; (Stauffer et al., 2000)), 5 μg of the ERβ-selective agonist diarylpropionitrile (DPN; Tocris Bioscience; (Meyers et al., 2001)), or sesame oil vehicle (Sigma). All injections were 25 μl in volume and given subcutaneously. Doses were based on average weight of PD8 vole (~8 g) and are within the range of doses used in other studies (Clipperton-Allen et al., 2011; Landau et al., 1978; Uban et al., 2011). The treatment period (PD8–14) was selected because it has been shown to be a sensitive period for estrogenic manipulations in voles, unlike the first postnatal week (Kramer et al., 2009; Lonstein and De Vries, 2000a; Sullivan et al., 2014), and corresponds to the developmental stage in which ERα expression begins to increase and become sexually dimorphic (Yamamoto et al., 2006). It also precedes the period during which males presumably become less sensitive to ERα activation due to their reduction in ERα expression in the MEApd and BSTpm. Additional non-treated controls were obtained from breeders that were left undisturbed outside of routine cage changes to control for potential effects of the handling procedure required for PDs8–14 injections. As there were no differences between oil and non-treated controls, they were combined into a single control group.

Alloparental behavior

At PD21, subjects were removed from the home cage and allowed to acclimate to the testing apparatus for at least 45 min, during which time food and water were freely available. The testing apparatus consisted of two standard size mouse cages (29 cm × 19 cm × 13 cm) connected by an 8 cm long clear acrylic tube. After the acclimation period, food and water were removed and an unrelated pup (PD1–3) was introduced into the center of the unoccupied chamber. The 10-minute test began when the experimental subject placed both forepaws into the cage containing the pup and was terminated if this failed to happen within 30 min. The test was stopped immediately if at any time a pup was attacked and its wounds were treated, or euthanized if necessary. The primary variables of interest were the percentage of attackers in each group and the total duration of pup contact, which included huddling over the pup and licking and grooming the pup. Retrieval and pup carrying were relatively rare in all groups and were not included in the measure of total pup contact. Non-attacking individuals were further divided into two alloparental categories based on their total duration of pup contact, with individuals displaying 103 s or more of pup contact designated "high alloparental" and those with less than 103 s designated "low alloparental." The 103-second threshold was empirically derived from the lower quartile of the combined male and female controls in the present experiment ($n = 71$).

Immunohistochemistry and image analysis

Immediately after testing, experimental subjects were deeply anesthetized and their brains were removed following transcardiac perfusion with 4% paraformaldehyde and 2.5% acrolein (pH 7.4). Brains were post-fixed for 24 h in 4% paraformaldehyde and equilibrated in 25% sucrose. 30-μm sections were cut on a freezing sliding microtome and stored in cryoprotectant at −20 ° C. Standard avidin:biotinylated enzyme complex (ABC) immunohistochemistry was conducted on free-floating sections using anti-ERα IgG (Santa Cruz Biotechnology, MC-20, diluted 1:7500) generated in rabbit. Briefly, sections were treated with 1% sodium borohydride and 0.014% phenylhydrazine to quench unreacted aldehydes from the perfusion and inactivate endogenous peroxidases, respectively. Sections were incubated in the primary antibody solution for 1 h at room temperature, and then for an additional ~60 h at 4 °C. Sections were incubated in anti-rabbit IgG (Vector Laboratories, BA-1000, diluted 1:600) for 1 h at room temperature, followed by incubation in ABC solution (Vector Laboratories, Vectastain Elite PK-6100, prepared according to manufacturer's instructions) for 1 h at room temperature. ERα was visualized by incubation in nickel-enhanced diaminobenzadine (Ni-DAB) solution for 15 min at room