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Progesterone receptor in the forebrain of female gray short-tailed opossums: Effects of exposure to male stimuli

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ABSTRACT

Progesterone receptor immunoreactivity (PRir) in brain areas involved in reproductive behavior in eutherian species was examined for the first time in a female marsupial, the gray short-tailed opossum (*Monodelphis domestica,* hereinafter, opossum*).* PRir in nuclei of neurons, measured as area covered by stained nuclei, was seen in the arcuate nucleus (Arc); anteroventral periventricular nucleus (AVPv); bed nucleus of the stria terminalis (BST); medial preoptic area (MPOA), and ventromedial hypothalamus (VMH), but not in control areas adjacent to the hypothalamus or cortex. Female opossums are induced into cytological, urogenital sinus (UGS), estrus by male pheromones and into behavioral estrus, i.e., receptivity, by pairing with a male, and both estradiol (E) and progesterone (P) are involved in induction of receptivity in intact and ovariectomized females. PRir in the AVPv, MPOA, and VMH was very low in females that had never been exposed to males or their scent marks, i.e., naïve anestrous (NVA) females, and either previous or current exposure to males or their scent marks was associated with elevated PRir. PRir was significantly higher in the AVPv and MPOA of anestrous females with previous but no current exposure to males and their scent marks, i.e., experienced anestrous (EXPA) females, than in NVA females, but PRir was significantly lower in the MPOA and VMH of EXPA females than in females that were behaviorally receptive and had recently copulated, i.e., behavioral receptive estrous (BRE) females. PRir was higher in the VMH of both UGS estrous (UGSE) and BRE females compared to that in EXPA animals, but PRir did not differ between UGSE and BRE females in any of the 3 brain areas examined, including the MPOA These results provide evidence that pheromonal induction of estrus and sexual receptivity in opossums is associated with elevation of PRir in the VMH and MPOA and that prior exposure to males or their pheromones, even in the absence of current male stimuli, is associated with persistent elevation of PRir in the AVPv and MPOA.

Introduction

Marsupial female reproductive behavior and its hormonal control are similar to that of eutherian species. For example, in gray short-tailed opossums (*Monodelphis domestica* hereinafter, opossums), small (60–150 g) marsupials native to Brazil (Streilein, 1982), an anestrous female is highly aggressive toward the male and typically shows open-mouth threats, screeches, and escape behavior when he approaches her. Although lordosis is not seen, a sexually receptive opossum shows little aggressiveness, allowing the male to mount, grasp her back feet with his hind feet and intromit (Fadem et al., 1996; Fadem, 1989). Proceptive behavior has been demonstrated by the finding that when allowed to choose, sexually receptive female opossums prefer to spend time with intact rather than with castrated males (Fadem et al., 2000).

In eutherian species, display of female sexual behavior is induced by estradiol (E) alone (Morali and Beyer, 1979) or both E and progesterone (P) (Feder and Marrone, 1977; Erskine, 1989) secreted by the ovaries and is abolished by ovariectomy. Ovariectomized (OVX) female opossums are highly aggressive toward males, and like rodents (Fadem et al., 1979; Erskine, 1989), OVX females that have been treated with both E and P are more sexually receptive than those treated with E alone (Fadem et al., 1996; Fadem and Erianne, 1997). Estrogen receptor immunoreactivity (ERir) has been reported in the MPOA, AVPv, VMH, arcuate nucleus (Arc), bed nucleus of the stria terminalis (BSTM), and median eminence of the hypothalamus of opossums (Etgen and Fadem, 1989; Handa et al., 1991; Pearson et al., 1993), but to our knowledge, neural PRir has not been reported in opossums or in any other marsupial species.

Female opossums do not show spontaneous estrous cycles but are induced into urogenital sinus (UGS) estrus (cornification of epithelial cells in the sexual receptive organ) within 5 to 10 days after initial exposure to male pheromone. Females access this non-volatile,

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estrus-inducing pheromone by nuzzling scent marks from the suprasternal gland of males (Fadem, 1987; Harder et al., 2008), a behavior associated with delivery of the pheromone to the vomeronasal organ (Poran et al., 1993; Jackson and Harder, 1996). Mere exposure to these marks, even in the absence of the male, leads to a rise in serum estradiol (E) (Fadem, 1989; Jackson and Harder, 2000). Juvenile females develop this responsiveness and a critical body mass (60 g) for puberty at about 5 months of age (Harder and Jackson, 2003). Opossums are induced ovulators (Baggott et al., 1987; Hinds et al., 1992; Stonerook and Harder, 1992), and ovulation is induced by male stimuli, in advance of copulation, which occurs an average of 2 days after pairing males with females in UGS estrus (Harder et al., 1993). Estrous females show a near simultaneous rise in P and a preovulatory rise in LH 10–20 h after pairing with a male but 18–24 h before copulation (Jackson et al., 1999; Harder et al., 2005). Receptivity in estrous females is dependent upon a male-induced rise in P, and estrous females that do not exhibit this rise when paired with a male do not copulate (Harder et al., 2005).

Because receptivity in opossums is dependent upon sequential elevation of plasma E and P, it is likely that, as in rodents, elevated P in female opossums acts upon elevated levels of neural PR to induce the receptive behaviors that are necessary for copulation. The sequential action of E and P on female reproductive behavior in rodents occurs mainly by induction of P receptor (PR) synthesis in E-receptor (ER)-containing neurons in the medial preoptic area (MPOA) and the ventromedial hypothalamus (VMH) (Blaustein and Feder, 1979; Blaustein and Olster, 1989; Pfaff et al., 1994), although brain areas such as the anteroventral periventricular nucleus of the hypothalamus (AVPv) may also be involved (Wiegand and Terasawa, 1982; Gu and Simerly, 1994). Thus, differences between anestrous and estrous opossums in PR expression in those areas of the forebrain are likely. In this study, forebrains of female opossums were examined for PRir, which was quantified in the AVPv, MPOA, and VMH, and the effects of current or prior exposure to males or their pheromones on PRir in these brain areas were analyzed.

Methods

Animals and experimental design

Female opossums (n=19, 5–25 months of age) were individually housed in polycarbonate cages (30×30×15 cm) in a mixed sex colony with a 14/10 h light/dark cycle (on at 1400 h) and a room temperature of 25–28 °C. Food (Fox Reproduction Food; Milk Specialties Co., New Holstein, WI) and acidified water (pH=4.2) were provided *ad libitum*. Males were caged individually and held on a separate rack, 1.7 m away from the female cage rack. The estrus-inducing pheromone of male opossums is not volatile (Harder et al., 2008), i.e., females are induced to estrus only by direct, nuzzling contact with male scent marks. Moreover, the use of separate cage racks for males and females and standardized husbandry and animal handling procedures precluded incidental transfer of this pheromone to females in this study. Other details of husbandry for this colony have been described (Jackson and Harder, 2000; Harder and Jackson, 2003). The animal facility was approved by the American Association for Accreditation of Laboratory Animal Care, and procedures relating to care and use of opossums in this study were described in protocols approved by the Institutional Laboratory Animal Care and Use Committee at The Ohio State University.

Females were assigned to one of the 4 treatment groups: not previously exposed to males or their scent marks: 1) naïve anestrus (NVA, n=5); 2) previously but not recently exposed to males and their scent marks: experienced anestrus (EXPA, n=5); 3) previously and recently exposed to male scent marks and in UGS estrus (UGSE, n=4); and 4) recently exposed to male scent marks, paired with males when in UGS estrus, and copulated: behavioral receptive estrus (BRE, n=5). Reproductive status in females was monitored by cytology of the UGS, the sexual receptive organ of marsupials (Fadem and Rayve, 1985; Baggott et al., 1987). Estrus is indicated by the presence of numerous keratinized epithelial cells in smears of vaginal mucus and a 4–6% gain in body mass. Anestrus is characterized by scant basal cells suspended in significantly less viscous vaginal mucus. Females in the NVA group had been isolated from mature males and their scent marks since birth. Females in the NVA and BRE groups were the youngest (aged 5 to 6 months), but all exceeded the critical body mass of 60 g associated with sexual maturity and responsiveness to male pheromone (Stonerook and Harder, 1992; Harder and Jackson, 2003).

EXPA females were isolated from males and their estrus-inducing pheromone for at least 14 days prior to euthanasia and collection of neural tissue. Both NVA and EXPA females remained in anestrus during this interval, which was confirmed by repeated UGS cytology. Prior to this experiment, females in the EXPA and UGSE group had been induced to estrus and paired with mature males, but only one female (UGSE) was known to have produced a litter. Females in the BRE groups were 6 months of age and had not previously been induced to estrus. UGSE and BRE females were induced to estrus by exposure to male scent marks collected by rubbing 4 sides of a 7-ml glass vial on the suprasternal gland of an unrelated male. The vial was then screwed into a base and placed daily in each female's cage (Jackson and Harder, 2000). UGSE females were euthanized when smears indicated UGS estrus (5–9 days after initial pheromone exposure), i.e., at the same cytological stage as BRE females were in when paired with males. BRE females were paired with breeding males at 1300–1500 h on their first day of UGS estrus, observed (directly and by video recording) until copulation was confirmed (second or third day after pairing) by a characteristic penile lock, and euthanized within 60 min of copulation.

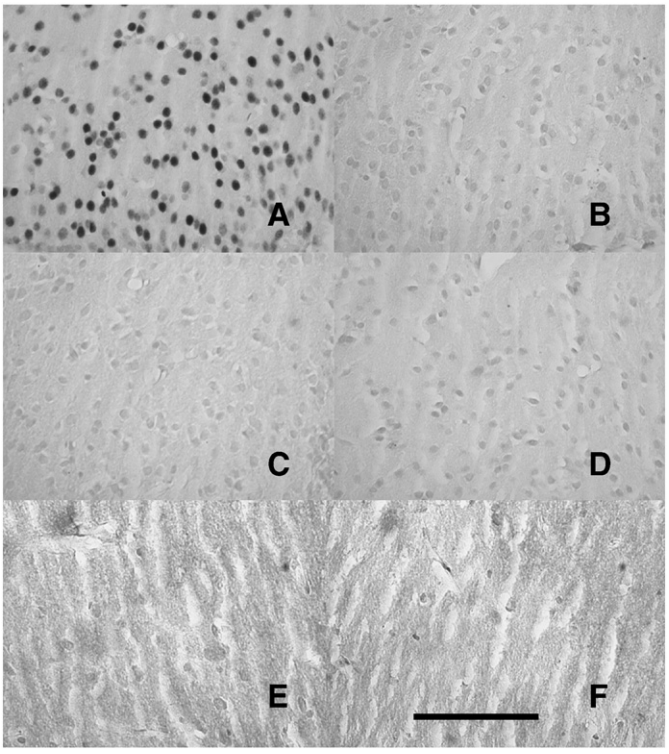

Fig. 1. Photomicrographs of female opossum brain sections following immunocytochemical control (ICC) procedures. MPOA from a receptive estrous female that received full ICC treatment (A) is compared to adjacent sections where primary antibody (B) or secondary antibody (C) was omitted or where the primary antibody was preabsorbed by its neutralizing peptide (D). Frontal cortex (E) and cortex adjacent to the hypothalamus (F) were selected as control areas of the brain that were not expected to show PR immunoreactivity. Scale bar=100 μm.