Available online at www.sciencedirect.com
SCIENCE DIRECT
Hormones and Behavior 50 (2006) 154–168
Hormones and Behavior
www.elsevier.com/locate/yhbeh

Progesterone receptor activation signals behavioral transitions across the reproductive cycle of the female rabbit

K.L. Hoffman *, G. González-Mariscal

Centro de Investigación en Reproducción Animal, CINVESTAV-Universidad Autónoma de Tlaxcala, Adpo Postal 62, Tlaxcala, Tlax. 90000 México

Received 11 October 2005; revised 27 January 2006; accepted 24 February 2006
Available online 11 April 2006

Abstract

The female rabbit is an exceptional experimental model to define mechanisms by which progesterone (P) controls the expression of reproductive behaviors. In the rabbit, the rise in P levels during pregnancy inhibits estrous scent marking ("chinning"), stimulates the excavation of a nest burrow ("digging"), and primes behaviors later used for nest construction. The pre-parturient fall of P triggers the construction of a straw nest ("straw carrying") that is lined with hair that she pulls from her own body ("hair pulling"). These behaviors can be replicated in ovariectomized (ovx) females given a schedule of estradiol (E) and P that mimics hormone levels during pregnancy (E from days 0 to 4, E + P from days 5 to 17, E from days 18 to 27). We administered PR antagonists RU486 or CDB(VA)2914 to ovx female rabbits during either the initial (days 5–11) or late (days 12–17) phases of P treatment, to determine the role of PR activation in coordinating the expression of these behaviors. Both antiprogestins attenuated the P-mediated decline in chinning and increase in digging when administered during days 5–11. When given across days 12–17, both antiprogestins triggered an early decline in digging, the onset of nest building in some Ss, and the reinstatement of chinning. These results point to a central role of PR activation for establishing and maintaining the behavioral phenotype of pregnancy, and for the behavioral transition from pregnancy to estrus.

Keywords: Maternal behavior; Rabbit; Nest building; Sexual behavior; Proceptive behavior; Scent marking; RU486; CDB(VA)2914; RTI3021-012

Striking changes in behavior are apparent across the reproductive cycle of many female mammals. For example, estrus is defined by enhanced sexual proceptivity and receptivity, whereas sexual behavior is generally inhibited during pregnancy. Parturition is sometimes preceded by nest building and followed by the onset of maternal behavior and eventually the reinstatement of estrus (for review, González-Mariscal and Poindron, 2002; Numan and Insel, 2003; Russel et al., 2001). The behavioral phenotypes typical of each phase of the reproductive cycle are established and maintained in large part by the endogenous hormonal milieu and by sensory stimuli originating from the offspring.

Progesterone (P) participates significantly in the regulation of female reproductive behavior in most mammalian species. In rodents, the preovulatory rise in P stimulates estrous behavior while establishing refractoriness to further P stimulation (sequential inhibition; Blaustein and Wade, 1977; González-Mariscal et al., 1993; Morin, 1977; Zucker and Goy, 1967); the molecular mechanisms underlying these effects are beginning to be elucidated (e.g., Beyer et al., 2003; González-Flores and Etgen, 2004; González-Flores et al., 2004a, 2004b). A similar biphasic action of P has been described in the ovariectomized hormone-treated horse (Asa et al., 1984), and in the dog a preovulatory rise in P normally stimulates estrous behavior (Concannon et al., 1979; Wildt et al., 1979). In other mammals, including primates, the cow, pig, cat, ferret, and rabbit, there is little evidence that P stimulates estrous behavior in either intact or ovariectomized hormone-treated females (Baum et al., 1976, 1977; Beyer and McDonald, 1973; Ford, 1985; Glencross et al., 1973; Johnson and Phoenix, 1978; Nadler et al., 1983; Parvizi et al., 1976; Slob et al., 1978; Valles et al., 1992; Villars et al., 1990; Wildt et al., 1981). By contrast, in rodents, primates, the rabbit, and the ferret, the action of P has been implicated in decrements in female attractivity, proceptivity, and/or receptivity during pregnancy or during the luteal phase of the menstrual cycle (Baum et al., 1976, 1977, 1986; Baum, 1979; Beyer and

* Corresponding author. Fax: +52 222 23 998 64.
E-mail address: rexvitro@hotmail.com (K.L. Hoffman).

0018-506X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2006.02.007

McDonald, 1973; Goy et al., 1966; Graham et al., 1972; Morin, 1977; Powers and Zucker, 1969). In some cases, chronically elevated P levels act to "prime" behaviors that are not expressed until after P levels decline. For example, in the rat and rabbit, chronically elevated P during pregnancy (in concert with E) primes maternal behaviors that are subsequently disinhibited when P levels fall prior to parturition (Bridges, 1984; González-Mariscal et al., 1994, 1996; Rosenblatt and Siegel, 1981). Similarly, in the ewe the expression of estrous behavior in response to E requires a prior rise and fall in P (Fabre-Nys and Martin, 1991a,b). Thus, P exerts stimulatory, inhibitory, and/or priming effects on female reproductive behavior across the reproductive cycle of most mammalian species.

Many of the biological effects of P involve the binding of this hormone to its intracellular receptor (PR). PR is a ligand-activated transcriptional modulator present in brain regions associated with reproductive behavior, and its expression changes across the reproductive cycle (e.g., Caba et al., 2004; Guerra-Araiza et al., 2003; Numan et al., 1999). Independent of PR activation, P also modulates the action of the 5HT3, nicotinic, and oxytocin receptors, and its ring-A reduced metabolite (3-alpha hydroxy-5-alpha-pregnan-20-one; 3-alpha-THP) enhances the activity of GABA-A channels, essentially mimicking the effects of benzodiazepenes (for reviews, see Rupprecht, 2003, Schumacher et al., 1999). In the female rodent, the stimulatory effect of P on sexual proceptivity and lordosis involves PR activation and also the action of ring A-reduced progestins on other receptors (e.g., GABA-A; Beyer et al., 1989, 2003; Frye, 2001). Given its many modes of action, it is a significant challenge to define mechanisms by which P exerts its behavioral effects.

The female rabbit is an exceptional model to investigate mechanisms by which P exerts inhibitory, stimulatory, and priming effects on reproductive behaviors. During pregnancy, P inhibits estrous scent marking ("chinning") and sexual receptivity (Beyer and Rivaud, 1969; González-Mariscal et al., 1990), while stimulating the excavation of a nest burrow ("digging"). Prior to parturition, digging declines concomitantly with P levels, the doe collects straw and builds a nest inside her burrow ("straw carrying"), pulls hair from her own body ("hair pulling"), and lines her nest with it (González-Mariscal et al., 1994; Myers and Poole, 1961). The central role of P in controlling these behaviors is apparent in ovx, E-primed females: chronic administration of E + P inhibits chinning, stimulates digging, and primes straw carrying and hair pulling, which are subsequently expressed after the withdrawal of P (González-Mariscal et al., 1996; Hudson et al., 1990).

We examined the role of PR activation for the behavioral effects of P in the ovx female rabbit. Previous immunohistochemical studies indicated that in both intact estrous and in ovx does primed for 5 days with E, PR was expressed in the infundibular nucleus, nucleus X [Wahren (1957); a nucleus located lateral to the ventromedial hypothalamic nucleus in the rabbit], preoptic region (periventricular, medial, and principal preoptic nuclei), and the paraventricular nucleus (Caba et al., 2003). After 3 days of E + P administration to E-primed ovx does, a treatment that inhibits chinning and stimulates digging, PR immunoreactivity had disappeared in all of these regions except the infundibular nucleus (Caba et al., 2003). Similarly, PR immunoreactivity was absent in the forebrain of late pregnant does (Caba et al., 2004). Thus, the behavioral phenotype of estrus was associated with increased PR immunoreactivity in several forebrain nuclei, while the behavioral phenotype of pregnancy was associated with the absence of PR immunoreactivity in the forebrain. Our initial hypothesis, based on these immunohistochemical data, was that PR activation would be necessary to *establish* the behavioral phenotype of pregnancy (inhibited chinning, increased digging), but that mechanisms independent of PR (such as chronic stimulation of GABA-A receptors) were responsible for *maintaining* this phenotype. To test this hypothesis, we treated ovx females with a schedule of E and P that mimics the changes in hormone levels during pregnancy (E from days 0 to 4, E + P from days 5 to 17, E from days 18 to 27). We predicted that PR antagonists administered during the initial phase of E + P treatment (days 5–11) would prevent the onset of behaviors associated with pregnancy, whereas PR antagonists administered during the late phase E + P treatment (days 12–17) would have no effect on the ongoing expression of these behaviors. Portions of this work have been published in abstract form (Hoffman and González-Mariscal, 2004, 2005).

Methods

Animals

Adult female New Zealand white rabbits (Ss) were used for all experiments. Ss were housed individually in wire cages (90 cm L, 64 cm W, 40 cm H) that contained a wooden nest box (50 cm L, 29 cm W, 29 cm H). Ss were maintained at ambient temperature, on a long day light schedule (14 h:10 h) in an animal housing facility managed by the Centro de Investigación en Reproducción Animal, Universidad Autónoma de Tlaxcala, and were given Purina rabbit chow and water ad libitum. Ss were ovariectomized after 6 months of age, and allowed to recover at least 3 weeks before being used in an experiment. In these experiments, both nulliparous and uniparous females were used, randomly distributed among the experimental groups. In order to minimize the number of animals, ovx Ss were used in two or more consecutive experiments, and were allowed at least 3 weeks of recovery between experiments. For these experiments, we used ovx Ss treated exogenously with hormone rather than intact pregnant females in order to more fully control hormone levels and to eliminate the hormonal contribution from the fetus and placenta. Animal care adhered to the Law for Protection of Animals (México).

Hormones and antiprogestins

Estradiol benzoate (E; Sigma) and progesterone (P; Sigma) were first suspended in a small amount of dichloromethane (<1.5% of final volume), sunflower oil was added to obtain the final concentration, and the solution was heated with stirring to completely dissolve the hormone. Mifepristone (RU486, Sigma) and 17-α-acetoxy-11β-(4-*N,N*-dimethylaminophenyl)-19-norpregna-4,9-diene-3,20-dione [CDB(VA)2914, also known as RTI 3021-012, generously provided by E. Gainer, HRA Pharma, Paris] were first suspended in a small amount of sunflower oil:benzylbenzoate:benzyl alcohol solution (8:1.5:0.5). Sunflower oil containing E and P at the appropriate concentrations was then added, and the solution was heated with stirring to fully dissolve the antiprogestin. The control (vehicle) solutions were prepared in tandem, in exactly the same fashion, except antiprogestin was not added.