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The inhibition of female rabbit sexual behavior by progesterone: Progesterone receptor-dependent and-independent effects

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ABSTRACT

In the pregnant domestic rabbit, scent marking ("chinning") and sexual behavior are inhibited by ovarian-derived progesterone (P). In order to distinguish behavioral effects of P that are PR-dependent from those mediated by its ring A reduced metabolites, we administered P, P+RU486 (PR antagonist), chlormadinone acetate (CA, synthetic progestin that does not form ring A reduced metabolites), or vehicle to ovariectomized (ovx) estradiol-benzoate (EB)-treated female rabbits, via sc injection, on experimental day 0. Chinning was quantified daily, and mating tests were done on days -1, 1, 3, 5, and 7. On day 1, chinning was significantly decreased, and the latency to be mounted by the male was significantly increased (indicating decreased sexual attractivity of the female) in P-treated females. The effect of P on chinning, but not its effect on sexual attractivity, was completely blocked by RU486 and replicated by CA. Although CA had no effect on attractivity on day 1, it decreased both sexual receptivity and attractivity on day 3. In a preference test in which the male could interact with either an ovx EB-treated female or an ovx female that had received one of the above hormone treatments 24 h earlier, P decreased sexual attractivity and increased aggression. The effect of P on aggression, but not its effect on attractivity, was blocked by RU486 and replicated by CA. These results indicate that both PR-dependent and PR-independent mechanisms decrease sexual attractivity, whereas PR activation is necessary for the inhibition of chinning and sexual receptivity, and for the stimulation of aggression.

Introduction

In female mammals, progesterone (P) and its receptor (PR) modulate many components of sexual and maternal behavior. With respect to sexual behavior, P can have stimulatory or inhibitory effects. Thus, in the estrogen-primed ovariectomized (ovx) female rat, P acutely enhances sexual attractivity, and stimulates proceptivity and receptivity (lordosis) (Albert et al., 1991; Nadler, 1970). However, 24 h after the administration of P, ovx female rats are behaviorally unresponsive to a second administration of this hormone (a phenomenon called sequential inhibition; Nadler, 1970). Likewise, "biphasic" effects of P (first stimulatory, then inhibitory) have been observed in the in ovx, estrogen-primed female guinea pig (Zucker, 1968), hamster (Debold et al., 1976), mouse (Edwards, 1970), and mare (Asa et al., 1984). Estrus in the female dog and elephant are also associated with elevations in circulating P (Beach et al., 1982; Brannian et al., 1988; Carden et al., 1998; Hodges, 1998).

However, in many other mammals, including several primate species, the cow, pig, cat, ferret, and rabbit, there is little evidence that P stimulates estrous behavior in either intact or ovx 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). In the estrogen-primed ovx female rhesus monkey, for example, exogenous administration of P decreases her sexual attractivity, apparently through its action on vaginal and/or perineal tissue (Baum et al., 1976, 1977; Zehr et al., 1998). Indeed, sexual behavior in the rat is most likely inhibited during pregnancy by high circulating levels of P (Powers and Zucker, 1969). In sheep, a decline in circulating P levels is necessary to prime estradiol-stimulated estrous behavior (Fabre-Nys and Gelez, 2007; Fabry-Nys and Martin, 1991a,b).

In addition to stimulating sexual behavior, P has also been shown to modulate the expression of agonistic behaviors that discourage the male's attempts to mate. For example, in the Syrian hamster, in which P also has a biphasic effect on female sexual behavior, P was observed to first decrease, then increase the frequency of attacks toward a stimulus female (Meisel and Sterner, 1990). During diestrus (when circulating P levels are high), the mare displays a threatening facial expression, squeals, or kicks when approached by a courting stallion (Crowell-Davis; 2007), or walks out from under the stallion as he tries to mount her (Asa et al., 1979).

In the female rabbit, a reflex ovulator, circulating P levels are negligible during estrus but begin to increase approximately 4 days after mating-induced ovulation, and are maintained at high levels through most of pregnancy (Challis et al., 1973; Hilliard and Eaton, 1971; Hilliard et al., 1968; Mikhail et al., 1961), when sexual receptivity

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and scent-marking behavior ("chinning") are inhibited (Beyer and Rivaud, 1969; González-Mariscal et al., 1990). In ovx females of this species, P has been shown to exert clear inhibitory effects on chinning and on sexual receptivity (Beyer et al., 1969; Hudson et al., 1990; Beyer et al., 2007).

In many cases, the behavioral effects of P have been shown to require ligand-dependent or-independent activation of the PR, although this question has been addressed in a very limited number of species. Thus, the PR antagonist mifepristone (RU486) and PR antisense RNA have been used experimentally to implicate PR activation in the stimulatory effect of P on estrus behavior in the rat and guinea pig (Brown and Blaustein 1984; Etgen and Barfield 1986; Mani et al., 1994; Ogawa et al., 1994; Pollio et al., 1993). In knockout mice lacking the A isoform of the PR, P fails to stimulate lordosis (Mani et al., 2006; White et al., 2007). In the ferret (a reflex ovulator), the experimental administration of RU486 to the female after mating has implicated PR activation in the post-coital inhibition of proceptivity (Villars et al., 1990), and in the ovx estradiol benzoate (EB)-primed rabbit, RU486 reversed the inhibitory effect of P on chinning (Hoffman and González-Mariscal, 2006).

However, P also can exert behavioral effects independently of the PR, through the action of its metabolites, pregnanolone (3α-hydroxy-5α-pregnan-20-one; 3α,5α-THP) and allopregnanolone (3α-hydroxy-5β-pregnan-20-one; 3α,5β-THP), which are formed from P by two successive biochemical reductions in ring A, catalyzed by 5α-reductase and 3α-hydroxysteroid oxidoreductase. These "neuroactive" metabolites of P modulate the activity of GABA-A receptors (for review, see Rupprecht, 2003), and stimulate sexual behavior in the EB-primed ovx female rats (Beyer et al., 1989, 1995, 1999; Frye et al., 1998; Frye, 2001).

In the present experiments, we sought to further characterize the effects of P on sexual behavior in the female rabbit, and determine which of these effects require PR activation and/or ring A reduction of this hormone. In Experiment 1, we examined the effect of a single dose of P on chinning and several variables related to female sexual behavior of ovx EB-primed females, and, using RU486, tested whether PR activation was necessary for the behavioral effects of this hormone. In Experiment 2, we determined which of these effects of P were replicated by the synthetic progestin chlormadinone acetate (CA), which strongly activates the PR, yet its ring A reduction is hindered due to the presence of a double bond at C6 and a 17α-acetoxy group (González-Flores et al., 1998; Thijssen, 1972; Raynaud et al., 1982). We used this compound to test whether ring A reduction was necessary for the behavioral effects of P. Experiment 3 was designed to confirm the effects of P on sexual attractivity that were observed in Experiment 1: to this end we administered a preference test, in which a trio of animals (a male, an EB-treated OVX female, and a test female) were allowed to interact freely in an open field arena.

Methods

Animals

Ovx, nulliparous female New Zealand white rabbits were used in these experiments. Females were housed individually in wire cages (46×40×61 cm) maintained at ambient temperature (15–25 °C) on a long-day light cycle, and given rabbit chow (Purina) and water ad libitum. Females were ovx after 6 months of age, and allowed to recover at least 3 weeks before being used in an experiment. Animal care adhered to the Law for Protection of Animals (México).

Steroid solutions

EB, P, or CA was suspended in a small amount of dichloromethane (100–200 μL). Sunflower oil was then added to yield the appropriate hormone concentrations (EB: 0.01 mg/mL; P: 20 mg/mL; CA: 6 mg/mL), and the steroids were then fully dissolved by heating. RU486 was first suspended in a small volume (approximately 1 mL) of benzyl benzoate:benzyl alcohol:sunflower oil (1.5:0.5:8 mL), sunflower oil was then added to make a solution of 40 mg/mL, and then the steroid was fully dissolved by heating. All steroids were injected sc, in 0.5 mL sunflower oil. Vehicle solutions were prepared in tandem, in exactly the same manner, except the steroid was omitted.

Experiments 1 and 2

Seventeen nulliparous adult ovx females were used in these experiments. They were given once-daily injections of 5 μg EB, at approximately 18:00 h, on experimental days 0–12. On experimental day 5, P or CA were given as a single injection (P: 10 mg, CA: 3 mg) at approximately 15:00 h. A third treatment group received RU486 (20 mg) on days 4 and 5, in addition to receiving P on day 5. The control groups received the EB injections only, plus the appropriate vehicle, on days 4 and/or 5. The doses of EB, P, and RU486 were chosen based on previous studies (Caba et al., 2003; Hoffman and González-Mariscal, 2006, 2007; Rauch et al., 1985). The dose of CA was chosen based on the binding affinity of this

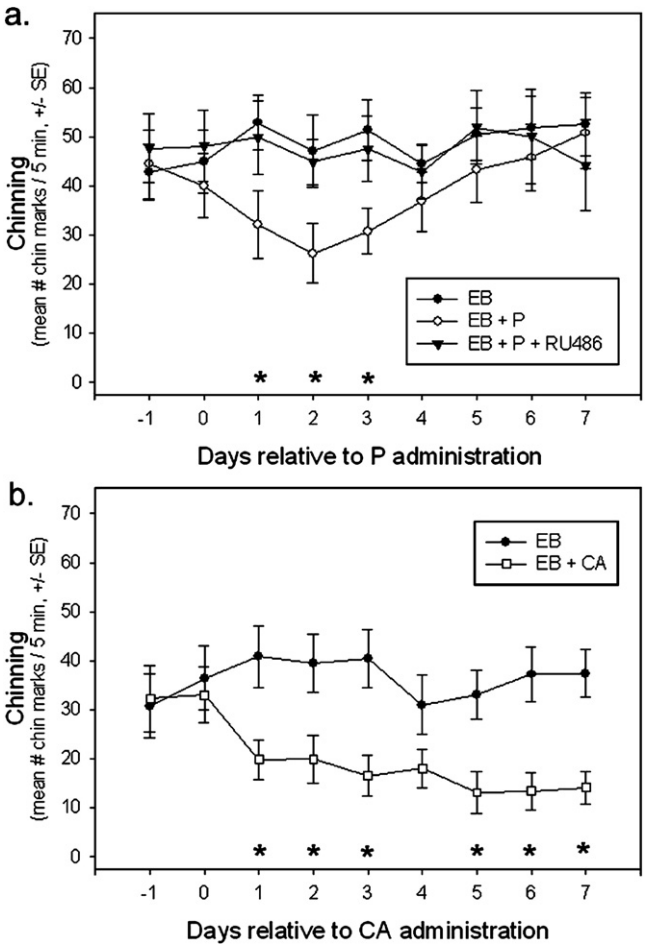

Fig. 1. Chinning in ovx female rabbits that received daily injections of estradiol benzoate (EB), and a single injection of progesterone (P), P+RU486, chlormadinone acetate (CA; a synthetic progestin), or vehicle on day 0. Data shown in (a) are from EB+vehicle, EB+P, and EB+P+RU486 groups; $n=8$ for each group. Asterisks denote days on which EB+P group differed significantly from the other two groups (Friedman test, post-hoc Wilcoxon, $p<0.05$). Data shown in (b) are from EB+vehicle and EB+CA groups; $n=9$ for each group. Asterisks denote significant differences between EB and EB+CA groups (Wilcoxon, $p<0.05$).