Hormones and Behavior **34,** 294–302 (1998)
Article No. HB981485

Regulation of Male Sexual Behavior by Progesterone Receptor, Sexual Experience, and Androgen

Steven M. Phelps,* John P. Lydon,† Bert W. O'Malley,† and David Crews*,[1]

**Institute of Reproductive Biology, Department of Zoology, University of Texas at Austin, Austin, Texas 78712; and †Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030*

Received March 9, 1998; revised June 18, 1998; accepted September 15, 1998

Recent studies have demonstrated that physiological doses of progesterone may facilitate the androgen-dependent display of male sexual behavior in laboratory rats and three species of lizard. We used mice with a targeted disruption of the progesterone receptor to investigate whether such interactions exist in male mice and whether they may be modified by sexual experience. We found that naive intact male progesterone receptor knockout (PRKO) mice exhibit reduced mount frequencies compared to wild-type (WT) mice. Also unlike WT mice, sexually experienced PRKO males show profound losses in many measures of sexual behavior following castration. In a second experiment, we tested whether male mice heterozygous for a null mutation at the progesterone receptor locus were responsive to testosterone and progesterone treatment. We found that heterozygous males showed a reduced response to testosterone. The data are consistent with experiments indicating that the progesterone receptor is able to facilitate male-typical sex behaviors in other species and suggest novel mechanisms underlying the interaction of androgens and experience.

Key Words: **progesterone receptor; male; sexual behavior; castration; knockout; mouse.**

The sources and mechanisms of individual variation represent a major challenge to the behavioral sciences. Within the study of masculine sexual behavior, there are two related examples of individual differences that are as salient as they are poorly understood: variation in the capacity of experienced males to exhibit sexual behaviors following castration, and variation in the sensitivity of males to androgen treatment (Sachs and Meisel, 1994). It is well documented that testosterone (T) may elicit male-typical behaviors by binding androgen receptor (AR) or by being aromatized and binding one of the estrogen receptor subtypes (ER) (Baum, Tobet, Starr, and Bradshaw, 1982; Christensen and Clemens, 1974). Although receptor abundance relates to the performance of male behaviors (Clark, Davis, and Roy, 1985), there is a great deal of variability that does not seem explicable simply in terms of circulating androgens (Beach and Holz-Tucker, 1949; Beach and Fowler, 1959; Damassa, Smith, Tennent, and Davidson, 1977) or receptor expression (Clemens, Wee, Weaver, Roy, Goldman, and Rakerd, 1988). What mechanisms are responsible for intraspecific variation in the display of male sexual behaviors, and how do they interact with androgen-dependent processes?

One obvious line of inquiry is to determine how variation in past experience might shape sexual behaviors. As in many vertebrate species, sexually experienced male mice exhibit sexual behavior following castration at much higher levels than do sexually naive males (Sachs and Meisel, 1994). Classic work in behavioral genetics has shown heritable strain differences in the influence experience exerts over androgen dependence (reviewed in McGill, 1965).

We sought to investigate a novel potential source for individual differences—the progesterone receptor (PR)—using male mice with a null mutation at the PR locus. This model system allowed us to investigate the contributions PR makes to the sex behavior of males with varied experience and plasma steroids.

Named for its central role in female reproduction,

[1]To whom correspondence should be addressed. Fax: (512) 471-6078. E-mail: crews@mail.utexas.edu.

0018-506X/98 $25.00

progesterone (P) traditionally has been thought to have little or no function in the control of sexual behavior in males. Indeed, early experiments indicated that administration of P to male birds and rats will inhibit their sexual behavior (Erickson, Bruder, Komisaruk, and Lehrman, 1967; Erpino, 1967, 1973; Bottoni, Lucini, and Massa, 1985). This viewpoint has become so accepted that it serves as a rationale for the use of progestins in the "chemical castration" of sex-offenders (Bradford, 1988; Lehne, 1988). However, the physiology of P secretion reveals a marked diurnal rhythm in P secretion in male rats (Kalra and Kalra, 1977) and humans (Vermeulen and Verdonck, 1976; Kage, Fonner, Weber, and Schoneshofer, 1982; Opstad, 1994). In addition, work with several species of reptiles has demonstrated that exogenous P, whether administered systemically or directly into the brain, will stimulate courtship and copulatory behavior in castrated males (Lindzey and Crews, 1986, 1988, 1992; Young, Greenberg, and Crews, 1991; Crews, Godwin, Hartman, Grammer, Prediger, and Sheppherd, 1996) and that T and P can synergize in stimulating sexual behavior in males (Young *et al.*, 1991; Lindzey and Crews, 1988, Lindzey and Crews, 1992) much as estrogen (E) and P synergize in stimulating sexual behavior in female rodents (Pfaff, Shwartz-Giblin, McCarthy, and Kow, 1994). These data prompted a reassessment of the evidence gleaned from mammalian work and the discovery that most data were derived from pharmacological doses of P and from the administration of synthetic progestins that have antiandrogenic properties (reviewed in Witt, Young, and Crews, 1994). Recent studies demonstrate that P administered systemically to produce physiological titers, or directly into the preoptic area (POA), stimulates the expression of sexual behavior of intact and castrated male rats (Witt, Young, and Crews, 1995; Witt, Reigada, and Wengroff, 1997); further, as in reptilian studies, T and P treatments synergize to stimulate male sexual behavior in castrated rats (Witt *et al.*, 1995).

Progesterone's role in the control of male sexual behavior has been most closely examined in reptilian models; these studies indicate that P may function by enhancing the responsiveness of the POA to androgens (Crews *et al.*, 1996). Although this has yet to be established in rodents, it is worth noting that researchers working with rats have recently found that injections into the POA of P enhance male-typical sex behavior, while injections of a P antagonist, RU 38486, impair performance (Witt *et al.*, 1997). Finally, neonatal male rats have substantially higher levels of PR in the medial POA than do female rats (Wagner, Nakayama, and De Vries, 1998). Taken together, these facts are consistent with the hypothesis that P plays a functional role in the regulation of sexual behavior in males.

We investigated the role of PR in the regulation of male sexual behavior with two studies. The first compared the sexual behavior of male mice that were homozygous for either a wild-type (WT) allele or a progesterone receptor knockout (PRKO) allele. For both genotypes, the sexual behaviors of naive and experienced intact males were compared to the behaviors of naive and experienced castrated males.

Previous studies have demonstrated that female mice heterozygous for a PR knockout allele show impaired PR expression in response to estrogen treatment, but have not been shown to exhibit deficiencies in intact behaviors (Mani, Blaustein, and O'Malley, 1997). In the second study, we sought to test whether subtle genetic deficits in PR were capable of impairing the responses of males to T replacement. To assess this possibility, we tested the sexual behaviors of male mice that were either WT or heterozygous for a PR knockout allele (HTZ). Males were tested when intact, castrated, and treated with some combination of T, P, and blank (Bl) capsules. Because intact mice heterozygous for disruptions at other loci may show normal levels of behaviors (e.g., Ogawa, LuBahn, Korach, and Pfaff, 1997), which presumably reflect a capacity to compensate for long-term deficits through regulatory mechanisms, we expected that the effects of genotype would be weak or absent from intact and castrated animals, but would be plainly manifest in response to P and T treatment.

METHODS

Generation of Knockout Mice

Generation of the progesterone receptor-deficient mouse model has previously been described (Lydon, DeMayo, Funk, Mani, Hughes, Montgomery, Shyamala, Conneely, and O'Malley, 1995). The animals used in this study were approximately F_8 of a "mixed" 129SvEv × C57BL6 background from an initial cross between an F_0 male chimera (generated by gene-targeting) and a C57BL6 female. This cross generated heterozygotes (F_1) that were 50% 129SvEv/50% C57BL6 and were subsequently crossed to generate F_2. Subsequent to F_2, either cousins or siblings were mated to generate the next generations. Since all sub-