Hormonal Regulation of Progesterone Receptor mRNA Expression in the Hypothalamus of Whiptail Lizards: Regional and Species Differences

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ABSTRACT: The effects of gonadal steroid hormones on steroid receptor mRNA expression vary across nuclei within the brain, between the sexes, and between species. We report that exogenous estrogen increases progesterone receptor (PR) mRNA levels in the periventricular preoptic area in an ancestor and descendant species pair of whiptail lizards, and also that this effect of estrogen is significantly stronger in females of the descendant species. Second, while progesterone strongly decreases PR mRNA in the ventromedial hypothalamus of whiptail lizards and rodents, we find that there is no discernible effect of progesterone on PR mRNA levels in the periventricular preoptic area in females of the ancestral member of this species pair. These findings are a further demonstration of the variability of steroid effects on steroid receptor mRNA levels across brain nuclei. This variability may be important both in behavioral transitions over the course of the ovarian cycle in this ancestor-descendant species pair of lizards and in the evolution of pseudosexual behavior in the descendant parthenogen species. J Neurobiol 39: 287–293, 1999

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Sexual behaviors in vertebrates are primarily regulated by gonadal steroid hormones acting on specific brain nuclei in the limbic system. Gonadal steroid effects on steroid receptor mRNA expression show great variation across different nuclei in the brain, between the sexes, and across species (Young and Crews, 1995). We have been studying steroid effects on steroid receptor mRNA expression in the hypothalamus of whiptail lizards (genus *Cnemidophorus*), a group native to the desert grasslands of southwestern North America. Of particular interest is the evolution of a novel behavior pattern in an ancestor-descendant species pair of these lizards. The desert grasslands whiptail, *Cnemidophorus uniparens*, is a triploid unisexual species that originated in a hybrid mating between the little striped whiptail, *Cnemidophorus inornatus*, and another *Cnemidophorus* species (probably *Cnemidophorus burti*) (Wright, 1993). This first generation hybrid then backcrossed with *C. inornatus* to create the triploid parthenogen *C. uniparens*. In the ancestral species *C. inornatus*, males display mounting and intromission behavior, while females exhibit receptivity in response to this male-typical behavior. In the descendant parthenogenetic species, however, each individual regularly and reliably exhibits both female- and male-like pseudosexual behaviors which are indistinguishable from the sexual behavior shown by their direct sexual ancestor, *C. inornatus*.

In both species, mounting behavior is mediated by the preoptic area (POA) as in other vertebrates (Kingston and Crews, 1994; Mayo and Crews, 1987; Rand

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and Crews, 1994; Rozendaal and Crews, 1989; reviewed for other vertebrates by Sachs and Meisel, 1994). Sexual behavior in male *C. inornatus* is dependent on testicular androgens, but several lines of evidence suggest that male-like pseudocopulatory behavior in *C. uniparens* is instead stimulated by the periovulatory surge in progesterone. This evidence includes the following: (a) Male-like pseudosexual behavior is most commonly observed during the luteal phase of the ovarian cycle when progesterone is elevated (Moore et al., 1985a), while androgens are uniformly undetectable through the ovarian cycle (Moore et al., 1985b). (b) Exogenous progesterone stimulates the expression of male-like pseudocopulatory behavior in ovariectomized animals (Grassman and Crews, 1986). (c) A potential evolutionary precursor to this stimulation of male-like pseudosexual behavior is seen in male *C. inornatus*, where male-typical sexual behavior can be reinstated in a proportion of castrated males by exogenous progesterone administered either systemically (Lindzey and Crews, 1986, 1992, 1993) or intracranially (Crews et al., 1996). Male *C. inornatus* with different sensitivities to progesterone administered systemically also differ in sensitivity to progesterone administered intracranially and in progesterone regulation of progesterone receptor (PR) and androgen receptor (AR) mRNA abundances (Crews et al., 1996).

This study examined patterns of steroid hormone receptor regulation in female *Cnemidophorus.* The general objectives are to better understand PR mRNA regulation in the hypothalamus and explore potential evolutionary changes in the nature of this regulation. We addressed two specific questions. Since female *C. inornatus* do not display male-typical sexual behaviors, while *C. uniparens* display male-like pseudosexual behavior, we first asked whether females of this ancestor–descendant pair differ in estrogen effects on PR mRNA levels in the preoptic area. Second, since a progesterone surge occurs at the time of ovulation and marks a behavioral transition, we asked whether progesterone effects on estrogen-induced PR mRNA levels differ between the preoptic area which mediates male-typical sexual behavior, and the ventromedial hypothalamus, which mediates female-typical sexual behavior.

MATERIALS AND METHODS

Lizards were captured near Sanderson, Texas (female *C. inornatus*) or Portal, Arizona (*C. uniparens*), transported to the University of Texas, and maintained in environmental chambers under breeding season conditions as described previously (Wade and Crews, 1991). Experimental animals were taken from group-housed conditions, ovariectomized, and held in social isolation as described previously (Wade and Crews, 1991) for 1 week to allow metabolic clearance of endogenous gonadal steroids.

In the first experiment, each female *C. inornatus* or *C. uniparens* was given a subcutaneous injection of either 0.5 μg estradiol benzoate (EB; Sigma) suspended in 10 mL steroid suspension vehicle (SSV) or 10 μL of vehicle alone between 1200 and 1500 h. This dosage of EB strongly and reliably stimulates receptive behavior in whiptail lizards (Young et al., 1995b). Experimental animals were sacrificed 24 h after injection between 1200 and 1500 h.

In the second experiment, female *C. inornatus* were given one of five different hormonal treatments intended to test a variety of hypotheses about estrogen and progesterone effects on PR mRNA abundance in both the periventricular and medial preoptic areas (Table 1). These treatments included blank-injected–blank-implanted animals intended to provide measures of PR mRNA abundances in the absence of hormonal stimulation, estrogen-injected–blank-implanted animals intended to provide measures of estrogen effects on PR mRNA abundances, and estrogen-injected and progesterone-implanted animals for assessing the effect of progesterone on estrogen-stimulated PR mRNA abundances. Implants were given either before or after estrogen injection to determine whether order of hormonal treatment (injections vs. implants) or anesthesia and surgery influenced PR mRNA levels. The estrogen injections were 0.5 μg EB in 10 μL SSV as above. The progesterone treatment consisted of subcutaneous silastic implants containing crystalline progesterone, while control blank capsules were empty. The implants were prepared as described in Lindzey

Table 1 Design for Experiment 2

Treatment	Days since Ovariectomy			
	6	7	8	9
Blank capsule, blank injection (blank then blank)	Blank capsule	Blank injection	Sacrifice	
Blank capsule, EB injection (blank then EB)	Blank capsule	EB injection	Sacrifice	
Progesterone capsule, EB injection (P then EB)	P capsule	EB injection	Sacrifice	
EB injection, blank capsule (EB then blank)		EB injection	Blank capsule	Sacrifice
EB injection, progesterone capsule (EB then P)		EB injection	P capsule	Sacrifice

Descriptions in parentheses are those used in Figure 2.

and Crews (1986) [13 mm outside length, 10 mm inside length (packed hormone), 1.47 mm inner diameter, 1.96 mm outer diameter]. The hormone administration methods for Experiment 2 are also detailed in Godwin et al. (1996).

For tissue harvesting, brains were rapidly removed, frozen on dry ice, and stored at −80°C . These brains were cryosectioned at 20 μm onto poly-L-lysine–coated slides and again stored at −80°C with dessicant until processing by *in situ* hybridization. The *in situ* hybridization and silver grain quantification procedures were identical to those described previously for *Cnemidophorus* lizards (Young et al., 1994,1995b,c; Godwin and Crews, 1995; Crews et al., 1996). We used a cloned segment of the PR from *C. uniparens* to generate the 394-bp riboprobe as described previously (LYCUPR 3a) (Young et al., 1995a). *Cnemidophorus inornatus* is the maternal ancestor of *C. uniparens*, and this portion of the PR gene shares >99% nucleotide sequence homology between these two species (Young et al., 1995a). Because of this high sequence homology, we assume no significant difference in strength of hybridization or differential binding of the riboprobe–PR mRNA complexes that would confound interpretation of hybridization signals between the two species. The slides are briefly exposed with this method to achieve an approximate minimum hybridization signal of three times background silver grain density and simultaneously prevent overexposure. Overexposure can obscure differences between cells through a ceiling effect on silver grain density. The light exposure makes quantification of absolute numbers of cells positive for a given mRNA species unreliable in comparison to immunocytochemical methods. Data on numbers of cells positive for PR mRNA are therefore not presented here.

Mean silver grain densities were compared within the medial and periventricular regions of the POA across species and hormone treatment groups by analysis of variance. Silver grain densities are expressed as grains per cluster, where clusters are aggregations of grains over single cells. Post hoc comparisons were performed using Tukey–Kramer HSD tests [one-way analysis fo variance (ANOVA)] or preplanned orthogonal contrasts (two-way ANOVA). Data were $\log_{10}$ transformed to reduce heteroscedasticity. Statistical analyses were performed using SAS JMP 3.1.6 (Cary, NC) on an Apple Macintosh computer.

RESULTS

Figure 1 shows the relative abundances of PR mRNA in the periventricular region of the POA (PvPOA) of both *C. uniparens* and *C. inornatus* females in response to EB and SSV alone. The overall pattern of response was similar in that EB appears to increase PR mRNA in both species. This was indicated by the lack of a significant interaction term between species and treatment (two-way ANOVA with species and treatment as factors, $p = .78$). There was a significant effect of hormone treatment across the two species, with EB increasing PR mRNA abundances in this brain area (treatment effect: $p < .05$). Most interestingly, there was also a significant difference in the relative abundances of PR mRNA between the species (species effect: $p < .005$). These results indicate that although the overall pattern of response in PR mRNA abundances is similar between females of the two species (both appear to show increases with estrogen stimulation), *C. uniparens* females have higher PR mRNA levels overall. In addition, although the lack of a significant interaction term for species by treatment suggests no species difference in response to treatment, this response does appear stronger in *C. uniparens*. This interpretation is supported by preplanned orthogonal contrasts within and between the two species. Progesterone receptor mRNA abundances in the PvPOA were increased by EB treatment in *C. uniparens* (EB > control: orthogonal contrast, $p < .05$), but not in female *C. inornatus* in this experiment (EB = control: $p = .17$, but see Experiment 2 below). Levels of PR mRNA were also greater in EB-treated *C. uniparens* than in EB-treated *C. inornatus* females ($p = .01$). The medial POA did not show significant differences in PR mRNA abundance across species or treatments (data not shown).

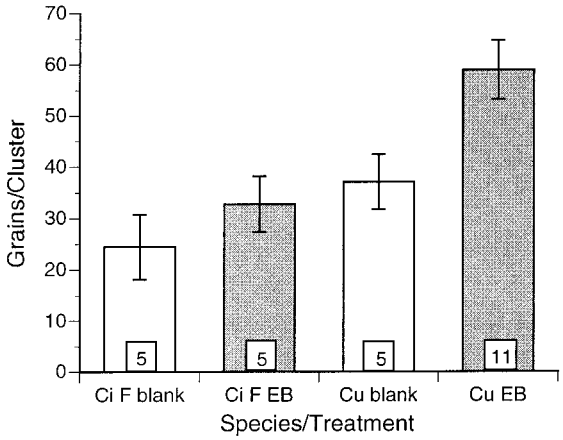

Figure 1 Progesterone receptor (PR) mRNA levels in the periventricular region of the preoptic area (PvPOA) for female *C. inornatus* (Ci F) and *C. uniparens* (Cu) given either blank or EB injections. Depicted is the abundance of PR mRNA measured as average number of silver grains per cluster (mean ± S.E.M.) in the PvPOA of the ancestral sexual (*C. inornatus*) and descendant parthenogenetic (*C. uniparens*) whiptail lizards.

In Experiment 2, giving EB injections to *C. inornatus* females either before or after they received a blank intraperitoneal implant increased PR mRNA abundances in the PvPOA relative to vehicle-injected controls (Fig. 2) (one-way ANOVA for PvPOA: $p < .005$; post hoc comparisons: EB-injected animals > blank-injected animals, $p < .05$). This result differs

from that of Experiment 1 in finding a significant effect of EB treatment in *C. inornatus* females, but sample sizes were larger and brains were taken at 48 rather than 24 h postinjection. Progesterone implants had no discernible effect on PR mRNA abundance in the PvPOA, with levels in females given progesterone implants either before or after EB injections being higher than blank-injected animals ($p < .01$) and not different from females receiving EB injections and blank implants ($p > .05$). This contrasts strongly with patterns in the VMH, where a progesterone implant given either before or after estrogen strongly decreases PR mRNA abundances (Fig. 2) (data from Godwin et al., 1995).

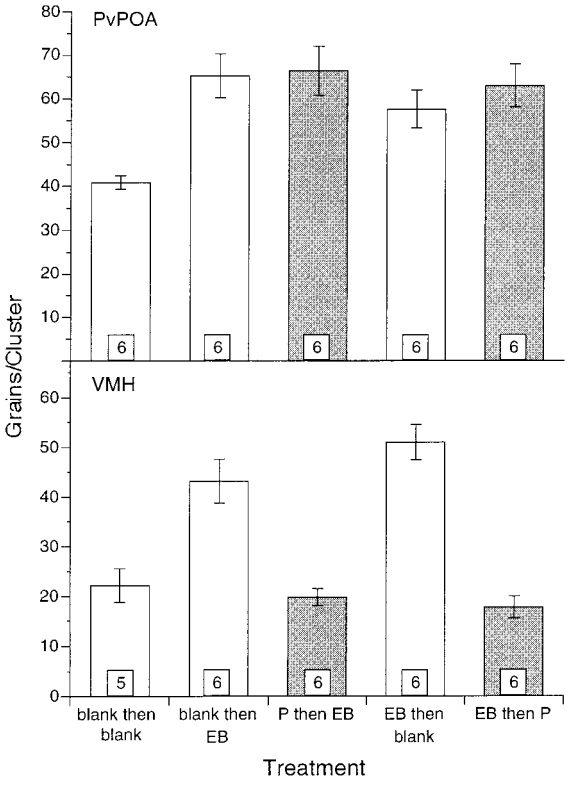

Figure 2 Estrogen and progesterone effects on progesterone receptor (PR) mRNA levels in the diencephalon of female *C. inornatus* given one of five different hormonal treatments. Depicted are the abundances of PR mRNA measured as average number of silver grains per cluster (mean ± S.E.M.) in the periventricular region of the preoptic area (PvPOA) and the ventromedial hypothalamus (VMH) of the same individual females (data from Godwin et al., 1996). Bars shown in gray are treatments in which females received progesterone. Detailed treatment descriptions are given in Table 1.

DISCUSSION

We found that estrogenic stimulation of PR mRNA levels in the PvPOA is stronger in the descendant parthenogen species than in females of its sexual ancestor in *Cnemidophorus*. We also found that exogenous progesterone does not appear to influence PR mRNA abundances in the preoptic area of females in this sexual ancestor. Previous work in this laboratory showed that this dose of EB increased PR mRNA abundances in the PvPOA of *C. uniparens* (Young et al., 1995a), but did not explore the possibility that females of these two species might differ in estrogen sensitivity in this brain area. This species difference in estrogen sensitivity may be important with respect to species differences in reproductive physiology and behavior in this ancestor–descendant pair.

Progesterone receptor expression appears to be importantly linked to sexual behavior in a variety of animal models and behavioral paradigms. Progesterone receptor protein or mRNA levels in discrete hypothalamic nuclei are increased in a number of species by exposure to estrogen (Pfaff et al., 1994; Young et al., 1995a,b; reviewed in Young and Crews, 1995). In *Cnemidophorus* lizards, this regulation of PR mRNA abundance differs by species and sex. The VMH is the primary integrative area in the brain for receptive behavior in both species based on lesion and intracranial hormone implantation studies (Wade and Crews, 1991; Kendrick et al., 1995). Female *C. inornatus* show increased PR mRNA levels in the VMH in response to a receptivity-inducing dose of EB, while males show neither an increase in PR mRNA nor receptive behavior (Godwin and Crews, 1995) (Fig. 2 for females). However, ovariectomized female *C. inornatus* are less sensitive to exogenous EB than *C. uniparens* both in terms of the dosages necessary to induce receptive behavior and increases in PR mRNA in the VMH (Young et al., 1995b). This relationship of female-typical receptive behavior to estrogenic regulation of PR mRNA in *Cnemidophorus* has parallels in rats (reviewed in Pfaff et al., 1994). These findings also strongly suggest that the estrogen sensitivity of PR abundance in discrete brain nuclei may have important behavioral consequences.

A sensitivity compensation model proposed by Young and Crews (1995) postulates that differences in circulating levels of steroid hormones between species are paralleled by compensatory changes in sensitivity to these steroid hormone stimuli in discrete brain nuclei which control reproductive function and behavior. These sensitivity differences would be mediated primarily by differences in steroid hormone

receptor concentrations. *Cnemidophorus uniparens* have lower circulating estradiol levels during the vitellogenic phase of the ovarian cycle than females of their ancestor *C. inornatus* (Moore et al., 1985b). However, PR mRNA levels in the PvPOA do not differ between intact females of the two species and PR mRNA levels are actually higher in the mPOA of *C. uniparens* (Young et al., 1995c). Both this study and a previous one (Young et al., 1995a) showed that estradiol can increase PR mRNA levels in this brain area in *C. uniparens*. The results presented here also show that *C. uniparens* are more sensitive to a controlled estrogenic stimulation of PR mRNA in the PvPOA than are females of the ancestral species *C. inornatus*. Together with the patterns described above for intact females in these two species across the ovarian cycle, the species difference in estrogen sensitivity shown here is consistent with predictions of the sensitivity compensation model.

As with female-typical sexual behavior, the display of male-typical sexual behavior has been linked to steroid hormone receptor expression. For example, sexually active male rams have higher estrogen receptor levels in the hypothalamus than sexually inactive rams (Alexander et al., 1993; Perkins et al., 1995). Male *C. inornatus* differing in sensitivity to progesterone also differ in progesterone regulation of AR- and PR mRNA abundances in the POA (Crews et al., 1996). Several lines of evidence summarized above indicate that progesterone acts in the AH-POA to stimulate male-like pseudosexual behavior in *C. uniparens*. In contrast to the lower circulating estradiol levels in *C. uniparens* than female *C. inornatus*, circulating progesterone levels are at least as high in *C. uniparens* (Moore et al., 1985b). We find that *C. uniparens* are more sensitive to estrogenic stimulation of PR mRNA abundances in the PvPOA than female *C. inornatus*, This finding is consistent with the hypothesis that responsiveness to progesterone in the AH-POA during the luteal phase of the ovarian cycle underlies the display of the male-like pseudocopulatory behavior in the descendant *C. uniparens*.

Progesterone does not affect estrogen-induced PR mRNA abundances in the PvPOA of female *C. inornatus* in our experimental design. This is true when progesterone is given either before or after an estrogen stimulus. The absence of a discernible progesterone effect on PR mRNA in the PvPOA contrasts with patterns observed in the VMH, the brain area critical for female-typical receptive behavior. The progesterone dosage used here strongly decreases EB-induced PR mRNA levels in the VMH of female *C. inornatus*, and this decrease in PR mRNA is correlated with a total loss of estrogen-induced sexual receptivity (Godwin et al., 1996). Very similar decreases of either PR immunoreactivity or progestin binding sites in response to progesterone administration are also seen in the mediobasal hypothalamus of female rodents (Blaustein and Feder, 1980; Blaustein and Turcotte, 1990; Blaustein et al., 1994). This down-regulation is likely important in the behavioral refractoriness these animals show to estrogenic stimulation shortly following ovulation or progesterone exposure. Rising progesterone levels around ovulation very likely decrease progestin sensitivity in the VMH in whiptail lizards, but the data presented here suggest this decrease in progestin sensitivity does not occur in the POA. Indeed, PR mRNA abundances decrease dramatically in the postovulatory period in the VMH of intact female *Cnemidophorus*, but not in the PvPOA (Young et al., 1995c). A change in progestin sensitivity of the POA relative to the VMH could be important for the behavioral transition from female-like pseudosexual behavior to male-like pseudosexual behavior that occurs around this time in *C. uniparens*.

The progesterone receptor exists as two isoforms (A and B) in humans and chickens but not, apparently, rabbits, and there is evidence of functional differences between these two isoforms (Horwitz and Alexander, 1983; Conneely et al., 1987; Loosfelt et al., 1986; Giangrande et al., 1997). The two isoforms are transcribed from distinct estrogen-inducible promoters within the same PR gene in humans, producing a full-length form (hPR-B) and a truncated form lacking the first 164 N-terminal amino acids (hPR-A) (Kastner et al., 1990). This truncated N-terminal portion is in the A and B transactivation domains of the protein and does not include any part of the DNA-binding, hinge, or hormone-binding domains (C, D, and E, respectively). We have no information regarding the possible existence of PR isoforms in *Cnemidophorus*. The 394-bp riboprobe used here (LYCUPR 3a) (Young et al., 1994, 1995a) is complementary to a region spanning part of the DNA-binding domain (C), the hinge region (D), and a portion of the hormone-binding domain (E). Since the region which is truncated to give the PR-A isoform is upstream of these regions in other species and their mRNAs are otherwise identical, our probe should bind the mRNAs encoding both PR isoforms if these are present in *Cnemidophorus*.

We measured levels of PR mRNA rather than PR protein. The working assumption here is that PR mRNA levels reflect levels of functional PR protein. This correspondence has not been shown in the hypothalamus of *Cnemidophorus*. However, studies in rats do suggest that PR mRNA is a good predictor of

PR protein levels and behavioral responsiveness (lordosis) to progesterone (Parsons et al., 1980, 1982; Romano et al., 1989). Specifically in *Cnemidophorus* lizards, the responsiveness of steroid receptor mRNA abundances in discrete brain nuclei to exogenous steroids correlates well with behavioral responsiveness to the same hormonal treatments in several studies (Young et al., 1995b; Godwin and Crews, 1995; Crews et al., 1996; Godwin et al., 1996). Taken together, these observations provide a basis for addressing the steroid hormone sensitivity of nuclei in the preoptic area of this species pair of lizards and especially species differences in this sensitivity as these may relate to behavior.

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REFERENCES

Alexander BM, Perkins A, Van Kirk EA, Moss GE, Fitzgerald JA. 1993. Hypothalamic and hypophyseal receptors for estradiol in high and low sexually performing rams. Horm Behav 27:296–307.

Blaustein JD, Feder HH. 1980. Nuclear progestin receptors in guinea pig brain measured by an *in vitro* exchange assay after hormonal treatments that affect lordosis. Endocrinology 106:1061–1069.

Blaustein JD, Turcotte JC. 1990. Down-regulation of progestin receptors in guinea pig brain: new findings using an immunocytochemical technique. J Neurosci 21:675–685.

Blaustein JD, Tetel MJ, Nielsen Ricciardi KH, Delville Y, Turcotte JC. 1994. Hypothalamic ovarian steroid hormone-sensitive neurons involved in female sexual behavior. Psychoneuroendocrinology 19:505–516.

Conneely OM, Maxwell BM, Toft DO, Schrader WT, O'Malley BW. 1987. The A and B isoforms of the chicken progesterone receptor arise by alternate initiation of translation of a unique mRNA. Biochem Biophys Res Commun 149:493–501.

Crews D, Godwin J, Hartman V, Grammer M, Prediger EA, Shephard R. 1996. Intrahypothalamic implantation of progesterone in castrated male whiptail lizards (*Cnemidophorus inornatus*) elicits courtship and copulatory behavior and affects androgen- and progesterone receptor-mRNA expression in the brain. J Neurosci 16:7347–7352.

Giangrande PH, Pollio G, McDonnell DP. 1997. Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. J Biol Chem 272:32889–32900.

Godwin J, Crews D. 1995. Sex differences in estrogen and progesterone receptor messenger ribonucleic acid regulation in the brain of little striped whiptail lizards. Neuroendocrinology 62:293–300.

Godwin J, Hartman V, Grammer M, Crews D. 1996. Progesterone inhibits female-typical receptive behavior and decreases hypothalamic estrogen and progesterone receptor messenger ribonucleic acid levels in female whiptail lizards. Horm Behav.30:138–144.

Grassman M, Crews D. 1986. Progesterone induction of pseudocopulatory behavior and stimulus-response complementarity in an all-female lizard species. Horm Behav 20:327–335.

Horwitz KB, Alexander PS. 1983. *In situ* photolinked nuclear progesterone receptors of human breast cancer cells: subunit molecular weights after transformation and translocation. Endocrinology 113:2195–2201.

Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P. 1990. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J 9:1603–1614.

Kendrick AM, Rand MS, Crews D. 1995. Electrolytic lesions of the ventromedial hypothalamus abolish sexual receptivity in female whiptail lizards, *Cnemidophorus uniparens*. Brain Res 680:226–228.

Kingston PA, Crews D. 1994. Role of the AH-POA in the control of courtship and copulatory behavior in sexual and unisexual whiptail lizards. Brain Res 643:349–351.

Lindzey J, Crews D. 1986. Hormonal control of courtship and copulatory behavior in male *Cnemidophorus inornatus*, a direct sexual ancestor of a unisexual, parthenogenic lizard. Gen Comp Endocrinol 64:411–418.

Lindzey J, Crews D. 1992. Interactions between progesterone and androgens in the stimulation of sex behaviors in male little striped whiptail lizards, *Cnemidophorus inornatus*. Gen Comp Endocrinol 86:52–58.

Lindzey J, Crews D. 1993. Effects of progesterone and dihydrotestosterone on stimulation of androgen-dependent sex behavior, accessory sex structures, and *in vitro* binding characteristics of cytosolic androgen receptors in male whiptail lizards (*Cnemidophorus inornatus*). Horm Behav 27:269–281.

Loosfelt H, Atger M, Misrahi M, Guichon-Mantel A, Meriel C, Logeat F, Benarous R, Milgrom E. 1986. Cloning and sequence analysis of rabbit progesterone-receptor complementary DNA. Proc Natl Acad Sci USA 83:9045–9049.

Mayo ML, Crews D. 1987. Neural control of male-like pseudocopulatory behavior in the all-female lizard, *Cnemidophorus uniparens*: effects of intracranial implantation of dihydrotestosterone. Horm Behav 21:181–192.

Moore MC, Whittier JM, Billy AJ, Crews D. 1985a. Male-

like behavior in an all-female lizard: relationship to ovarian cycle. Anim Behav 33:284–289.

Moore MC, Whittier JM, Crews D. 1985b. Sex steroid hormones during the ovarian cycle of an all-female, parthenogenetic lizard and their correlation with pseudosexual behavior. Gen Comp Endocrinol 60:144–153.

Parsons B, MacLusky NJ, Krey L, Pfaff DW, McEwen BS. 1980. The temporal relationship between estrogen-inducible progestin receptors in the female rat brain and the time course of estrogen activation of mating behavior. Endocrinology 107:774–779.

Parsons B, Rainbow TC, MacLusky NJ, McEwen BS. 1982. Progestin receptors in rat hypothalamic and limbic nuclei. J Neurosci 2:1446–1452.

Perkins A, Fitzgerald JA, Moss GE. 1995. A comparison of LH secretion and brain estradiol receptors in heterosexual and homosexual rams and female sheep. Horm Behav 29:31–41.

Pfaff DW, Schwartz-Giblin S, McCarthy MM, Kow L-M. 1994. Cellular and molecular mechanisms of female reproducive behaviors. In: Neill JD, Knobil E, editors. The physiology of reproduction, Vol. 2. New York: Raven Press. p 107–220.

Rand MS, Crews D. 1994. The bisexual brain: sex behavior differences and sex differences in parthenogenetic and sexual lizards. Brain Res 663:163–167.

Romano GJ, Krust A, Pfaff DW. 1989. Expression and estrogen regulation of progesterone receptor mRNA in neurons of the mediobasal hypothalamus: an *in situ* hybridization study. Mol Endocrinol 3:1295–1300.

Rozendaal JC, Crews D. 1989. Effects of intracranial implantation of dihydrotestosterone on sexual behavior in male *Cnemidophorus inornatus*, a direct sexual ancestor of a parthenogenetic lizard. Horm Behav 23:194–202.

Sachs B, Meisel RL. 1994. The physiology of male sexual behavior. In: Neill JD, Knobil E, editors. The physiology of reproduction, Vol. 2. New York: Raven Press. p 3–106.

Wade J, Crews D. 1991. Effects of intracranial implantation of estrogen on receptivity in sexually and asexually reproducing female whiptail lizards, *Cnemidophorus inornatus* and *C. uniparens*. Horm Behav 25:342–353.

Wright JW. 1993. Evolution of the lizards of the genus *Cnemidophorus*. In: Wright JW, Vitt LJ, editor. Biology of whiptail lizards *(Genus cnemidophorus)*. Herpetologist's League Special Publication No. 3. Norman, OK: Oklahoma Museum of Natural History. p 27–82.

Young LJ, Lopreato GF, Horan K, Crews D. 1994. Cloning and *in situ* hybridization of estrogen receptor, progesterone receptor, and androgen receptor expression in the brain of whiptail lizards (*Cnemidophorus uniparens* and *C. inornatus*). J Comp Neurol 347:288–300.

Young LJ, Crews D. 1995. Comparative neuroendocrinology of steroid receptor gene expression and regulation: relationship to physiology and behavior. Trends Endocrinol Metab 6:317–323.

Young LJ, Godwin J, Grammer M, Gahr M, Crews D. 1995a. Reptilian sex steroid receptors: amplification, sequence and expression analysis. J Steroid Biochem Mol Biol 55:261–269.

Young LJ, Nag PK, Crews D. 1995b. Regulation of estrogen receptor and progesterone receptor messenger ribonucleic acid by estrogen in the brain of the whiptail lizard (*Cnemidophorus uniparens*). J Neuroendocrinol 7:119–125.

Young LJ, Nag PK, Crews D. 1995c. Species differences in behavioral and neural sensitivity to estrogen in whiptail lizards: correlation with hormone receptor messenger ribonucleic acid expression. Neuroendocrinology 61:680–686.

Young LJ, Nag PK, Crews D. 1995d. Species differences in estrogen receptor and progesterone receptor mRNA expression in the brain of sexual and unisexual lizards. J Neuroendocrinol 7:567–576.