0013-7227/04/$15.00/0
Printed in U.S.A.

Endocrinology 145(3):1046–1049
Copyright © 2004 by The Endocrine Society
doi: 10.1210/en.2003-1219

Neonatal Mice Possessing an *Sry* Transgene Show a Masculinized Pattern of Progesterone Receptor Expression in the Brain Independent of Sex Chromosome Status

CHRISTINE K. WAGNER, JUN XU, JENNIFER L. PFAU, PRINCY S. QUADROS, GEERT J. DE VRIES, AND ARTHUR P. ARNOLD

Department of Psychology and Center for Neuroscience Research (C.K.W., J.L.P., P.S.Q.), University at Albany-State University of New York, Albany, New York 12222; Department of Physiological Science and Laboratory of Neuroendocrinology of the Brain Research Institute (J.X., A.P.A.), University of California, Los Angeles, California 90095; and Center for Neuroendocrine Studies (P.S.Q., G.J.D.), University of Massachusetts, Amherst, Massachusetts 01003

To assess the relative roles of sex chromosome genes and gonadal steroid hormones in producing sex differences in progesterone receptor (PR) expression in the forebrain of neonatal mice, we used mice in which the *Sry* gene had been deleted from the Y-chromosome and inserted as a transgene on an autosome in both XX and XY genotypes. Levels of PR immunoreactivity (PRir) in the anteroventral periventricular nucleus, the medial preoptic nucleus, and the ventromedial nucleus were significantly higher in mice that possessed an *Sry* transgene compared with mice that lacked an *Sry* transgene, regardless of their complement of sex chromosomes (XX *vs.* XY). This result suggests that sexual differentiation of PR expression in these regions is likely controlled mainly by gonadal hormones, not by the genetic sex of the brain cells. No differences in PRir were detected between wild-type XY mice with the *Sry* gene on the Y-chromosome and XY mice with the *Sry* transgene, suggesting that testicular hormones produced in these two genotypes have comparable effects on neural tissue. (*Endocrinology* 145: 1046–1049, 2004)

THE TENET THAT gonadal steroid hormones are solely responsible for sexual differentiation of nongonadal tissues, including the brain, has endured for several decades. However, possible exceptions to the rule have been reported for neural and nonneural tissues of birds and mammals (1–4). Recent evidence suggests that the complement of sex chromosomes (XX *vs.* XY) in brain cells may also contribute to sex differences in the brain. Sex differences in the expression of X and Y genes have been reported in the neonatal mouse brain (5). The relative contributions of gonadal hormones and genetic sex of brain cells can be assessed in mice in which gonadal sexual phenotype is independent of genetic sex of the brain, as for example in mice in which the testis-determining gene, *Sry*, is deleted from the Y-chromosome and inserted as a transgene into an autosome in both XX and XY genotypes (6–8). These mice produce offspring that include XX and XY males (*i.e.* mice that have the *Sry* transgene and therefore develop testes) and XX and XY females (*i.e.* mice that lack the *Sry* transgene and therefore develop ovaries). Comparing these mice in adulthood has shown a sex chromosomal effect on vasopressin innervation independent of gonadal sex (9). However, it is difficult in the case of sex differences observed in adulthood to determine whether sex chromosomes influenced the development of neural tissue directly. Sex chromosome complement may alter circulating levels of testicular hormones during development or may render the brain differentially sensitive to gonadal hormones during periods of sexual differentiation. A sex difference present during neural development that is sensitive to the effects of gonadal hormones could shed light on this issue. The expression of the progesterone receptor (PR) gene in developing brain lends itself well to this question

During perinatal life, there is a dramatic and transient sex difference in the expression of PR within several regions of the rat and mouse forebrain (10–13). PR expression is significantly higher in fetal and neonatal male mice in the anteroventral periventricular nucleus (AVPv), the medial preoptic nucleus (MPN), and the ventromedial nucleus (VMN) (11). PR expression is induced in the developing male brain by estradiol, a metabolite of testosterone, acting via estrogen receptor α (11–13). Although the higher levels of testosterone in neonatal males appear to be the main factor in the sex difference in PR expression, whether sex chromosome genes contribute directly to this sex difference has not been explored.

The present study examined PR immunoreactivity in the brains of neonatal XX and XY mice that either possessed or lacked an *Sry* transgene. In this regard, PR expression provides a unique marker of sexual differentiation because the sex difference lies in the expression of a single gene that is directly regulated by estradiol and its nuclear receptor. In addition, the sex differences in PR are not permanent but are

Abbreviations: AVPv, Anteroventral periventricular nucleus; MPN, the medial preoptic nucleus; PR, progesterone activity; PRir, PR immunoreactivity; VMN, ventromedial nucleus.

Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Downloaded from endo.endojournals.org at Univ. of Massachusetts University Library Serials Dept on November 7, 2007

transitory during development (13), offering a model of developmental plasticity not provided by other sexually differentiated systems of the central nervous system.

Materials and Methods

Animals

All mice, gift of Dr. Paul Burgoyne, were of the random-bred MF1 strain, except for the Y-chromosome, which derived from strain 129 (129/SvEv-Gpil^c Y) (14). In some mice, the Y-chromosome was deleted for the testis-determining gene *Sry* [Tdy^{m1} mutation of Lovell-Badge and Robertson (6) and Gubbay *et al.* (7)], forming the Y^--chromosome, so that XY^- mice have ovaries and thus are called females. In some mice, an *Sry* transgene was inserted into an autosome, creating XX*Sry* and XY^- *Sry* mice that develop testes (8). XY^- *Sry* males were bred with normal MF1 XX females to produce four genotypes in which gonadal status (ovaries *vs.* testes) was independent of sex chromosome status (XX *vs.* XY^-) (*i.e.* XX and XY^- have ovaries, XX*Sry* and XY^- *Sry* have testes). In addition, wild-type MF1 XY^{129} males were compared with XY^- *Sry* males to test for any differences attributable to the transgenic *vs.* the endogenous *Sry* gene.

Mice were housed and bred at the University of California, Los Angeles (UCLA) and all experimental procedures were approved by the UCLA Institutional Animal Care and Use Committee. Genotype was determined by PCR using analysis of the presence of Y long arm gene family *Ssty* and of the *Sry* transgene (14). Pregnant females were allowed to deliver normally. On the day of birth (postnatal d 0; P0) neonates were killed by decapitation. Brains were removed and immediately immersion fixed in 5% acrolein in 0.1 M phosphate buffer for 6 h at 4 C, then immersed in 30% sucrose in 0.1 M PBS overnight at 4 C. Brains were shipped to the University of Massachusetts, where they were sectioned coronally at 50 μm thickness on a sliding microtome and stored in cryoprotectant (30% sucrose, 0.1% polyvinyl-pyrrolidone-40 in ethylene glycol and 0.1 M phosphate buffer) at −20 C until immunocytochemical processing.

Immunocytochemistry

Immunocytochemistry was performed on free-floating sections according to previously published methods (11, 13). The primary antibody was a rabbit polyclonal antiserum (Dako Inc., Glostrup, Denmark) directed against the DNA binding domain of the human PR. This antiserum detects both the A and B isoforms of PR (15). PR antiserum was diluted to 1:1000 in TBS (pH 7.6) containing 2% normal goat serum, 0.3% Triton X-100, and 0.02% sodium azide for 72 h at 4 C. After 60 min in biotinylated goat antirabbit IgG (5 μg/ml; Vector Laboratories, Burlingame, CA) and incubation in ABC reagent (Vectastain Elite Kit, Vector Laboratories) for 60 min, tissue was incubated in TBS containing 0.05% diaminobenzidine, 0.75 mM nickel ammonium sulfate, 0.15%-β-D-glucose, 0.04% ammonium chloride and 0.001% glucose oxidase for approximately 20 min. The sections were rinsed and mounted onto gelatin-coated slides and coverslipped. Preabsorption of the PR antiserum overnight with human PR (A and B isoforms) at 10 times higher molar concentration than that of the primary antiserum, or omission of the PR antiserum abolished all immunoreactivity (11, 12).

Analysis

Representative sections in the AVPv, the MPN, and the VMN for each animal were selected for image analysis by an experimenter blind to the genotype of the animals. Sections for analysis were chosen within the rostral MPN, the mid-AVPv and the caudal aspect of the ventrolateral VMN, which most closely corresponds with plate nos. 8/9 for the AVPv, plate no. 9 for the MPN and plate no. 17 for the VMN of the E22 rat brain atlas of Altman and Bayer (16). Microscopic images of the PRir in the AVPv, MPN and VMN were captured with an Olympus BH-2 microscope fitted with a CCD72 (Dage MTI, Michigan City, MI) camera that was connected to a QuickCapture frame grabber board (Data Translation Inc., Marlboro, MA) in a MacIntosh IIfx computer. National Institutes of Health (NIH) Image software (W. Rasband, NIH, Bethesda, MD) was used to analyze captured images. The total amount of PRir was determined for each region by measuring the area (μm^2) covered by thresholded pixels [*i.e.* those pixels with a gray level higher than a defined threshold density (specific immunoreactive staining)]. Threshold was defined as the mean OD five times the SD higher than the mean background density. The mean background density was measured in a region devoid of PRir immediately lateral to the analyzed region containing PRir. Statistical analysis was performed using separate two-way ANOVAs (gonadal status × sex chromosome status) for each region. Preplanned comparisons were made using Student-Newman-Keuls ($P < 0.05$). Direct comparisons between wild-type males and transgenic XY^- Sry mice were made using Student's *t* test ($P < 0.05$).

Results

There was no difference in PRir levels in the AVPv, MPN, and VMN of wild-type XY males and XY^- *Sry* mice ($P = 0.53$ for AVPv; $P = 0.47$ for MPN; $P = 0.70$ for VMN; Fig. 1). Levels of PRir were significantly higher in all three brain regions examined in mice that possessed an *Sry* transgene compared with mice that lacked an *Sry* transgene in both XX and XY^- sex chromosomal genotypes (Fig. 2). Two-way ANOVA revealed a significant main effect of *Sry* transgene in the AVPv ($F_{1,31} = 36.091$, $P < 0.0001$), the MPN ($F_{1,34} = 116.466$, $P < 0.0001$) and the VMN ($F_{1,34} = 201.301$, $P < 0.0001$). There was no significant main effect of sex chromosome complement

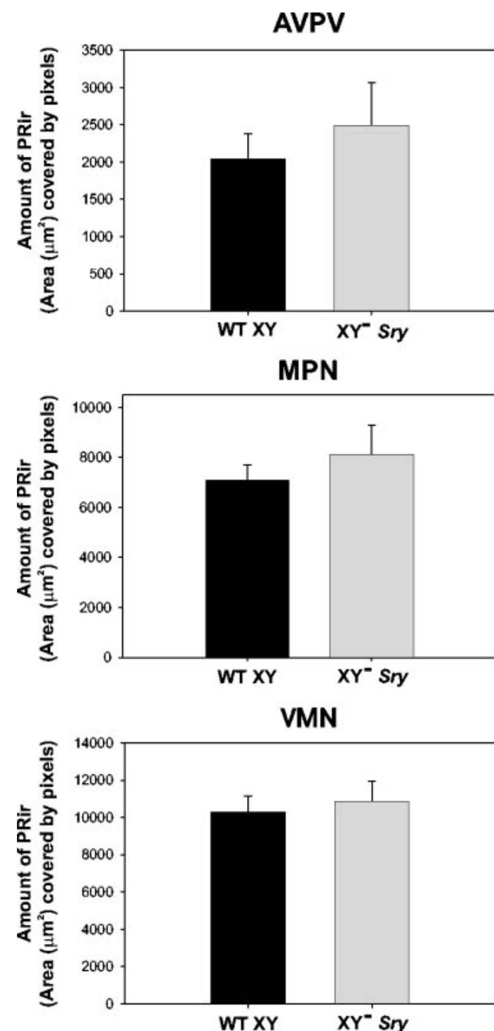

FIG. 1. Relative amount of PRir in the AVPv, the MPN, and the VMN in wild-type XY and XY^- *Sry* male mice.

Downloaded from endo.endojournals.org at Univ. of Massachusetts University Library Serials Dept on November 7, 2007

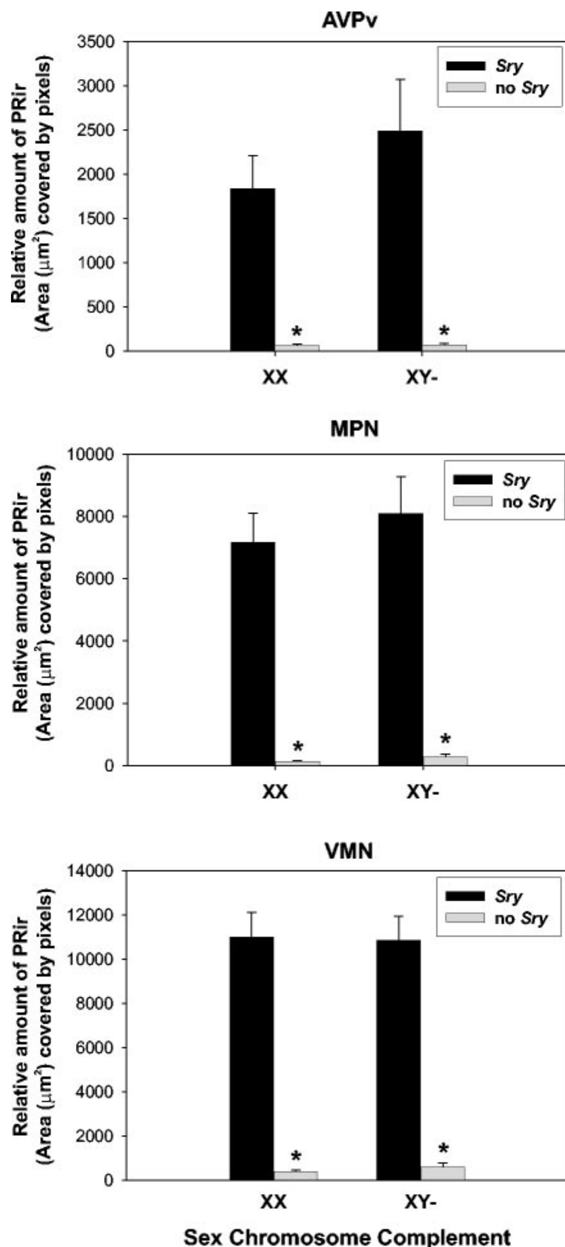

FIG. 2. Relative amount of PRir in the AVPv, the MPN, and the VMN in XX and XY neonatal mice that either lack or possess an *Sry* transgene on an autosome. *, Significantly different from *Sry* ($P < 0.05$).

(XX *vs.* XY$^-$), nor a significant interaction between *Sry transgene* (*i.e*. gonadal status) and sex chromosome status in any of the regions. *Post hoc* analysis revealed that male XX*Sry* and XY$^-$ *Sry* male mice had significantly higher levels of PRir compared with XX and XY$^-$ female mice respectively in all three regions ($P < 0.05$). In no region was there a significant difference between XX*Sry* and XY$^-$ *Sry* or XX and XY$^-$.

Discussion

The present study tested the influence of gonadal secretions as well as genetic sex on the expression of PR in perinatal brains. Mice with testes (XY, XY$^-$ *Sry*, and XX*Sry*) differed dramatically from the two groups without testes (XX and XY$^-$) in all three regions examined. In no case was there a group difference attributable to the complement of sex chromosomes. Thus, XY$^-$ *Sry* males did not differ from XX males and XY$^-$ females did not differ from XX females. In addition, there was no interaction between sex chromosome complement and the presence or absence of testes. These results indicate that XX and XY$^-$ cells in these brain regions can respond fully, at least in terms of PR expression, to the differentiating influences of gonadal steroid hormones irrespective of their complement of sex chromosome genes.

The present results suggest that males with XX*Sry* and XY$^-$ *SRY* genotype have functionally equivalent levels of gonadal hormones prenatally as do females with XX and XY$^-$ genotype. Sex differences in the expression of PR on the day of birth depend on the presence of gonadal steroids prenatally. Treatment of females prenatally with either testosterone or estrogen increased PR expression and abolished the sex difference in PR seen in fetal rats (12). Likewise, the aromatase inhibitor, 1,4,6-androstatriene-3,17-dione, decreased PR in fetal male rats to levels normally seen in females (12). Moreover, the sex difference in PR is abolished in estrogen receptor α knockout mice. PR expression in knockout males is not different from that seen in wild-type females (11). In this regard, PR expression on the day of birth is a very sensitive barometer of the prenatal actions of gonadal steroids, particularly the testosterone metabolite estradiol.

The present study reveals that the expression of PR is not different between any of the groups with testes, whether they possessed the endogenous *Sry* gene, or the *Sry* transgene on either an XY- or an XX-chromosome background. Similarly, the two female groups were indistinguishable from one another in PR expression. These results suggest that sex chromosome complement does not significantly influence prenatal gonadal hormone levels or the ability of the fetal brain to respond to estradiol. These findings are consistent with the idea that the effects of sex chromosome complement on sex differences in vasopressin innervation in adult mice (9) cannot simply be explained by differences in prenatal levels of testosterone or its metabolite, estradiol. Similarly, the present results demonstrating that males with the endogenous *Sry* gene and males with the *Sry* transgene do not differ in the expression of PR on the day of birth suggest that differences in sexual behavior and neural morphology found previously between these groups (9) are more likely attributable to differential genetic effects of the transgene *vs.* the wild-type gene rather than to differences in prenatal levels or actions of gonadal steroid hormones.

Acknowledgments

We thank Dr. Paul Burgoyne for the gift of the mice.

Received September 15, 2003. Accepted November 18, 2003.

Address all correspondence and requests for reprints to: Christine K. Wagner, Department of Psychology, Social Science 369, 1400 Washington Ave, University at Albany, Albany, New York 12222. E-mail: cwagner@albany.edu.

This work was supported by NIH Grants MH59268 and NS43196 (to A.P.A.), NIH Grant HD37244 (to C.K.W.), and National Research Service Award MH65874 (to P.S.Q.).

Downloaded from endo.endojournals.org at Univ. of Massachusetts University Library Serials Dept on November 7, 2007

References

1. **Arnold AP** 2002 Concepts of genetic and hormonal induction of vertebrate sexual differentiation in the twentieth century, with special reference to the brain. In: Pfaff DW, Arnold AP, Etgen A, Fahrbach S, Rubin R, eds. Hormones, brain, and behavior. San Diego: Academic Press; 4:105–135
2. **Renfree MB, Wilson JD, Shaw G** 2002 The hormonal control of sexual development. Novartis Found Symp 244:136–152
3. **Reisert I, Pilgrim C** 1991 Sexual differentiation of monoaminergic neurons—genetic or epigenetic. Trends Neurosci 14:467–473
4. **Agate RJ, Grisham W, Wade J, Mann S, Wingfield J, Schanen C, Palotie A, Arnold AP** 2003 Neural, not gonadal, origin of brain sex differences in a gynandromorphic finch. Proc Natl Acad Sci USA 100:4873–4878
5. **Xu J, Burgoyne PS, Arnold AP** 2002 Sex differences in sex chromosome gene expression in mouse brain. Hum Mol Genet 11:1409–1419
6. **Lovell-Badge R, Robertson E** 1990 XY female mice resulting from a heritable mutation in the primary testis-determining gene, Tdy. Development 109:635–646
7. **Gubbay J, Vivian N, Economou A, Jackson D, Goodfellow P, Lovell-Badge R** 1992 Inverted repeat structure of the *Sry* locus in mice. Proc Natl Acad Sci USA 89:7953–7957
8. **Mahadevaiah SK, Odorisio T, Elliot DJ, Rattigan A, Szot M, Laval SH, Washburn LL, McCarrey JR, Cattanach BM, Lovell-Badge R, Burgoyne PS** 1998 Mouse homologues of the human AZF candidate gene RBM are expressed in spermatogonia and spermatids, and map to a Y chromosome deletion interval associated with a high incidence of sperm abnormalities. Hum Mol Genet 7:715–727
9. **De Vries GJ, Rissman EF, Simerly RB, Yang LY, Scordalakes EM, Auger CJ, Swain A, Lovell-Badge R, Burgoyne PS, Arnold AP** 2002 A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. J Neurosci 22:9005–9014
10. **Wagner CK, Nakayama AY, De Vries GJ** 1998 Potential role of maternal progesterone in the sexual differentiation of the brain. Endocrinology 139: 3658–3661
11. **Wagner CK, Pfau JL, De Vries GJ, Merchenthaler IJ** 2001 Sex differences in progesterone receptor immunoreactivity in neonatal mouse brain depend on estrogen receptor α expression. J Neurobiol 47:176–182
12. **Quadros PS, Pfau, JL, Goldstein AY, De Vries GJ, Wagner CK** 2002 Sex differences in progesterone receptor expression: a potential mechanism for estradiol-mediated sexual differentiation. Endocrinology 143:3727–3739
13. **Quadros PS, Goldstein AY, De Vries GJ, Wagner CK** 2002 Regulation of sex differences in progesterone receptor expression in the medial preoptic nucleus of postnatal rats. J Neuroenocrinol 14:761–767
14. **Simpson EM, Linder CC, Sargent EE, Davisson MT, Mobraaten LE, Sharp JJ** 1997 Genetic variation among 129 substrains and its importance for targeted mutagenesis in mice. Nat Genet 16:19–27
15. **Traish AM, Wotiz HH** 1990 Monocolonal and polyclonal antibodies to human progesterone receptor peptide-(533–547) recognize a specific site in unactivated (8S) and activated (4S) progesterone receptor and distinguish between intact and proteolyzed receptors. Endocrinology 127:1167–1175
16. **Altman J, Bayer SA**1995 Atlas of prenatal rat brain development. Boca Raton, FL: CRC Press; 485

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Downloaded from endo.endojournals.org at Univ. of Massachusetts University Library Serials Dept on November 7, 2007