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Oxytocin is required for nursing but is not essential for parturition or reproductive behavior

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ABSTRACT **Oxytocin, a neurohypophyseal hormone, has been traditionally considered essential for mammalian reproduction. In addition to uterine contractions during labor and milk ejection during nursing, oxytocin has been implicated in anterior pituitary function, paracrine effects in the testis and ovary, and the neural control of maternal and sexual behaviors. To determine the essential role(s) of oxytocin in mammalian reproductive function, mice deficient in oxytocin have been generated using embryonic stem cell technology. A deletion of exon 1 encoding the oxytocin peptide was generated in embryonic stem cells at a high frequency and was successfully transmitted in the germ line. Southern blot analysis of genomic DNA from homozygote offspring and *in situ* hybridization with an exonic probe 3′ of the deletion failed to detect any oxytocin or neurophysin sequences, respectively, confirming that the mutation was a null mutation. Mice lacking oxytocin are both viable and fertile. Males do not have any reproductive behavioral or functional defects in the absence of oxytocin. Similarly, females lacking oxytocin have no obvious deficits in fertility or reproduction, including gestation and parturition. However, although oxytocin-deficient females demonstrate normal maternal behavior, all offspring die shortly after birth because of the dam's inability to nurse. Postpartum injections of oxytocin to the oxytocin-deficient mothers restore milk ejection and rescue the offspring. Thus, despite the multiple reproductive activities that have been attributed to oxytocin, oxytocin plays an essential role only in milk ejection in the mouse.**

Extracts from the posterior pituitary were first noted to have "oxytocic" and "galactogogic" effects at the turn of this century (1). Nearly 50 years later, Du Vigneaud *et al.* (2) sequenced the endogenous 9-amino acid peptide and synthesized oxytocin as well as the related nonapeptide vasopressin. Molecular confirmation of the oxytocin sequence came in the early 1980s upon the cloning of cDNAs and genes encoding the oxytocin–neurophysin preprohormone (3–5). The linked oxytocin and arginine vasopressin (AVP) genes from mouse strain B10.A were first described by Gainer and colleagues (6). Similar to other species, exon 1 encodes the signal peptide, the oxytocin peptide, the 3-amino acid endoprotease recognition sequence, and the first 9 amino acids of the neurophysin. Exons 2 and 3 encode the majority of the neurophysin sequence.

Oxytocin has been postulated to facilitate mammalian reproduction at several levels. In addition to milk ejection in response to suckling and uterine contractions during labor (1), oxytocin is believed to play important roles elsewhere in both males and females. Oxytocin is synthesized in the hypothalamic paraventricular and supraoptic nuclei during late embryogenesis (7) and is released into the circulation via the posterior pituitary. Oxytocin is also synthesized in the corpus luteum (8, 9), uterus (10, 11), placenta (12), amnion (13), and testis (14, 15) although the mouse testis does not appear to be a source of oxytocin (15). Whereas posterior pituitary-derived oxytocin appears to regulate milk ejection, production of oxytocin in the uterus and/or placenta is believed to function to initiate and maintain parturition (10–12, 16). Oxytocin is the strongest uterotonic substance known and has been used widely to induce labor (1, 17). In particular, oxytocin and oxytocin receptor mRNA levels in the rodent uterus increase dramatically during gestation and at parturition, and the myometrium is more sensitive to oxytocin around the time of parturition (10, 16, 17). Oxytocin is also believed to regulate reproductive function at the level of the pituitary via a positive effect on luteinizing hormone release at proestrus (18) and at the level of the gonads (8, 9, 14, 15, 19). In the testis, oxytocin is believed to regulate Leydig cell steroidogenesis in some species, while in the ovary, oxytocin synthesis may be important for steroidogenesis, luteinization, and luteal regression. Oxytocin synthesis in the brain is postulated to play an important role in male and female mating behavior including a function in lordosis (20) and in male copulation and ejaculation (21). After intraventricular infusion, oxytocin appears to be the most potent stimulator of "spontaneous" erections in rats (21). In addition, oxytocin is believed to be necessary for the onset of maternal behavior in rodents (22–27). Central injection of oxytocin induces the full range of maternal behaviors (i.e., retrieval and grouping of pups, licking of pups, nest building, and crouching) in ovariectomized estrogen-primed virgin rats (22). In support of a physiologic role for oxytocin, central injection of an oxytocin antagonist or lesion of oxytocin-producing cells in the paraventricular nucleus suppresses the onset of the maternal behavior in postpartum female rats (23, 24). Similar maternal functions appear to be present in mice (27).

A receptor for oxytocin has been cloned and sequenced (28) and found to be expressed in many of the tissues implicated in the effects of endogenous and exogenous oxytocin (29). This receptor is notable for its exquisite responsiveness to gonadal and adrenal steroids (29) and for its down-regulation by exogenous oxytocin administration (30).

To determine the essential role(s) of oxytocin in mammalian reproductive physiology, we have generated mice carrying a deletion of the oxytocin-coding region using homologous recombination in embryonic stem (ES) cells. *In situ* hybridization of brains of oxytocin-deficient mice verified the absence of oxytocin/neurophysin mRNA expression in the paraventricular nucleus (PVN) and supraoptic nucleus (SON), the main sites of central oxytocin synthesis. Male and female mice deficient in oxytocin have been generated and used to study the functional consequences of oxytocin absence on reproductive behavior, fertility, parturition, and nursing. Because previous studies with the vasopressin-deficient Brattleboro rat have

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Abbreviations: ES, embryonic stem; AVP, vasopressin; PVN, paraventricular nucleus; SON, supraoptic nucleus.

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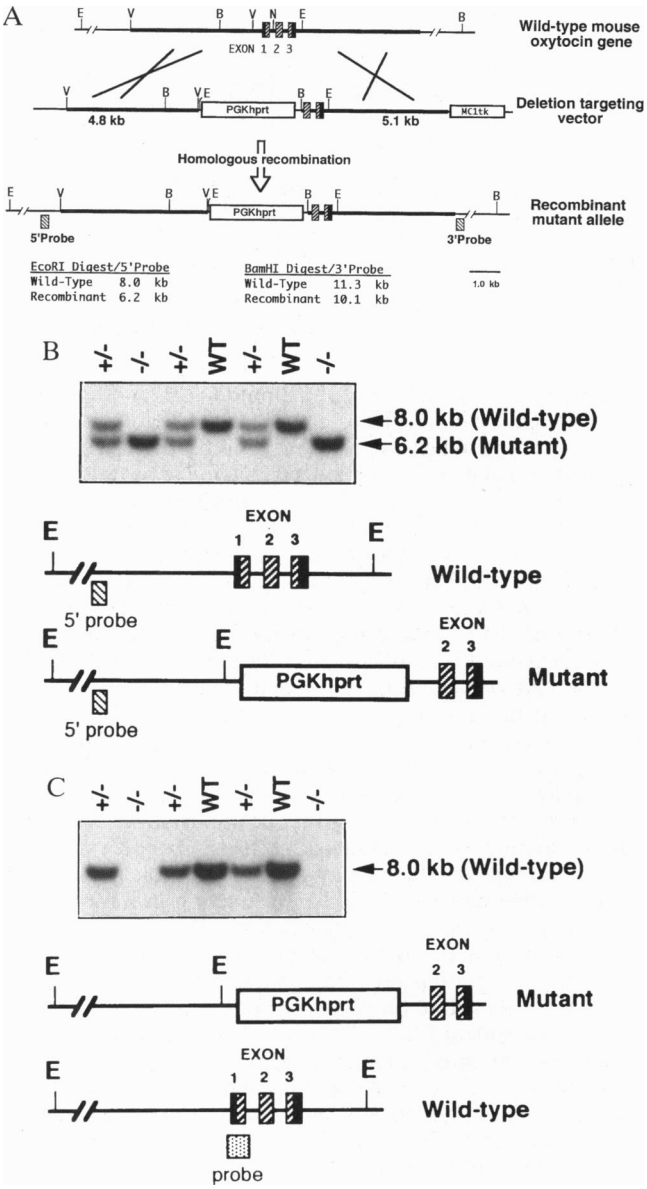

FIG. 1. Targeting of the oxytocin gene in ES cells and Southern blot analysis of DNA from offspring derived from heterozygote intercrosses. (*A*) The targeting vector to delete exon 1 [690 bp of sequence; the *Eco*RV (V) to *Nar*I (N) fragment] of the oxytocin gene is shown. This mutation deletes the coding sequence of exon 1 encompassing the initiation ATG codon, the signal peptide sequence, and the oxytocin nonapeptide sequence and also deletes some upstream regulatory sequences. The targeting vector contains 5.3 kb of sequence upstream of exon 1, 4.5 kb of sequence downstream of exon 1, the PGK-hprt expression cassette, and an MC1-tk (thymidine kinase) expression cassette. Homologous recombination between this targeting vector and the endogenous oxytocin locus (top) would enlarge the locus by 2.95 kb. The recombinant allele was detected by restriction endonuclease digestion of ES cell DNA with either *Eco*RI (E) or *Bam*H1 (B) and Southern blot analysis using 5′ or 3′ external probes, respectively. The presence of a 6.2-kb mutant allele versus a wild-type 8.0-kb fragment upon digestion with *Eco*RI is diagnostic of the correct recombination on the 5′ arm. A 10.1-kb fragment versus a wild-type 11.3-kb fragment upon digestion with *Bam*HI is diagnostic of the mutant allele when using a 3′ external probe. (*B*) The crosshatched boxes of the oxytocin gene denotes protein coding region; the solid boxes are untranslated regions. Genomic DNA (≈5 mg), isolated from the tails of offspring from 1 litter generated from a heterozygote intercross, was digested with *Eco*RI and analyzed as described (33) using the 5′ probe. The presence of a single 6.2-kb fragment indicates a homozygote (−/−) genotype. WT, wild-type; +/−, heterozygote. (*C*) The blot in *B* was stripped and rehybridized with the 690-bp oxytocin-encoding fragment that was predicted to be deleted. DNA from mice that were genotyped as homozygote (−/−) in *B*, are now devoid of any hybridizable signal, whereas wild-type (WT) and heterozygote (+/−) lanes continue to show a 8.0-kb wild-type fragment.

reported abnormalities in vasopressin receptor binding and oxytocin mRNA (31, 32), we have also investigated the effects of oxytocin deficiency on vasopressin mRNA and oxytocin receptor binding.

MATERIALS AND METHODS

Targeted Deletion of the Oxytocin Gene in ES Cells. Using a genomic probe from the oxytocin gene isolated from mouse strain B10.A (6), >21 kb of sequence encompassing the mouse oxytocin gene from strain 129SvEv (Stratagene) was isolated. An oxytocin targeting vector was constructed to delete exon 1 that encodes the oxytocin peptide (Fig. 1*A*). The oxytocin targeting vector (25 μg) was electroporated into hprt-negative AB2.1 ES cells and selected in HAT (hypoxanthine/aminopterine/thymidine) and 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU). Enrichment in HAT and FIAU was 18.9-fold compared with HAT alone. Forty-seven percent of the clones (41 out of 87 clones) screened by Southern blot analysis were correctly targeted. Targeted ES cell clones were injected into blastocysts to generate chimeras as described (34). Germ-line transmission of the deleted oxytocin allele from chimeric males derived from two ES cell clones was achieved. Screening by Southern blot analysis was as described (33).

Mice. Breeding pairs of mice heterozygous or homozygous for the deleted oxytocin allele were mated at approximately 6 weeks of age. Whereas the correct Mendelian frequency was observed from matings of heterozygote mice derived from ES cell line OT19-F7, there was a decrease in the number of homozygotes born to some heterozygotes derived from ES cell line OT19-B1. This is likely due to a linked mutation in ES cell line OT19-B1 that was unrelated to the targeted oxytocin mutation.

For the timed mating experiments, male and female mice homozygous for the oxytocin deletion were >6 weeks old. Female mice that had copulation plugs were removed from the breeding cage and observed until delivery.

For the oxytocin injections, oxytocin at 600 milliunits/kg (Sigma) was injected every 2–6 h intraperitoneally for several days after delivery. The mothers who received the oxytocin injections were present in normal breeding cages. Offspring were observed to have milk in their stomachs soon after the maternal injections were begun.

Gross and Histologic Analysis. Tissues were processed and analyzed as described (33). Testis weights were recorded on heterozygote and homozygote mice 81–98 days of age.

***In Situ* Hybridization and Radioligand Receptor Autoradiography.** For the *in situ* hybridization and receptor autoradiographic studies, brains from five heterozygote and five homozygote oxytocin mutant mice (three male and two female each) were removed, flash frozen on dry ice, and stored at −70 C. The brains were sectioned at 20 μm and thaw-mounted onto Superfrost plus slides (Fisher), to perform *in situ* hybridization and receptor autoradiography on adjacent sections. The sections were stored with dessicant at −70°C until used.

For *in situ* hybridization, the sections were fixed in 4% paraformaldehyde (pH 7.2) and treated as described (35). Antisense oligonucleotide probes [oxytocin, 42 bp complementary to the rat mRNA encoding amino acids 82–96 of the oxytocin-associated neurophysin peptide (36); AVP, 48 bp complementary to the rat mRNA encoding amino 129–144 of the AVP precursor peptide] were labeled with ^{35}S-labeled dATP by using terminal deoxyribonucleotidyltransferase to a

specific activity of 1×10^6/pmol and applied to the sections at a concentration of 4.3 pmol/ml in hybridization solution (50% formamide/10% dextran sulfate/0.3 M NaCl/10 mM Tris·HCl, pH 8.0/1 mM EDTA/1× Denhardt's solution/10 mM dithiothreitol/tRNA at 0.5 mg/ml). After hybridization overnight at 37°C, unhybridized probe was removed by rinsing four times in 1× standard saline citrate (SSC) (60°C) followed by a 1-h stirring rinse in 1× SSC at room temperature. The slides were then dehydrated in ethanol, exposed to Kodak BioMax MR film for 6 h to obtain images for quantification, then dipped in autoradiographic emulsion (NTB2, Kodak), and exposed for 3 days before developing and staining with cresyl violet.

For the radioligand receptor binding, alternate slide-mounted sections were processed for receptor autoradiography using ^{125}I-labeled d$(CH_2)_5$[Tyr(Me)$_2$,Tyr^9-NH_2]ornithine vacotocin (NEN) as described for the mouse (30). After a prewash in 50 mM Tris·HCl (pH 7.4), slides were exposed to a 60-min incubation (at room temperature) of 60 pM ^{125}I-labeled d$(CH_2)_5$ [Tyr(Me)$_2$ Tyr^9-NH_2]ornithine vasotocin in Tris with MgCl (10 mM), BSA (0.1%), and bacitracin (0.05%). One modification from the original protocol was that the final washing procedure was followed by a 1-min fixation in 0.1% paraformaldehyde and a 35-min room temperature stirring rinse in 50 mM Tris·HCl, pH 7.4/100 mM $MgCl_2$ to reduce background. Nonspecific binding was defined in adjacent sections by coincubation with 1 μM of the selective oxytocin ligand [Thr^4,Gly^7]oxytocin. After air drying, the slides were exposed to BioMax MR film (Kodak) for 48 h. ^{125}I autoradiographic standards (Amersham) were included in the cassette for quantification.

Data and Statistical Analysis. Film autoradiograms were analyzed using the IMAGE program (National Institutes of Health). For the receptor binding studies, optical densities were converted to dpm/mg of tissue equivalents using ^{125}I autoradiographic standards (Amersham). Specific binding was calculated by subtracting nonspecific binding from the total binding for each area. *In situ* hybridization images were quantified by measuring optical density on x-ray film standardized using a Kodak density step wedge. All sections were coded to obscure the identity of the tissue and each region of interest for binding was measured bilaterally from at least two sections. Values from heterozygote and homozygote oxytocin mutant mice were compared using Student's *t* test ($\alpha = 0.05$).

RESULTS

Targeting of the Oxytocin Gene in ES Cells. The structure of the mouse oxytocin gene from strain B10.A has been published (6). Using a region of the gene that lacked repetitive sequences, >21 kb of DNA encompassing the mouse strain 129SvEv oxytocin gene was cloned. DNA from mouse strain 129SvEv is isogenic to the AB2.1 ES cell line used in the gene targeting experiment. Using the 129SvEv oxytocin gene sequences, a targeting vector to delete exon 1 of the oxytocin gene was constructed (Fig. 1*A*). Exon 1 encodes the active oxytocin nonapeptide as well as the translation initiating ATG codon. The targeting vector contained both positive and negative selectable markers (Fig. 1*A*). Since the targeting vector was designed to delete the exon encoding the oxytocin peptide, no oxytocin protein would be synthesized.

The oxytocin targeting vector was electroporated into the hprt-negative AB2.1 ES cell line and HAT- and 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU)-resistant colonies were screened by Southern blot analysis using 5′ and 3′ external probes. Forty-seven percent of the ES cell clones demonstrated the correctly targeted oxytocin allele. Two of these ES cell clones, OT19-F7 and OT19-B1, were used to generate male chimeras that transmitted the deleted oxytocin allele.

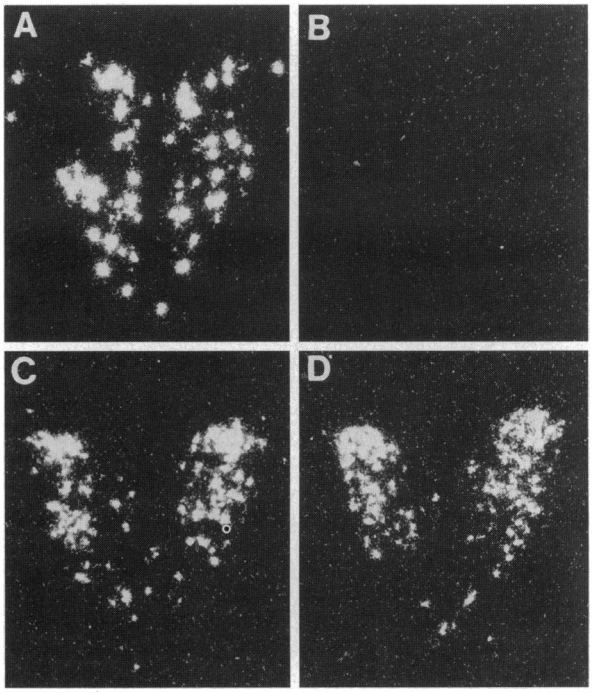

FIG. 2. Darkfield photomicrographs of oxytocin (*A* and *B*) and AVP (*C* and *D*) mRNA expression. White areas represent regions of *in situ* hybridization of ^{35}S-labeled oligonucleotide probes in oxytocin heterozygote (*A* and *C*) and homozygote (*B* and *D*) mutant mice. Depicted is the PVN of the hypothalamus surrounding the third ventricle, appearing as a dark line in the center in each panel. Note the absence of oxytocin mRNA and the normal pattern of AVP mRNA in the homozygous mutant (*B* and *D*). Quantitative optical density measures from film autoradiograms reveal no group differences (n = 5 per group) in AVP mRNA (PVN, 0.128 ± 0.013 versus 0.114 ± 0.010; SON, 0.190 ± 0.028 versus 0.212 ± 0.034 optical density units in homozygous versus heterozygous mice, respectively).

Generation of Oxytocin-Deficient Mice. Male and female mice heterozygous for the deleted oxytocin allele were produced at the expected frequency. Heterozygotes (C57Bl/6/129SvEv genetic background) were intercrossed to investigate the developmental consequences of oxytocin deficiency. Litter sizes from these intercrosses were normal (mean = 7.5 offspring per litter; n = 14 litters). Out of 98 progeny genotyped from these matings (derived from ES cell line OT19-F7), 27 were homozygotes (27.5%), 42 were heterozygotes (42.9%), and 29 were wild type (29.6%), consistent with the expected Mendelian frequency of 1:2:1 (Fig. 1*B*). In addition, similar numbers of male (13) and female (14) homozygotes were produced. Thus, oxytocin-deficient male and female mice are viable and appear to have normal sexual differentiation.

To confirm that the mice genotyped as oxytocin homozygote mutants lacked exon 1 that encodes the oxytocin peptide, the above described genomic Southern blot was stripped and rehybridized with the 690-bp region that was predicted to be deleted. As expected, the genomic probe detected a 8.0-kb fragment in mice genotyped as either wild type or heterozygote but failed to hybridize to any fragments in the two mice genotyped as homozygotes (Fig. 1*C*). This confirms that the oxytocin coding region was deleted. As a further confirmation that no oxytocin/neurophysin mRNA was synthesized, *in situ* hybridization of the PVN and SON was performed using a 42-bp oligonucleotide complementary to rat mRNA encoding amino acids 82–96 of the oxytocin-associated neurophysin peptide. Whereas oxytocin mRNA was abundant in the PVN (Fig. 2*A*) and SON (data not shown) of heterozygotes, no oxytocin/neurophysin mRNA was present in the PVN (Fig. 2*B*) and SON (data not shown) of homozygote mutants. In

contrast, there was no difference in the AVP mRNA content in the PVN (Fig. 2 *C* and *D*) or SON (data not shown) between heterozygote and homozygote mice ($P > 0.05$). Thus, the sequences encoding the oxytocin peptide are absent from the genome of homozygote mice and the mRNA encoding the oxytocin/neurophysin precursor is not synthesized, confirming that the mutant allele is null for oxytocin and neurophysin function. Although this mutant allele does not synthesize oxytocin-associated neurophysin, there is no alteration in the synthesis of AVP mRNA.

Oxytocin-Deficient Mice Are Fertile and Females Show No Gestation or Parturition Defects. Oxytocin has been postulated to play important roles in both male and female reproductive tracts (8–19, 37, 38). Since oxytocin-deficient male and female mice were healthy and had overtly normal external genitalia, we mated homozygote males with heterozygote females, homozygote females with heterozygote males, and homozygote males with homozygote females. Homozygote males mated with heterozygote females gave rise to 122 progeny, 50% of which (61 progeny) were homozygotes. Litters were born every 31.5 days ($n = 27$ litters; $n = 5$ males mated to 10 females). There appeared to be no defects in reproductive behavior of homozygote males nor did they display any defects in the reproductive axis either grossly or histologically (Fig. 3 *A* and *B* and data not shown). Testes from homozygote adult males (99.8 ± 13.8 mg; $n = 10$) were statistically similar in size to heterozygote adult males (111.6 ± 15.9 mg; $n = 9$). There were no gross or histologic defects in the testes (Fig. 3 *A* and *B*) consistent with the observed normal fertility of the homozygote males. Thus, oxytocin is not essential for male reproductive behavior or function.

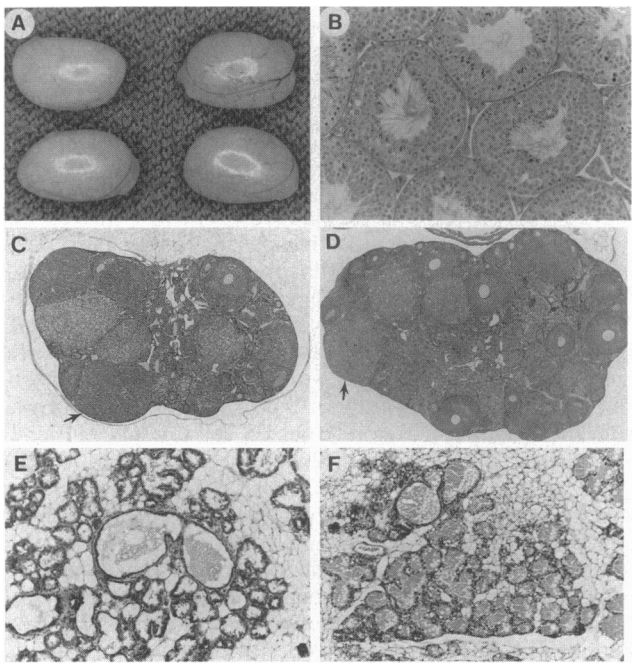

FIG. 3. Morphological and histological analysis of the gonads and breast tissue of oxytocin-deficient and control mice. (*A*) Gross analysis of the testes of 42-day-old heterozygote (left) and homozygote (right) littermate mice. There is no gross difference in the testes of these mice. (*B*) Histological analysis through the seminiferous tubules of the testis of an adult homozygous mutant mouse (high power). Stages of spermatogenesis are normal, spermatozoa are present in the two tubules in the center, and Leydig (interstitial) cell islands are obvious between the tubules. (*C* and *D*) Histology of ovaries from adult oxytocin heterozygote (*C*) and homozygote (*D*) mutant mice photographed at the same low power magnification. All stages of folliculogenesis including multiple corpora lutea (arrows) are present in both ovaries. (*E* and *F*) Post-partum histology of mammary gland tissue from oxytocin heterozygote (*E*) and homozygote (*F*) mutant mice photographed at the same magnification. Ducts continue to accumulate milk in the mammary tissue of the homozygous mutant.

Similar to the oxytocin-deficient males, oxytocin-deficient females are fertile. Homozygote females mated with heterozygote males demonstrated normal copulation behavior, became pregnant, and delivered their offspring. Consistent with the normal fertility of the homozygote females, histologic analysis of the ovaries of adult homozygote females showed normal stages of folliculogenesis and the presence of multiple corpora lutea similar to heterozygote controls (Fig. 3 *C* and *D*). Pregnancies and deliveries were observed at regular intervals (litters born every 26.3 days; births of 22 litters observed from a total of nine females).

To confirm that the placenta of heterozygote offspring (i.e., capable of synthesizing oxytocin) was not responsible for inducing labor, matings of homozygote females and homozygote males were performed. Similarly, homozygote females from these matings became pregnant and delivered their offspring at regular intervals (litters born every 30.2 days; observations from 12 litters born to six females). To further confirm that gestation time and labor were normal, timed matings of homozygote females with homozygote males were performed. Deliveries from these matings ($n = 8$ homozygote females) were timely and occurred approximately 18.5–19.5 days post coitum. Thus, despite the presence of oxytocin receptors in the uterus (28) and despite an important function of oxytocin in the induction of labor in humans (1), mice lacking oxytocin show no obvious deficits in fertility, gestation, or parturition.

Oxytocin-Deficient Female Mice Fail to Nurse Their Offspring. Oxytocin has been postulated to play an important role in maternal postpartum behavior (22–25) and in milk ejection in response to suckling (1, 37, 38). In all cases, pregnant homozygote female mice were observed to build a typical nest, and after delivery, the offspring were cleaned and present in the nest. Offspring that were moved outside of the nest were quickly retrieved by the mother. Thus, maternal behavior appeared normal in the absence of oxytocin. In tests of maternal behavior with newborn pups, retrieval was 86 ± 28 sec versus 125 ± 105 sec, time in nest was 1304 ± 291 sec versus 1228 ± 209 sec, and time grooming pups was 485 ± 129 sec versus 631 ± 151 sec for heterozygote versus homozygote females, respectively.

Despite ostensibly normal maternal behavior, offspring of homozygous mutant females offspring died within 24 h of delivery. Milk was not observed in the stomachs of the offspring born to the homozygous females. Offspring were observed to "latch-on" to the mothers' nipples and the mothers appeared to encourage this behavior since live offspring were found in the nest. However, this behavior by the offspring failed to elicit milk release, leading to pup death.

To confirm that there were no defects in milk production in the absence of oxytocin and that the only defect was in milk ejection, histologic analysis of the breast tissue from control and homozygote postpartum females was performed. Despite the inability to nurse their offspring, oxytocin-deficient females continued to have milk in their mammary glands similar to heterozygote control females which could nurse their offspring (Fig. 3 *E* and *F*). Furthermore, postpartum intraperitoneal injections of oxytocin given to the oxytocin homozygote females every few hours produced enough milk ejection to keep several offspring from each litter alive as long as the injections continued (7–12 days postnatally). In one case in which the injections continued until 12 days postpartum, the offspring eventually survived to the adult stage. Thus, oxytocin is required for milk let-down, and exogenously supplied oxytocin can rescue the defect.

Distribution of Oxytocin Receptors. To determine whether the absence of oxytocin conferred any effects on oxytocin receptor binding, radioligand receptor autoradiography was

performed. Unlike the vasopressin-deficient Brattleboro rat that shows an abnormal pattern of brain AVP receptors (31), the distribution of oxytocin receptors in the brain appeared identical in oxytocin heterozygous and homozygous mutant mice (Fig. 4). Furthermore, no quantitative differences were detected in any of the regions analyzed. Note that the ventromedial nucleus of the hypothalamus, a region implicated in reproductive behavior in the rat, has a relatively low level of oxytocin receptors in the mouse brain.

DISCUSSION

Multiple functions have been attributed to oxytocin in mammals. These putative functions include local roles in the anterior pituitary (18), gonads (8, 9, 14, 15, 19), uterus (10, 11), placenta (12), and amnion (13). The most convincing studies have implicated oxytocin in the induction of labor (1) and in milk let-down in response to suckling (1). In addition to its putative roles in parturition and lactation, oxytocin has also been implicated in the control of a number of behaviors associated with reproduction including sexual receptivity (39, 40), maternal behavior (41), and pair bonding (25). However, despite the many functions attributed to oxytocin, male mice lacking oxytocin show no functional defects and oxytocin-deficient female mice only fail to nurse their offspring. Induction of labor and parturition proceed normally in the absence of oxytocin synthesis. Clearly, although oxytocin can affect many processes, oxytocin *per se* is not essential for maternal behaviors in mice. So why are oxytocin receptors induced in the uterus at the time of parturition, why is the myometrium more sensitive to oxytocin at parturition, and what significance can be attributed to oxytocin as the most potent uterotonic substance (1, 10, 16, 17)? One hypothesis is that oxytocin secreted either locally or from the posterior pituitary can aid in the induction of labor and parturition but is not necessary because other uterotonic substances (known or unknown) are more potent and essential in this process. Alternatively, another oxytocin-like ligand may bind to identical oxytocin receptors and act as a uterotonic inducer, thereby replacing oxytocin function in oxytocin-deficient mice. Our studies show that AVP does not appear to fulfill this role since synthesis of this oxytocin-like peptide is not increased in the oxytocin-deficient mice (Fig. 2). However, if there is another oxytocin-like peptide in mammals, tissue extracts (e.g., uterine extracts at labor) derived from our female oxytocin-deficient mice may be a rich source of this activity, especially if it is upregulated in the absence of oxytocin.

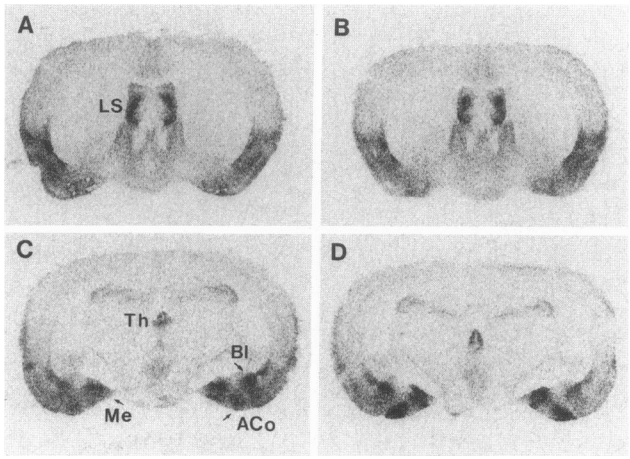

FIG. 4. Bright-field autoradiograms of oxytocin receptor distribution in oxytocin heterozygote (*A* and *C*) and homozygote (*B* and *D*) mutant mice. The pattern observed is identical to previous descriptions of oxytocin receptor distribution in the mouse brain (30). Dark regions represent regions of specific binding. No differences are evident in binding (dpm/mg tissue equivalents) between the two groups: lateral septum, 5477 ± 880 versus 4019 ± 467; thalamus, 5453 ± 669 versus 4954 ± 630; medial amygdala, 9004 ± 1172 versus 8464 ± 1054 comparing heterozygote versus homozygote, respectively. ACo, anterior cortical amygdala; Bl, basolateral amygdala; LS, lateral septum; Me, medial amygdala; Th, thalamus.

The effects of central oxytocin release are mediated by receptors localized in specific brain regions. We hypothesized that the pattern of oxytocin innervation and secretion could shape the pattern of adult oxytocin receptor expression, which could in turn contribute to the species diversity in receptor distribution. This notion is supported by the observation that chronic oxytocin treatment modulates oxytocin receptor concentrations in the rat brain (42). However, our analysis of brain oxytocin receptors revealed no effects of oxytocin deficiency on the distribution or abundance of receptor expression, consistent with an earlier report demonstrating that PVN lesions did not influence the expression of oxytocin receptor expression in the rat brain (42). It should be noted that the neuroanatomical distribution and regulation of these receptors is extremely variable among species (43). In fact, each species analyzed to date exhibits a unique pattern of receptor distribution in brain and presumably a different functional response to central oxytocin release (30). It is therefore possible that the absence of oxytocin in other species (with different patterns of brain receptors) could result in altered reproductive behaviors as suggested from previous pharmacologic and lesion studies.

As shown in this report, the only functional defect in our mice is an inability of oxytocin-deficient female mice to nurse their offspring. Since oxytocin is only essential for nursing, it is possible that mutations in the oxytocin or oxytocin receptor genes in humans are present and that individuals with nursing defects may be homozygous mutant at the oxytocin locus. Clearly, analysis of women or families of women with nursing defects would be a first step in determining whether any such mutations are present in humans. Lastly, the mutation in the oxytocin gene not only abolishes expression of oxytocin but also the synthesis of the oxytocin-associated neurophysin. Since absence of this form of neurophysin does not result in any additional unexpected defects in either males or females, this neurophysin does not play any additional roles in mammals other than its role in oxytocin synthesis.

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