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Centrally administered oxytocin elicits exaggerated grooming in oxytocin null mice

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Abstract

Experiments were conducted to determine if the chronic absence of the neurotransmitter oxytocin (OT) in null mice resulted in alterations in the responsiveness and abundance of central OT receptors. Self-grooming elicited by intracerebroventricularly administered OT was studied as an indicator of the activation of central OT receptors and autoradiography was used to map the distribution and density of OT receptors in OT null and wild type mice. The intracerebroventricular administration of OT, but not vehicle, artificial cerebrospinal fluid (aCSF), produced a robust increase in grooming behavior in both OT null and wild type animals, $P < .001$. However, OT-induced grooming was significantly greater in OT null than wild type mice, $P < .005$. The enhanced grooming was selective to OT as indicated by the finding that grooming to intracerebroventricular arginine vasopressin (AVP) was of the same magnitude in both OT null and wild type mice. OT-induced grooming appears to be mediated through the activation of OT receptors because pretreatment of animals with an OT antagonist, Atosiban, abolished OT-induced grooming, but not AVP-induced grooming. OT receptor distribution and binding in brains of OT null and wild type mice were examined by autoradiography and were not significantly different. The results indicate that the chronic absence of OT in null mice leads to an increase in OT receptor responsiveness that contributes to the augmented grooming activity elicited by centrally administered OT.

Keywords: Grooming; Mice; Oxytocin; Oxytocin receptor; Neurohypophysis; Vasopressin

1. Introduction

Mice that lack oxytocin (OT null mice) provide a novel model to examine the hormonal and behavioral effects of this peptide. OT that is released from the posterior pituitary into the peripheral circulation facilitates uterine contractility during labor and milk ejection during lactation (Higuchi et al., 1985). Although OT null mice are able to deliver young and produce milk (a prolactin-dependent function), the mice are unable to eject milk (Young et al., 1996; Nishimori et al., 1996), which is an OT-dependent function (Higuchi et al., 1985). OT also functions as a neurotransmitter within the brain and appears to be involved in the regulation of a variety of behaviors. Studies in rats suggest that OT modulates the onset of maternal behavior (Pedersen et al., 1982), grooming (Caldwell et al., 1986; Drago et al., 1986a), anxiety (Windle et al., 1997; Bale et al., 2001), stress (Windle et al., 1997; Neumann, 2002), and intake of food and sodium (Verbalis et al., 1993). OT null mice have alterations in several behaviors that appear to relate to deficiency of central OT pathways, such as social recognition (Ferguson et al., 2000, 2001), aggression (Winslow et al., 2000), stress-induced analgesia (Robinson et al., 2002), anxiety (Mantella et al., 2003), salt ingestion (Amico et al., 2001, 2003; Puryear et al., 2001), and responses to psychogenic stress (Amico et al., 2004). In several instances, central administration of OT to OT null mice corrects the abnormality (Ferguson et al., 2001; Mantella et al., 2003).

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In the present study, we conducted experiments in which self-grooming was used as a marker of central nervous system OT receptor activation. Central injection of OT induces self-grooming of the facial, truncal, and genital areas of the body in male and female rats (Caldwell et al., 1986; Drago et al., 1986a) and mice (Delanoy et al., 1978; Drago et al., 1999). In the rat, the OT receptor is believed to mediate grooming because the behavior can be inhibited by administration of an OT antagonist (Drago et al., 1991). Theoretically, the absence of OT in OT null mice may give rise to developmental differences and/or alterations in OT receptor responsivity as well as changes in the distribution and density of OT receptors. To examine these possibilities in the present study, the responsivity of OT receptors was assessed by comparing the grooming responses elicited by intracerebroventricular infusion of OT in OT deficient and wild type mice in the presence or absence of an OT antagonist, d[Dtyr(Et)^2,Thr^4] ornithine vasotocin, Atosiban (Ferring Pharmaceuticals, San Diego, CA). Autoradiography was used to assess the amount and distribution of OT receptors in OT null and wild type mice. To determine whether the grooming pattern elicited by OT in mice is specific for OT, the behavioral responses to arginine vasopressin (AVP), a neuropeptide structurally related to OT, was also tested in OT null mice. Both OT and AVP elicit well-defined behavioral effects following intracerebroventricular injection. Centrally administered AVP induces grooming in rodents, but the grooming has been described as much more frenetic and genitally oriented than OT-induced grooming (Drago et al., 1986a; Caldwell et al., 1986).

2. Methods

2.1. Animals

Male wild type and OT null mice of C 57BL/6 background were used for these studies. Dr. Scott Young, National Institutes of Mental Health, generated the OT-deficient mice (Young et al., 1996) and breeding pairs were purchased from Jackson Laboratories (Bar Harbor, ME). We maintain a breeding colony of the mice in the viral-free quarters of the University of Pittsburgh Central Animal Facility. Animals used for the studies were from the F5 generation and ranged in age from 8 to 10 months. Mice were housed in a room with a 12-h light/12-h dark cycle (lights on at 0700 h). Food (Prolab RMH 3000 5 P00, LabDiet/Purina) and water were provided ad libitum.

OT null mice are unable to nurse young because of inability to eject milk (Young et al., 1996). Therefore, we bred heterozygous female with OT null male mice to generate OT-deficient mice and bred wild type male and female mice to produce wild type progeny (Amico et al., 2001). OT null and wild type offspring from the matings were used for these studies. Pups were weaned at approximately 24–26 days postnatal and housed in standard rodent cages in same-sex groups of four per cage. Mice were caged individually for the week prior to, and during, an experiment. All experiments were conducted between 0900 and 1200 h. Experiments were conducted in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

To identify the genotype of each mouse, DNA from a small sample of tail was extracted and prepared for polymerase chain reaction (PCR) using primers that were synthesized at the University of Pittsburgh sequence facility (Amico et al., 2001). Pairs of primers were designed for PCR that either detected the wild type allele (OT, 332 bp) or the mutant allele (neomycin resistance cassette, 430 bp). The primers for the wild type allele were (forward) TCG CTC TGC CAC AGT CCG GAT TC and (reverse) TCA GTG TTC TGA GCT GCA AAC C, and for the mutant allele, these were (forward) AGA GGC TAT TCG GCT ATG ACT G and (reverse) TTC GTC CAG ATC ATC CTG ATC. The absence of an OT DNA band and the presence of a neomycin DNA band confirmed the OT null genotype, whereas the presence of an OT DNA band and absence of a neomycin DNA band confirmed the wild type genotype.

2.2. Insertion of lateral ventricular cannulae and injection of synthetic peptides

Mice were anesthetized with ketamine (0.75 mg/10 g body weight) and xylazine (0.025 mg/10 g body weight) for stereotaxic placement of cannulae into the lateral ventricle. The mice were placed in a stereotaxic apparatus and a 1-cm midline incision was made across the top of the skull. After cleansing the periosteum, a 1-mm hole was drilled 0.1 mm lateral to and 0.5 mm caudal to the bregma. A 26-gauge stainless steel guide cannula was placed 2.6 mm below the skull surface into the lateral ventricle. The cannula was secured to the skull with dental cement and a stylus was inserted to maintain patency. Animals were allowed to recover for 7 days after surgery prior to testing. Injections were made by inserting a 33-gauge stainless steel injector tube into the guide cannula. The injector tube was attached to PE-10 tubing fitted to a 10-μl Hamilton syringe. Patency of the injection system was confirmed by evidence of movement of an air bubble placed in the PE-10 tubing.

OT (1 or 10 ng, Bachem, San Carlos, CA), OT antagonist (Atosiban, 100 ng), or AVP (1 or 10 ng, Bachem) was dissolved in artificial cerebrospinal fluid (aCSF) and injected as a 2-μl volume over 2 min to awake, restrained mice. In experiments in which mice were pretreated with the OT antagonist, the antagonist was injected 15 min before OT or AVP.

After the completion of all experiments, India ink in aCSF was injected as a 2-μl volume over 2 min. The mice were rapidly killed by scissors decapitation and the brains were removed and inspected for ink in the ventricles. Only

mice showing ink in the ventricles were included in the analysis.

2.3. Behavioral observations

Behavioral observations were initiated 5 min after injection of aCSF, OT, AVP, or OT antagonist and recorded for the first minute of every 5 min. The occurrence of grooming was recorded every 5 min in a 30-min session. A single observer, blinded to the genotype and treatment, recorded behavioral measurements. A grooming score was derived for each animal with a maximum score of 1.0 if grooming was observed in every 5-min observation period. The minimum was 0 if an animal did not groom in any of the 5-min intervals. The grooming behaviors that were observed and scored were derived from published guidelines (Drago et al., 1986a,b, 1991) and were as follows: washing (e.g., movements of the forepaws in front of the snout and licking of the forepaws); scratching the face, ears, head, or body by one of the limbs; licking of the body fur, limbs, tail, or genital area; and intense grooming (e.g., persistence of any of these grooming behaviors for 30 s or longer without interruption).

2.4. Experiment 1: behavior following OT or AVP injections

Mice of both genotypes in which catheter patency was verified were used in these experiments. A within-subjects crossover design was used whereby each mouse served as its own control. Half of the mice received aCSF in the first experimental test, and received peptide (OT) 1 week later in the second experimental test. The other half of the mice received the same treatments in reverse (peptide in the first test and aCSF in the second).

In a pilot study, a 1-ng dose of OT was tested for its ability to induce grooming because this dose had been reported to restore social memory in OT null mice (Ferguson et al., 2000). The 1-ng dose of OT failed to induce grooming. Therefore, a 10-fold greater dose of OT, 10 ng, was tested and found to elicit grooming. The 10-ng dose of OT was used in this study.

AVP was tested for behavioral effects in the mice after a rest period of 2 weeks. Doses of AVP of 1 and 10 ng were selected to be equimolar to the OT doses that we found to elicit grooming.

2.5. Experiment 2: administration of an OT antagonist prior to OT or AVP

The ability of an OT antagonist to block OT-induced grooming was tested in OT null mice that received 100 ng of Atosiban ($n=6$) or aCSF ($n=6$) into the lateral ventricle 15 min prior to injection of 10 ng of synthetic OT. The ability of an OT antagonist to block AVP-induced grooming was tested in OT null ($n=6$) and wild type ($n=4$) mice that received 100 ng of Atosiban or aCSF into the lateral ventricle 15 min prior to injection of 10 ng of synthetic AVP. Behaviors were scored every 5 min for 15 min before and 30 min after the injection of synthetic OT or AVP.

2.6. Experiment 3: OT receptor distribution and binding in the brains of OT null and wild type mice

Animals were killed by scissors decapitation. Brains from OT null and wild type mice were rapidly removed, fresh frozen, and sectioned at a 20-μm thickness. Coronal sections were placed onto organosilane subbed slides and stored at −80 °C before being shipped on dry ice to the University of Maryland, Baltimore for OT receptor autoradiography.

The assay of OT receptors by autoradiography was adapted from Francis et al. (2002). Slides were thawed to room temperature for 10 min and a Pap pen (Sigma-Aldrich, St Louis, MO) was used to encircle the outer perimeter of the tissue sections. The tissue was fixed by immersion in 4% paraformaldehyde for 2 min and rinsed in three washes of 50 mM Tris-buffered saline (TBS, pH 7.4) for 5 min at room temperature. Immediately following the third wash, tissue sections were incubated for 1 h with 50 pM of [125]I-labeled OVTA (Perkin Elmer Lifescience, Boston, MA), an OT receptor specific ligand, diluted in TBS containing 10 mM $MgCl_2$, 1% bovine serum albumin, and 0.05% bacitracin. Nonspecific binding was determined by adding 50 mM unlabeled Thr4,Gly7 OT (Bachem) to the incubation mixture. After incubation, slides were rinsed four times in cold 50 mM TBS containing 10 mM $MgCl_2$ for 5 min followed by a wash in the same chilled buffer for 30 min. Finally, slides were rapidly dried under a stream of cool air before being placed into autoradiographic cassettes and apposed to BioMax MR film (Kodak, Rochester, NY). [125]I microscale standards (Amersham Biosciences, Piscataway, NJ) were placed into film cassettes. Film was developed after 7 days of exposure using standard photographic techniques.

Films were analyzed by capturing images with a MTI CCD72 camera (DAGE MTI, Michigan City, IN) connected to a Macintosh computer. Densitometry was conducted using NIH Image public domain software (http://rsb.info.nih.gov/nih-image/) using a density step wedge for calibration and data were expressed as relative density. The region of interest was outlined and measured for 10–30 sections per animal. The mean of this value was then used for each animal.

2.7. Statistical analysis

Values are expressed as group mean ± S.E.M. Group data for observations of grooming behavior were analyzed by two-way analysis of variance (ANOVA) in which genotype and treatment were variables. For the densitometry of autoradiograms, data were analyzed by one-way ANOVA. When the overall F ratio was significant, pairwise comparisons were made with the Bonferroni/Dunn post hoc

comparison. Differences were considered significant when $P < .05$. Statistical analysis was performed using Sigma Stat software.

3. Results

Mice of both genotypes that received lateral ventricular injections of OT, 10 ng, exhibited greater grooming behavior than mice that were injected with aCSF, $P < .001$ (Fig. 1). Grooming induced by injection of OT, 10 ng, was greater in OT null mice ($n = 11$) than wild type mice ($n = 8$), $P < .005$ (Fig. 1). Injection of Atosiban, 100 ng, ($n = 6$), but not aCSF ($n = 6$), into the lateral ventricle of OT null mice 15 min before administration of OT, 10 ng, abolished OT-induced grooming behavior (Fig. 2). Atosiban alone did not induce grooming.

Injection of AVP, 1 ng (Fig. 3, upper panel) or 10 ng (Fig. 3, lower panel), into the lateral ventricle elicited grooming that was greater than in mice injected with aCSF, $P < .001$, but the intensity of the AVP-induced grooming was not significantly different between genotypes (Fig. 3). Injection of 100 ng of Atosiban or aCSF 15 min prior to injection of AVP into the lateral ventricle of OT null ($n = 6$) or wild type ($n = 4$) mice did not affect AVP-induced grooming (not shown).

Fig. 4 illustrates the time course of the grooming behavior following OT, 10 ng, (Fig. 4A) or AVP, 10 ng, (Fig. 4B) in mice of both genotypes. The percentage of OT null mice grooming after intracerebroventricular injection of OT remained high at the end of the 30-min observation period, whereas the percentage of wild type mice grooming was similar to that induced by aCSF (Fig. 4A). When the type of grooming pattern elicited by OT was compared between genotypes, OT null mice displayed more body ($P < .005$) and intense ($P < .005$) grooming, but not head grooming, than wild type mice following administration of OT (not shown). In contrast, the type of grooming pattern induced by AVP was not different between genotypes.

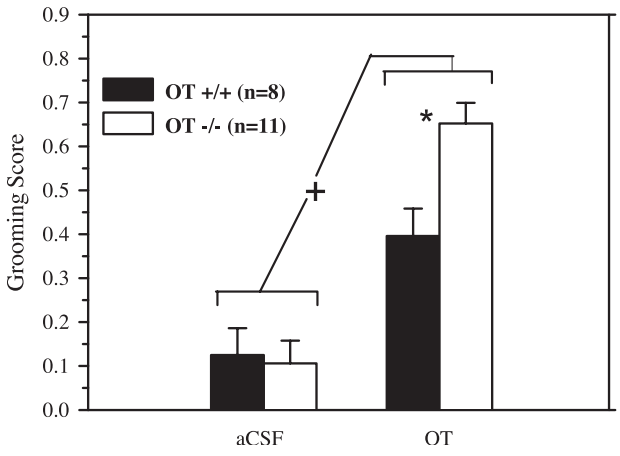

Fig. 1. Grooming behavior in OT −/− (null) and OT +/+ (wild type) mice treated with synthetic OT, 10 ng, or aCSF icv. OT produced grooming in both genotypes that was significantly greater than that induced by aCSF (two-way ANOVA, $P < .001$). However, there was a significantly greater grooming response to OT in the OT −/− than in the OT +/+ mice (ANOVA indicated a significant interaction between genotype and treatment, $P < .001$, $^*P < .005$ OT −/− versus OT +/+ mice, Bonferroni t test).

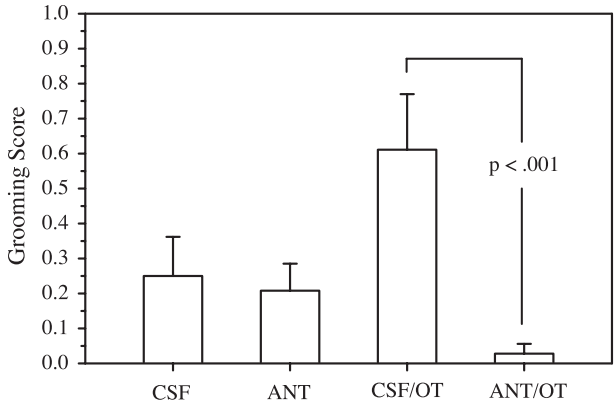

Fig. 2. Effect of pretreatment with an OT antagonist (Atosiban, ANT), 100 ng icv, or aCSF on grooming behavior to OT, 10 ng icv, in OT −/− (null) mice. Grooming behavior was significantly attenuated following pretreatment with the OT antagonist ($^*P < .001$ paired t test).

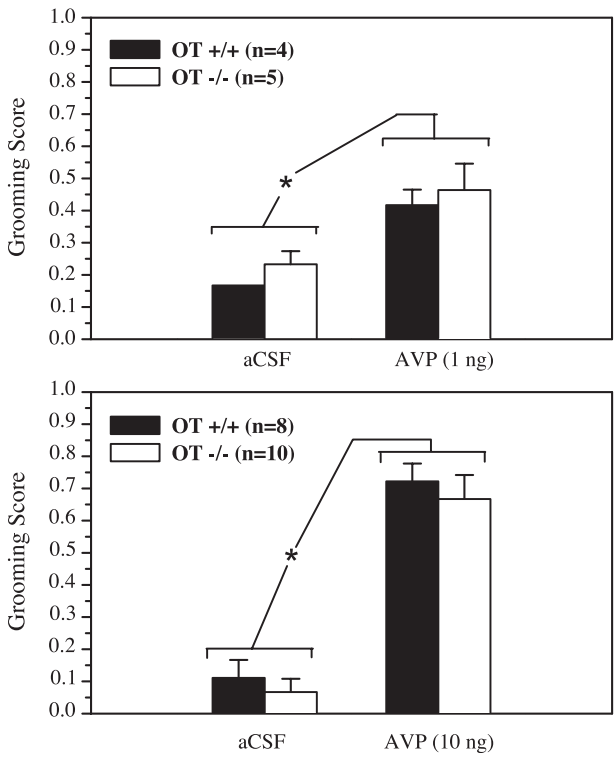

Fig. 3. Grooming behavior in OT −/− (null) and OT +/+ (wild type) mice treated with synthetic AVP, 1 ng (upper panel) or 10 ng (lower panel) icv. Intracerebroventricular aCSF was used as the control. Grooming produced by AVP was significantly greater than produced by aCSF (ANOVA, $^*P < .01$) for both 1 and 10 ng of AVP. There was no difference between genotypes in the magnitude of the grooming behavior to 1 or 10 ng of AVP.

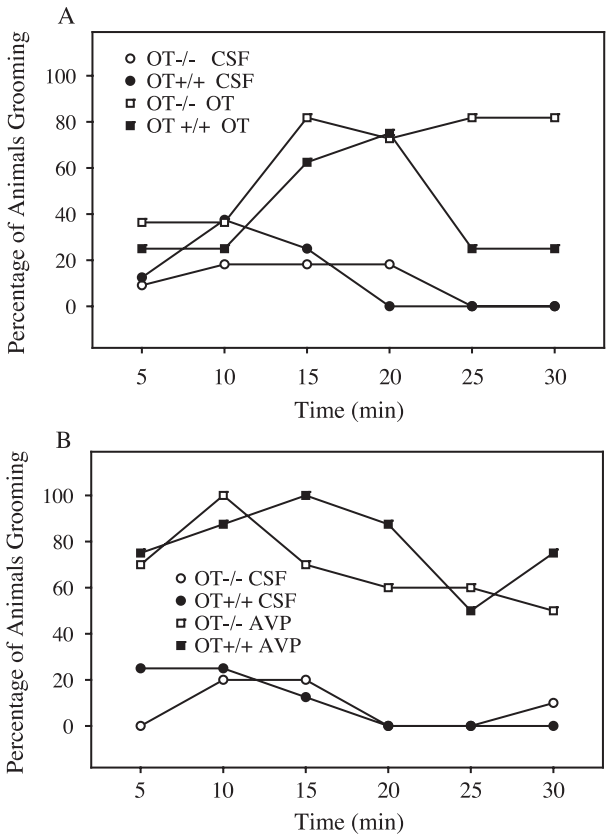

Fig. 4. Time course of grooming behavior produced by synthetic OT (A) or synthetic AVP (B) in OT −/− (null) and OT +/+ (wild type) mice. The *y*-axis is the percentage of the animals that demonstrated grooming in each 5-min time interval.

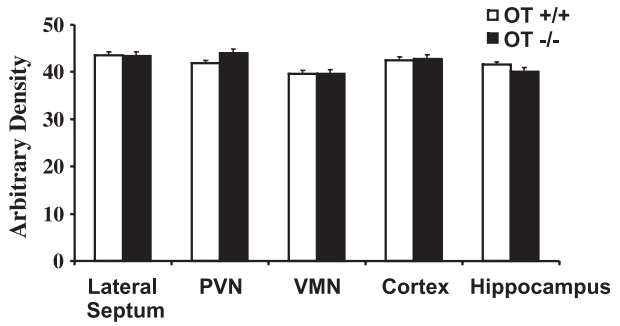

Fig. 6. [125]I-OVTA was used to assess OT receptor binding by autoradiography. One-way ANOVA indicated no significant differences in density signal between genotypes.

Representative autoradiograms of OT receptor binding for wild type and OT null mice are presented in Fig. 5. High levels of binding were observed in the lateral septum, paraventricular nucleus, cortex, ventromedial nucleus of the hypothalamus, and hippocampus. Low-level binding was also observed, although inconsistently, in the preoptic area, the bed nucleus of the stria-terminalis, and the amygdala. The latter regions were not analyzed because binding was only observed in some animals, and did not vary consistently with genotype. One-way ANOVA indicated no significant differences in OT receptor binding signal between wild type and OT null mice (Fig. 6). There also was no evidence of a difference in the distribution of the OT receptor between wild type and mutant types.

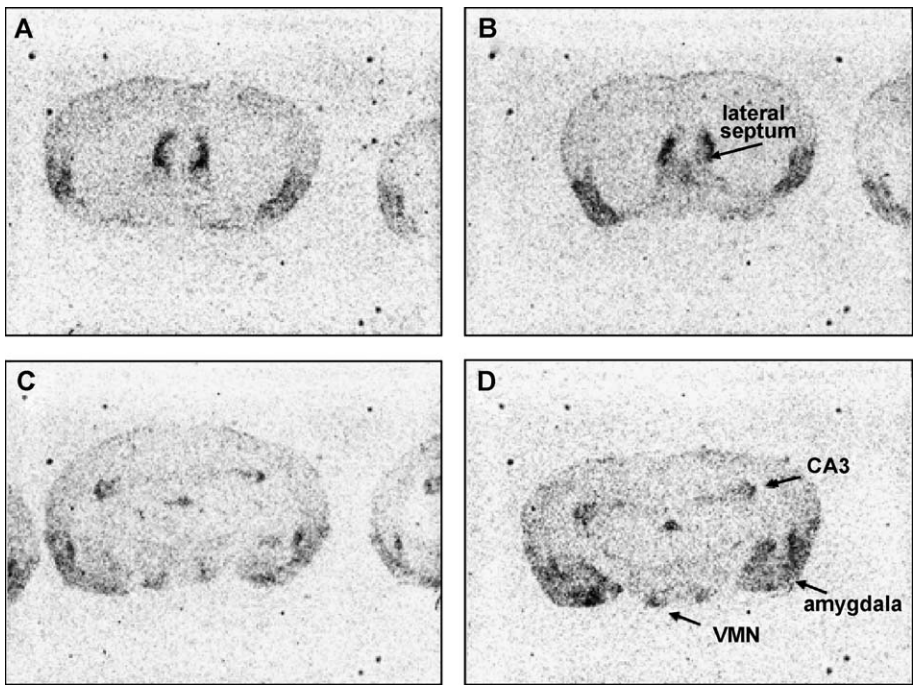

Fig. 5. Representative autoradiograms of OT receptor binding. (A) High levels of binding were observed in the lateral septum of wild type and (B) OT null mice. Binding was also consistently observed in the hippocampus (CA3), cortex and associated amygdala (subnuclei could not be delineated), and ventromedial nucleus (VMN) of both (C) wild type and (D) OT null mice. There were no significant differences in binding between genotype (ANOVA).

4. Discussion

This is the first study to investigate the responsiveness of central OT receptors in OT null mice by using grooming behavior as a marker of OT receptor activation. Robust grooming responses were elicited by intracerebroventricular administration of OT in both null and wild type mice; however, the magnitude of the grooming response was significantly greater in the mice lacking the OT gene. The augmented grooming was mediated through activation of OT receptors because the effect was inhibited by blocking OT receptors with an antagonist, Atosiban. In addition, the augmented grooming response was selective for OT because AVP-induced grooming was of similar magnitude in null and wild type mice. We also found, based upon autoradiographic determinations, that the distribution and density of OT receptors were similar in both genotypes, thereby indicating that alterations in receptor number or their location do not account for the increased responsiveness observed in OT-deficient mice. However, it is possible that the technique employed to measure OT receptors was not adequate to detect physiologic changes in receptor expression in every brain region that may be involved in grooming.

There are several possible mechanisms that may explain the augmented grooming behavior we observed in OT null mice. First, the OT receptors of OT null mice are not exposed to endogenous OT, whereas in wild type mice, endogenously released OT occupies a portion of the OT receptor population. Therefore, exogenously administered OT has fewer receptors available for activation in wild type animals. In contrast, the total population of OT receptors is available in OT null mice. Thus, injection of exogenous OT to OT null mice may result in enhanced receptor activation and grooming. Second, endogenous OT may regulate the abundance and/or binding affinity of the OT receptor. We therefore determined OT receptor distribution and binding in the brains of OT-deficient and wild type mice using autoradiography. However, we found no difference in the distribution or binding of brain OT receptors between OT null and wild type mice, with the distribution we observed being similar to that reported by Young et al. (1997). A third possibility is that differences exist between OT null and wild type mice in the downstream signal transduction pathways following receptor activation. A fourth possibility for enhanced OT-induced grooming is that in the absence of OT, developmental differences arise that result in enhanced grooming behavior when OT is reintroduced.

Studies in the rat suggest OT induces self-grooming by acting at the PVH (Van Erp et al., 1993) or ventral tegmental area (Stivers et al., 1988). Microinfusion of OT into the lateral hypothalamus, but not the preoptic area, has also been reported to increase grooming in the rat (Kaltwasser and Andres, 1989). We found no difference in OT binding in the PVN of null versus wild type mice, and there was no evidence of binding in the lateral hypothalamus. Gene expression for OTR has been reported in the laterodorsal tegmental nucleus of the mouse but at very low levels (Gould and Zingg, 2003). OTR distribution in the rat is distinctly different from the mouse, precluding our ability to draw conclusions regarding the potential site of action of OT on grooming behavior in the mouse. In rats, OT-induced grooming also likely involves dopaminergic and serotonergic systems in the ventral tegmentum (Kaltwasser and Crawley, 1987) and the nucleus accumbens (Drago et al., 1991). Dopamine systems also play a role in OT-induced grooming in mice because dopamine receptor deficient mice display significantly less grooming following injection of exogenous OT than wild type mice (Drago et al., 1999). Thus, a developmental change in the tone of the dopaminergic system, or perhaps another neurotransmitter system, may contribute to an enhanced response of OT null mice to exogenously administered OT, but we have not yet explored this possibility.

Although mice of both genotypes displayed grooming behavior following central injection of synthetic AVP, the pattern of AVP-induced grooming differed from OT-induced grooming. AVP-induced grooming in mice was much more frenetic and genitally oriented than OT-induced grooming. These observations in mice are in agreement with prior studies in rats, in which grooming elicited by central infusion of AVP had also been reported to differ from that induced by OT (Drago et al., 1986a; Caldwell et al., 1986). In contrast to OT-induced grooming, which was more intense in OT null than wild type mice, AVP-induced grooming was not different between genotypes. In addition, administration of an OT antagonist prior to AVP did not block AVP-induced grooming. This observation suggests that the enhanced grooming response to OT in OT null versus wild type mice is specific for OT. Also of interest is the fact that we were able to elicit grooming behavior with a dose of 10 ng of OT. The doses previously shown to elicit grooming effects in rats and mice were typically in excess of 100 ng (Drago et al., 1986a, 1999). Perhaps, methodological differences between studies account for the variance in effective doses.

In summary, male mice responded to administration of OT into the lateral ventricle with self-grooming behaviors that are similar to those induced in the rat with centrally administered OT. OT-induced self-grooming was blocked by an OT antagonist, indicating that the grooming response in OT null and wild type mice is mediated via the OT receptor. Additionally, the intensity of the self-grooming behavior in OT null mice was greater than in wild type mice following central administration of OT. The enhanced grooming in OT null compared to wild type mice following intracerebroventricular injection of OT was not observed following exogenous AVP. The ability of OT to induce self-grooming provides a behavioral index by which to monitor OT receptor activation in mice. The blockade of OT-induced self-grooming by an OT antagonist suggests that self-grooming can be used to monitor the efficacy of centrally

administered OT receptor antagonists in mice. Although the present study does not identify the area of the brain that mediates this behavior in mice or whether differences in neurotransmitter tone account for the greater grooming response in OT null versus wild type mice, these questions can be answered by future studies using mice of both genotypes.

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