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NEONATAL OXYTOCIN MANIPULATIONS HAVE LONG-LASTING, SEXUALLY DIMORPHIC EFFECTS ON VASOPRESSIN RECEPTORS

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Abstract—Developmental exposure to oxytocin (OT) or oxytocin antagonists (OTAs) has been shown to cause long-lasting and often sexually dimorphic effects on social behaviors in prairie voles (*Microtus ochrogaster*). Because regulation of social behavior in monogamous mammals involves central receptors for OT, arginine vasopressin (AVP), and dopamine, we examined the hypothesis that the long-lasting, developmental effects of exposure to neonatal OT or OTA might reflect changes in the expression of receptors for these peptides. On postnatal day 1, prairie voles were injected intraperitoneally with either OT (1 mg/kg), an OTA (0.1 mg/kg), saline vehicle, or were handled only. At approximately 60 days of age, vasopressin V1a receptors, OT receptors (OTR) and dopamine D2 receptor binding were quantified using receptor autoradiography in brain tissue taken from males and females. Significant treatment effects on V1a binding were found in the bed nucleus of the stria terminalis (BNST), cingulate cortex (CgCtx), mediodorsal thalamus (MdThal), medial preoptic area of the hypothalamus (MPOA), and lateral septum (LS). The CgCtx, MPOA, ventral pallidum, and LS also showed significant sex by treatment interactions on V1a binding. No significant treatment or sex differences were observed for D2 receptor binding. No significant treatment difference was observed for OTR receptor binding, and only a marginal sex difference. Changes in the neuropeptide receptor expression, especially the V1a receptor, may help to explain sexually dimorphic changes in behavior that follow comparable neonatal manipulations.

Key words: oxytocin, vasopressin, dopamine, monogamy, pair-bonding, development.

The neurobiology of social behavior has been shown to be intimately linked to two central neuropeptide hormones, oxytocin (OT) and vasopressin. OT is a nine-amino-acid peptide associated with labor and milk let-down, as well as the formation of pair-bonds (particularly in females) and mother–infant bonds (Carter, 1998; Williams et al., 1994; Zingg, 2002). The related neuropeptide, arginine vasopressin (AVP), well-known for its peripheral effects on blood pressure and water balance, also plays a role in pair-bond formation and parental care, especially in males (Berecek, 1991; Winslow et al., 1993; Wang et al., 1998). OT and AVP differ by two amino acids and may exhibit some receptor cross-reactivity (Barberis and Tribollet, 1996), allowing potential interactions between the two peptide systems. Changes in exposure to either OT or AVP during development may have significant potential to affect both social behavior and its regulation over a lifetime.

Variations in child-rearing practices have the capacity to affect OT exposure in offspring. Breast milk contains OT (Leake et al., 1981), and OT is also released by warmth and touch (Uvnas-Moberg, 1998). In dairy calves, suckling from the mother rather than drinking mother's milk from a bucket raises the calf's plasma level of OT (Lupoli et al., 2001). Developmental exposure to OT in rats is associated in later life with lower blood pressure (Holst et al., 2002), lower corticosterone levels (Sohlstrom et al., 2000), higher body weight (Sohlstrom et al., 2000), and can reverse the effects of maternal malnutrition (Olausson et al., 2003). In rodents, mothers that lick and groom their infants produced female offspring with significant increases in OT binding in the central amygdala and the bed nucleus of the stria terminalis (BNST). In contrast, male offspring of high licking and grooming mothers had higher AVP binding in the central nucleus of the amygdala (Francis et al., 2002). These neural differences were reflected in behavior, with offspring of high licking and grooming mothers also demonstrating high licking and grooming (Francis et al., 1999). Differences in perinatal exposure to OT, through breast-feeding or other forms of infant care, may thus have the ability to change neural systems in a long-term (perhaps lifelong) manner.

In addition to the OT and AVP systems, the dopamine system appears to be crucial to the expression of social behavior. Dopamine has recently been identified for its crucial role both in pair-bonding (Aragona et al., 2003, 2006; Liu and Wang, 2003; Wang et al., 1999) and parenting behavior (Lonstein, 2002). Access to dopamine type 2 receptors (D2) is necessary for formation of a pair-bond, while activation of D1 dopamine receptors blocks pair-bond formation. Following formation of the bond, D1 receptors are up-regulated, preventing formation of a second

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Abbreviations: AVP, arginine vasopressin; BNST, bed nucleus of the stria terminalis; CgCtx, cingulate cortex; CTL, control; DPM, disintegrations per minute; D2, dopamine type 2 receptors; HAN, handled only group; LS, lateral septum; MANOVA, multivariate analysis of variance; MeA, medial amygdala; MPOA, medial preoptic area; OT, oxytocin; OTA, oxytocin antagonist; OTR, oxytocin receptor; PND, postnatal day; SAL, saline; VP, ventral pallidum; V1a, vasopressin receptors type 1a.

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bond (Aragona et al., 2006). Essential to the actions of dopamine in pair-bonding is co-localization of D2 receptors with OT receptors in the nucleus accumbens (in females) and with vasopressin receptors type 1a (V1a) in the ventral pallidum (VP) (in males).

The prairie vole (*Microtus ochrogaster*), a socially monogamous rodent native to the Midwestern United States, is a well-studied model for sociality. Prairie voles exhibit selective pair-bonds (Williams et al., 1992) and high levels of both paternal and alloparental care (Roberts et al., 1998; Lonstein and De Vries, 2000). This species is sensitive to developmental manipulations in OT, showing long-lasting changes in behavior and physiology (Carter, 2003). In a series of experiments, prairie voles were injected on postnatal day 1 (PND1) with either 1 mg/kg OT, 0.1 mg/kg oxytocin antagonist (OTA), saline (SAL) vehicle, or were handled only. Developmental exposure to OT facilitated pair-bond formation in adulthood in male voles (Bales and Carter, 2003b), whereas OTA exposure on PND1 produced a marked reduction in alloparental behavior in males (Bales et al., 2004b). Manipulation of either OT or OTA on PND1 altered the subsequent patterning of male sexual behavior and reduced male reproductive potential (Bales et al., 2004a). Finally, in males treatment with OTA resulted in fewer AVP-immunoreactive cells, and did not significantly alter the number of OT-immunoreactive cells (Yamamoto et al., 2004).

In females in general, the behavioral effects of neonatal exposure to OT or OTA were less pronounced than in males. However, neonatal OT did increase the mate-guarding component of pair-bonding shown by adult females (Bales and Carter, 2003a). Females were capable of responding to OTA, since female pups exposed to 0.1 mg/kg OTA on PND1 emitted significantly fewer ultrasonic vocalizations upon separation from their parents on PND8 (Kramer et al., 2003). PND1 OTA-treated females showed, as adults, increased neural activation of the central amygdala when exposed to a member of the opposite sex (Kramer et al., 2006). Also in females, a single PND1 exposure to either OT or OTA resulted in higher numbers of OT-immunoreactive cells in the paraventricular nucleus of the hypothalamus on PND21 (Yamamoto et al., 2004).

The purpose of the present study was to examine the hypothesis that at least some of the functional changes that we observed following a single neonatal exposure to OT or OTA might be reflected in or due to the developmental capacity of these manipulations to influence receptor expression for neuropeptides or transmitters that have been previously implicated in social behavior. Oxytocin receptor (OTR), AVP V1a and dopamine (D2) receptor binding were measured in adulthood using quantitative autoradiography. The brain areas selected for study were those in which these receptors were abundant and for which there was prior evidence of relevance to social behavior. Based on the behavioral changes that we had observed, we predicted that the effects of OT and OTA would (a) differ from each other, (b) be regionally specific and (c) differ in males and females.

EXPERIMENTAL PROCEDURES

Neonatal treatments

Subjects were laboratory-bred male and female prairie voles, descendants of a wild stock originally captured near Champaign, IL, USA. Stock was systematically outbred. Animals were maintained on a 14 h light:10 h dark cycle and given food (Purina rabbit chow, Purina Mills, St. Louis, MO, USA) and water *ad libitum*. Breeding pairs were maintained in large polycarbonate cages (25×45×60 cm) and provided with cotton for nesting material. On PND21 offspring were removed and housed in same-sex sibling pairs in smaller (12×18×28 cm) cages. Sibling pairs were maintained in single-sex colony rooms. All studies were approved by the Animal Care and Use Committee of the University of Illinois at Chicago and complied with National Institutes of Health ethical guidelines as set forth in the Guide for Laboratory Animal Care. Every effort was made to minimize the number of animals used and their suffering.

Within 24 h of birth (PND1), test subjects randomly received either a single 1 mg/kg injection of OT (Bachem, San Carlos, CA, USA), a single 0.1 mg/kg injection of OTA, or were assigned to one of two control groups receiving either an injection of isotonic SAL, or handling without injection (HAN). The OT receptor antagonist ([d(CH_2)$_5$, Tyr(Me)^2, Orn^8]-vasotocin) (Bankowski et al., 1980) administered in this study also had been used in behavioral studies in this species, and is commercially available from Bachem. This antagonist is capable of affecting both OT and AVP V1a receptors, with a binding profile similar to Atosiban, the OTA most widely used to prevent premature labor (Bankowski et al., 1980; Manning et al., 1995). A lower dose of OTA than OT was used because in studies in rats, the OTA used here has been shown to be approximately 10–100 times more effective in receptor binding than the natural ligand (Barberis and Tribollet, 1996). In adult rodents, OT crossed the blood–brain-barrier (BBB) in small amounts (0.2–1.3%) when administered peripherally (Jones and Robinson, 1982; Ermisch et al., 1985; Banks and Kastin, 1985). The blood–brain-barrier of neonatal rodents should be more permeable than in adults (Vorbrodt, 1993). Finally, a study performed using these compounds and dosages in neonatal prairie voles showed specific and differential activations of c-Fos in various brain areas (Cushing et al., 2003); however, whether the consequences of neonatal OT or OTA were due to central or peripheral actions remains to be determined.

All injections were 50 μl in volume and administered intraperitoneally in 250 μl gas-tight Hamilton syringes. Infants were weighed and toe-clipped for identification on the day of birth. Although litters were not matched for pup number, infants were only used in the study if at least one control and one treatment animal of a given sex were available in the litter, and litters of more than six pups at birth were culled to six. Animals remained undisturbed in same-sex pairs until killing at 60 days of age, and were not used in any behavioral testing.

Receptor autoradiography

Following death, brains were quickly removed, flash-frozen on dry ice and stored at −80 °C. Brains were sectioned at 20 μm thickness, mounted onto Super-frost slides and stored at −80 °C until the time of assay. Sections were allowed to thaw to room temperature and then immersed in 0.1% paraformaldehyde for 2 min to optimize tissue integrity. Sections then were rinsed three times in 50 mM Tris–HCl (pH 7.4) at room temperature for 5 min and incubated for 60 min at room temperature in a solution of 50 mM Tris–HCl (pH 7.4) with 10 mM $MgCl_2$, 0.1% bovine serum albumin, and 50 pM of radiotracer. For OTR binding, [^{125}I]-ornithine vasotocin analog [(^{125}I)OVTA] was employed [vasotocin, d(CH_2)$_5$[Tyr(Me)^2, Thr^4, Orn^8, (^{125}I)Tyr^9-NH_2]; 2200 Ci/mmol] (NEN Nuclear, Boston, MA, USA). For V1a receptor binding, ^{125}I-lin-vasopressin [^{125}I-phenylacetyl-D-Tyr(ME)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2] (NEN Nu-