Oxytocin and Autistic Disorder: Alterations in Peptide Forms

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Background: *Oxytocin (OT) is synthesized as a prohormone that is sequentially processed to peptides. These peptides are the bioactive amidated form (OT) and the C-terminal extended peptides, OT-Gly, OT-Gly-Lys and OT-Gly-Lys-Arg, which are designated together as OT-X. As an extension of our previous study finding decreased plasma OT in autism, studies were conducted to determine whether there were changes in OT peptide forms in autistic children.*

Methods: *Twenty eight male subjects (97 ± 20 months; range, 70–139 months), diagnosed with DSM-IV autistic disorder through observation and semi-structured interview, were compared with 31 age-matched nonpsychiatric control subjects (106 ± 22 months; range, 74–140 months). Using OT antisera with different specificity for the peptide forms, we measured plasma OT and OT-X in each group.*

Results: T *tests showed that there was a decrease in plasma OT ($t = 4.4$, $p < .0001$), an increase in OT-X ($t = 2.3$, $p < .03$) and an increase in the ratio of OT-X/OT ($t = 4.5$, $p < .0001$) in the autistic sample, compared with control subjects.*

Conclusions: *The results suggest that children with autistic disorder show alterations in the endocrine OT system. Deficits in OT peptide processing in children with autism may be important in the development of this syndrome.* Biol Psychiatry 2001;50:609–613 © *2001 Society of Biological Psychiatry*

Key Words: Hypothalamus, peptide processing, behavior, pediatrics, neuroendocrinology

Introduction

Researchers have recently suggested that the social impairments found in autistic disorder are associated with changes in plasma oxytocin (OT) levels (Modahl et al 1998). A central OT system, projecting to limbic and other brain areas, has been implicated in maternal behavior, infant separation distress, sexual behavior, and other general aspects of affiliation in mammals (Insel 1992). Recent studies in an animal model that lack the ability to synthesize the hormone demonstrate changes in behavior, associated with emotionality and aggression (Lucot et al, 2000; Winslow et al., 2000). Based on the idea that social impairments in autism are primary symptoms (Fein et al 1996), it was suggested that this central OT system might be dysfunctional in this population (Modahl et al 1993; Waterhouse et al 1996). Our findings that plasma OT is lower in autistic children and has abnormal associations with sociality provide support for a possible role for OT in this disorder. The changes could reflect underlying alterations in the brain OT axis, alterations in peptide receptors, and/or peptide synthesis and processing.

Oxytocin is synthesized in neurons in the hypothalamus as part of a protein precursor, which is enzymatically cleaved and amidated as it is transported axonally (Gainer et al 1987). It is a product of the OT gene located at human gene locus 20p13 (Rao et al 1992). The processing cascade results in the production of neurophysin I and OT-extended form (OT-X), which is OT with a C-terminal, three-amino-acid extension (Gainer et al 1995). Oxytocin–extended form is further cleaved by enzymatic activity to yield the nine-amino-acid active peptide, OT. The proteolysis may involve several pro-hormone convertases, convertase 2 (PC2) (20p11-1-11.2) and convertase 5 (PC5) (9q21.3) (Gabreels et al 1998). Both enzymes are found in OT neurosecretory vesicles and are a part of a family of subtilisen/kexinlike convertases (Seidah et al 1994).

Alterations in OT prohormone processing are noted as a normal process of development. Studies in rats and sheep showed that processing of OT-X to OT occurs more completely as the fetus matures (Morris et al 1992; Whitnall et al 1985). Whitnall et al (1985) further found,

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Received September 12, 2000; revised March 2, 2001; accepted March 9, 2001.

0006-3223/01/$20.00
PII S0006-3223(01)01139-8

in examining the latency in OT processing compared with its structural relative, vasopressin, that the delay in OT-X conversion corresponded to delays in the appearance of OT in neurites. Altstein and Gainer (1988) determined that OT-X processing in the adult rat was high (over 99% peptide cleavage). In contrast, OT-X processing in the fetus was very low and incomplete, resulting in 40% unprocessed precursor and the accumulation of C-terminally extended nonamidated peptide forms (OT-Gly, OT-Gly-Lys, and OT-Gly-Lys-Arg). Morris and et al (1992) found that plasma levels of OT-X are elevated early in fetal sheep development and then show a decrease as levels of fully processed circulating OT begin to predominate later in gestation. Likewise, OT-X was present in human umbilical arterial and venous blood, with changes associated with labor and delivery (Mueller-Heubach et al 1995). It was suggested that the developmental change in OT processing may contribute to regulation of birth and fetal neural and endocrine development and are attributable to enzymatic activity.

Because our previous study showed that autistic children had lower levels of plasma OT (Modahl et al 1998), it was logical to explore the question as to whether there were alterations in the OT peptide forms.

Methods and Materials

Subjects

Data were derived from samples originating in the previous study (Modahl et al 1998). This study used 28 of the autistic subjects and 31 of the nonautistic, nonpsychiatric control males, between the ages of 6 and 11 years. Subjects with autism were recruited from autism schools and autism support groups in Massachusetts and Connecticut. An experienced child psychologist (DF) arrived at DSM-IV diagnoses for all subjects through interview using the Autistic Disorders Checklist (Rapin 1996) and behavioral observation. All subjects met full criteria for DSM-IV diagnosis of autistic disorder. Diagnoses were confirmed by an experienced child psychiatrist (CF) by case review. Control subjects were solicited through newspaper advertisements. All subjects and their families were informed of the procedures (i.e., venipuncture and psychological screening) and their purpose through IRB-approved information, both verbally and in writing. Informed consent was obtained from all non-autistic children directly; when direct informed consent was not possible with autistic subjects, consent was obtained from their parents. Subjects were group-matched for chronological age: mean age of the autistic group was 8.1 years versus 8.8 years for the control group (Table 1). Control subjects were screened for psychiatric conditions and potentially confounding medical conditions through parent interview. Female subjects were excluded because of the low incidence of autism in girls and the resultant difficulty in obtaining a gender-balanced sample.

Table 1. Sample Characteristics of Autistic and Normal Children

	Autistic (n = 28)	Control (n = 31)
Age (months)		
Mean	97	106
SD	20	22
Range	70–139	74–140
Stanford-Binet visual abstract reasoning prorated standard area scores		
Mean	61	105
SD	22	13
Range	36–99	80–139
PPVT-R standard scores		
Mean	54	111
SD	22	15
Range	29–99	86–143
Vineland standard scores		
Communication		
Mean	59	105
SD	16	10
Range	36–86	82–126
Socialization		
Mean	57	98
SD	10	14
Range	38–80	71–118
Daily living		
Mean	45	100
SD	14	11
Range	20–72	74–133

PPVT-R, Peabody Picture Vocabulary Test-Revised.

Procedure

Details of the testing procedure were provided in a previous publication (Modahl et al 1998). Briefly, after a rest and fasting period, a blood sample was taken, and a brief cognitive assessment was made. Ten mL of blood was collected into heparinized tubes, iced until processing within 15 min. Samples were centrifuged at 4°C at 3000 *g* for 20 min. The plasma was divided into 1 mL aliquots and stored at −70°C until shipment, on dry ice, to the Immunoassay Laboratory (MM). Autistic subjects were tested at Boston Children's Hospital, Boston, MA. Control subjects were tested at the University of Connecticut Health Center (Farmington, CT), Newington Children's Hospital (Newington, CT) or Boston Children's Hospital (Boston, MA).

The peptide radioimmunoassays (RIAs) for OT and OT-X were performed according to previously established techniques (Morris et al 1992). Plasma samples were thawed on ice, plasma proteins were precipitated with acetone, and the supernatant was extracted with petroleum ether. After lyophylization, the extracts were resuspended in assay buffer with duplicate measurements for both assays. ^{125}I oxytocin was purchased from Dupont, Inc. (Boston, MA). A nonequilibrium assay was used with an incubation volume of 500 μL and an incubation time of 4 days at 4°C. The OT antiserum, developed by Morris and colleagues, is specific for the amidated peptide. There is no cross-reactivity with vasopressin or other related peptides. The OT-X antiserum (VA-17) was supplied by Harold Gainer (National Institutes of

Health, Bethesda, MD). It cross-reacts with amidated OT as well as the three extended OT peptide forms (OT-G, OT-GK, and OT-GKR) (Altstein and Gainer 1988). The results were calculated using logit transformation of the data. Comparisons were made between OT, OT-X, and the ratio of OT-X/OT.

Behavioral and cognitive data were collected from all subjects using the following methods: 1) brief questionnaire regarding variables that could affect the measurement of peptide levels, such as medications and recent food intake; 2) Vineland Adaptive Behavior Scales (Sparrow et al 1984); 3) Stanford Binet fourth edition Copying and Pattern Analysis subtests (Thorndike et al 1986); and 4) Peabody Picture Vocabulary Test (PPVT; Dunn and Dunn 1981). Parents of subjects with autism were additionally interviewed using the Autistic Disorders Checklist (Rapin 1996) and an adaptation based on DSM-IV criteria. The Autistic Disorders Checklist (Rapin 1996) was adapted by Lorna Wing from DSM-III-R criteria and surveys a broad range of autistic symptoms in the DSM domains of social behavior, communication, and restricted repertoire through semi-structured interview.

Results

Statistical Methods

Group differences were examined using *t* tests. Associations between peptide levels and behavioral, cognitive, and physiologic variables were examined with Pearson correlations.

Group Characteristics

Descriptive statistics for background variables are provided in Table 1. The groups are well matched for chronological age. As expected, the control group showed superior performance in nonverbal intelligence quotient (IQ), verbal IQ, and Vineland Communication, Socialization, and Daily Living scores, compared to the autistic group.

Plasma OT and OT-X

Distributions of OT and of OT-X were overlapping for the autistic and control groups; however, there was a higher proportion of nondetectable levels for OT in the autistic group and a higher proportion of nondetectable levels for OT-X in the control group. Plasma levels of OT were lower in the autistic group (.8 ± .09) than in the control group (1.4 ± .10) (Figure 1; $t = 4.4$, $p < .0001$) whereas OT-X levels were higher (autistic 2.9 ± .29; control 2.0 ± .22; $t = 2.3$, $p < .03$). The ratio between OT-X and OT was much higher in the autistic group (4.7 ± .59) compared to the control group (1.8 ± .25; $t = 4.5$, $p < .0001$), with a more than two-fold difference.

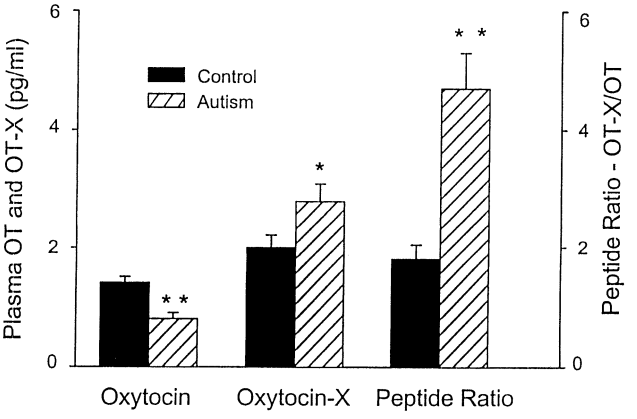

Figure 1. Mean plasma oxytocin (OT), OT-extended form (OT-X), and peptide ratio values in control ($n = 31$) and autistic ($n = 28$) children. * $p < .03$, ** $p < .0001$ (*t* tests; control vs. autistic).

Medication History

Ten of the autistic subjects were taking a variety of medications (including combinations), and their OT-X values were individually examined for potential associated differences. Of three children taking clonidine, one child (A) was .5 SD below the autistic mean, and two (B, C) were at the mean. One child (C) also taking divalproex sodium (Depakote) was at the mean, one (A) also taking carbamazepine (Tegretol) was .5 SD below, and one (G) on phenytoin (Dilantin) alone was at the mean. Three children were taking methylphenidate hydrochloride (Ritalin): one was .5 below the mean (D), and two were about 1 SD below the mean (E, F). One child taking a form of lithium (H) was less than .5 SD below the mean. One child taking amitriptyline was about .5 SD below the mean. An additional child (J), on a combination of clonidine, fluoxetine, Depakote and haloperidol (Haldol), was 1.6 SD above the mean. Just over half of the medicated subjects had OT-X values below the mean; however, *t* tests showed no significant differences in OT-X between these subjects and nonmedicated autistic subjects. The only pattern qualitatively observed was for Ritalin, showing a tendency for slightly lower OT-X values; however, no consistent effects were shown by Ritalin on OT in the previous study. In general, findings for the group are consistent with previous findings (Modahl et al 1998), which showed no consistent or specific effects of medication on OT.

Location Effects

Location effects were also examined. *T* tests revealed no significant difference in OT-X values between control subjects drawn in Boston (1.7 ± .29) and control subjects drawn in Connecticut (2.4 ± .34). The significant intergroup difference in OT-X was preserved when looking at

the Boston subgroup of control subjects verses the autistic subjects, also drawn at the same location (control 1.7 ± .29; autistic 2.9 ± .29; $t = 2.5$, $p < .01$).

Correlation between Peptide Levels and Behavior

All correlations reported are at or above the $p < .05$ level. As reported previously by Modahl et al (1998), age was positively correlated with OT in the normal group but not in the autistic group. Conversely, OT-X increased with age only in the autistic group ($r = .50$, $p < .01$).

As reported previously (Modahl et al 1998), OT was positively associated with level of daily living skills and interpersonal relations (Vineland Adaptive Behavior Scale scores) in the normal group but negatively associated with these variables in the autistic group. In the current study, OT-X was not significantly correlated with any of the Vineland domains or intellectual scores in either group. Whereas the previous study showed several correlations between OT and specific autistic symptoms, in the present study OT-X was correlated positively with an Autistic Disorders Checklist (ADC) item regarding presence of stereotypies ($r = .43$, $p < .05$) and negatively with an ADC item regarding abnormalities in comfort-giving ($r = -.40$, $p < .05$) within the autistic group.

Discussion

Our findings suggest that there are changes in brain oxytocin processing in autistic children. This was seen as reciprocal changes in the fully processed, amidated OT peptide as compared to the immature C-terminal extended forms. Plasma OT levels were reduced in autistic children, whereas OT-X levels were elevated, resulting in a more than two-fold difference in the peptide ratios. Taken with previous findings (Modahl et al 1998), OT-X showed few associations with physiologic and behavioral variables compared with OT. Whereas OT showed an age-related rise in control children, OT-X failed to show this pattern. In contrast, there was an age-related rise in OT-X levels in the autistic group. The failure to show an age-related decline in OT-X in control subjects may be an artifact of restricted variability. Plasma OT-X levels in this group for a majority of subjects were nondetectable (0), which would restrict correlational findings. It is also possible that developmental declines in OT-X may not be captured within the age range sampled for the present study; rather, declines may occur much earlier in childhood.

Some autistic subjects were taking psychotropic medications, and although no robust effects on OT or OT-X were found, this could present a potential limitation to interpretation of peptide findings. In addition, subjects were diagnosed using standard research practices of the time of this study, when instruments currently used for research were not available. Thus, it is possible that if current instruments, such as the Autism Diagnostic Interview (ADI), were employed, there might result a slightly different sample composition in the autistic group.

Although plasma measurements are not a direct index of central peptidergic function, they may reflect alterations in gene expression and prohormone/peptide production. The increase in the C-terminal OT peptides in the autistic group suggests incomplete processing of the OT prohormone. The amidated bioactive peptide is the result of a cascade of proteolytic processing, the last steps being the removal of the terminal Gly-Lys-Arg (Gainer et al 1987). In autistic children, the age-related rise in OT-X, in the absence of the normal rise in OT, suggests a failure of developmental progression in peptide processing. Indeed, there is much evidence to suggest that there are developmental changes in neuropeptide processing in animals and humans. Higher levels of OT-X were observed in the fetal brain and circulation (Altstein and Gainer 1988; Morris et al 1992). In humans there are high levels of OT-X in the fetal circulation and increased levels of an altered OT form in response to steroid stimulation (Amico et al 1985; Mueller-Heubach et al 1995).

The findings for the control group suggest that the metabolism of OT may involve the turnover of nearly all available OT-X, yielding OT-X measurements at very low or nondetectable levels. In comparison, data from the autistic group suggest that some of these children fail to completely process prohormone OT, with ratios of over 10:1 (OT-X/OT). This finding suggests that the immature OT forms serve as the primary circulating molecule in the absence of, and perhaps in compensation for, OT. However, there is little information on the functional role of OT-X. Indeed, binding and functional studies show that OT-X is not an effective agonist at OT sensitive sites (Mitchell et al 1998).

Deficiencies in conversion of OT-X to OT in autism is likely the result of alterations in the level or activity of several candidate OT-X/OT pro-hormone convertases, such as PC2 and PC5; however, the primary cause is probably the result of genetic defects. Indeed, there is evidence that in genetic syndromes such as Prader-Willi (15q13–14) there are central neuroendocrine changes, seen as a decrease in the number and volume of OT neurons (Swaab et al 1995). Interestingly, pro-hormone convertase 2 (PC2) (20p11–1-11.2) is dependent on a polypeptide (7B2) that shares the chromosomal site for Prader-Willi Syndrome (Braks and Martens 1994; Gabreels et al 1998). Chromosomal abnormalities on this site have also been implicated in many case studies of autistic individuals (Cook 1998; Szatmari et al 1998). Furthermore, the PC2 gene (20p11–1-11.2) is proximal to the oxytocin gene

(20p13). It is possible that faulty OT-X/OT processing could result from defects in the area of 20p11–13 affecting PC2 and the oxytocin gene itself. Given growing support in the literature that autism is a disorder of genetic origin (Szatmari 1998), replication of the plasma OT-X/OT abnormality and examination of the genes regulating oxytocin and OT-X/OT convertases in autistic individuals comprise the next step.

This study was supported by grants from the March of Dimes (DF) and National Institutes of Health HL43178 (MM).

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