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Research Article

Journal of Life Science and Biotechnology ISSN: 2456-1061

Decreased Oxytocin Associated with high mTOR in Individuals with Autism

A.J. Russo, PhD, Lakshmi Anubrolu, M.D.

Visiting Assistant Professor of Biology Hartwick College Oneonta, NY 13820

Research Director Pfeiffer Medical Center Health Research Institute Warrenville, Il 60555

Medical Director Pfeiffer Medical Center Warrenville, Il 60555

Correspondence

Russoa2@hartwick.edu

ARTICLE INFO

corresponding Author:
A.J. Russo, PhD

ABSTRACT

Abstract

Background: Autism Spectrum Conditions (ASC) are a group of neurodevelopmental conditions characterized by difficulties in social interaction and communication, alongside unusually repetitive behaviors and narrow interests. The neuropeptide oxytocin (OXT) has been implicated in the pathophysiology of behavioral deficits among patients with autism spectrum disorder (ASD).

Objectives: To test the hypothesis that decreased oxytocin levels are associated with high mTOR levels in individuals with autism.

Methods: We used ELISAs to measure plasma oxytocin and cellular phosphorylated mTOR in individuals with autism and age/gender similar controls, including mothers of autistic children, and non autistic siblings.

Results: We found that mean oxytocin levels of individuals with autism were significantly lower than oxytocin levels of mothers of autistic children and neurotypical, age and gender similar, controls. We also found that phosphorylated mTOR was significantly higher, and low oxytocin correlated significantly with high those high levels in the same autistic group.

Conclusions: This data supports the possibility that reduced oxtocin levels in individuals with autism results in high mTOR. High mTOR, in turn, may result in high protein translation in these patients, which is associated with changes in behavior.

KEYWORDS *Major bleeding, Net clinical outcomes, MACE, Bivalirudin, Heparin*

Introduction

Autism Spectrum Disorder (ASD) are a group of neurodevelopmental conditions characterized by difficulties in social interaction and communication, including unusually repetitive behaviors and narrow interests (1). The prevalence of ASC in the general population is around 1% [2], with a male:female sex ratio of 4:1 in classic autism [3], increasing to as high as 9:1 in AS [4]. ASC are highly heritable, as indicated in three different twin studies [5-7].

The neuropeptide oxytocin (OXT), a natural brain peptide produced in the hypothalamus, has been implicated in the pathophysiology, and possibly the etiology, of behavioral deficits among patients with autism spectrum disorder (ASD) (8), and there is a significant association of a sequence variant in the *OXTR* gene with Asperger's (9). However, the molecular mechanisms underlying the role of OXT in ASD remain unclear. Recent studies have shown that OXT plays an important role in ameliorating behavioral deficits in an ASD-like mouse model, mediated by inhibiting the ERK signaling pathway and its downstream proteins (10). Acute intranasal oxytocin temporarily enhances social cognition, empathy, and reciprocity in individuals with ASD (11). However, recent clinical trials have yielded mixed results, leaving the field questioning whether oxytocin can live up to the hype.

OXT increases the salience of social stimuli and promotes parental nurturing and social bonds, and therefore, has received considerable attention as a potential treatment for social deficits in ASD. Recent studies in mice have demonstrated that a mutation in *Cntnap2* (the gene that encodes contactin-associated protein-like 2), which, in humans, may result in ASD, displays robust social deficits and reduced amounts of brain OXT. In this model, daily intranasal OXT treatment over development improved later social engagement (13).

Acute intranasal OXT temporarily enhances social cognition, empathy, and reciprocity in individuals with ASD (11). In a recent small double blind study, those taking OXT spray therapy had significantly reduced autism core symptoms specific to social reciprocity (12), However, recent clinical trials have yielded mixed results (11, 26).

Inconsistent success of oxytocin treatment may be related to a possible genetic link associated with oxytocin deficiency. If so, knowledge of this association could provide a way to provide effective therapy to a more specific susceptible group of individuals with ASD.

Mammalian target of rapamycin complex-1 (mTORC1) is important in protein synthesis through its modulation of ribosomal biogenesis (14), cell proliferation and cell size (15) by way of sensing nutrient sufficiency signals (16) and cellular responses to stressors (17).

Recent studies suggest that, in addition to its role in the brain, OXT may also have an important neuromodulatory role in the gut by activating the phosphoinositide 3-kinase (PI3K)/Akt pathway in a dose and time-dependent manner (18). This pathway has mTOR downstream.

This study was designed to measure OXT levels in autistic children, mothers of autistic children and non-autistic siblings. It also searched for relationships between OXT and biomarkers of the Akt/mTOR pathway,

as well as other related biomarkers. These results may help elucidate subpopulations of autistic children who may benefit most by oxytocin therapy.

Materials and Methods

Subjects

We used ELISAs to measure plasma oxytocin and cellular mTOR in individuals with autism (N=85, mean age 7.2 years; 68 males) and age/gender similar controls (N=55, mean age 7.5 years; 44 males).

The diagnostic criteria used in this study were defined by DSM-IV criteria. In 2012, the separate diagnostic labels of Autistic Disorder, Asperger's Disorder, and Pervasive Developmental Disorder-not otherwise specified (PDD-NOS) were replaced by one umbrella termed "Autism Spectrum Disorder".

Plasma and white blood cells from consecutive individuals with diagnosed autism and controls were obtained from patients presenting at the Health Research Institute (HRI) * over a two year period. All autistic individuals who presented to HRI were asked to participate. Plasma and cells from patients who participated in this study were randomly chosen from all patients who volunteered. The autistic individuals in this study met the DSM-IV criteria and many were diagnosed using the Autism Diagnostic Interview-Revised - ADI-R before presenting to the HRI.

Patient consent was obtained from all patients involved in this study and the study was approved by the IRB of the HRI.

ELISAs used to measure plasma oxytocin (mybiosource, San Diego, CA)

1. All reagents and samples were brought to room temperature (18°C-25°C) naturally for 30min before starting assay procedures.

2. 50µl of standards, and samples were placed in the appropriate wells.

3. 100µl of HRP-conjugate anti-0xytocin IgG was added to each well, the plate was covered and incubated for for 60 minutes at 37°C.

4. The plate was washed 4 times. Each well received 300 µl of wash solution.

5. 50 µl Chromogen Solution A and 50µl Chromogen Solution B was added to each well. The plate was Gently mixed and then protected from light and incubated for 15 minutes at 37°C.

6. 50µl Stop Solution was added to each well.

7. The plate was read at 450 nm using an ELISA reader within 15 minutes after adding Stop Solution.

ELISAs used to measure cellular mTOR (eBiosciences, San Diego, CA).

1. 50 µL/well of 1X Cell Lysis Mix (negative control) and 50 µL/well Positive
Control Cell Lysate (positive control) to separate assay wells for controls.
2. 40 µl of lysis buffer (contains a combination of detergents, phosphatase inhibitors, salts and buffers) was added to each of the control and experimental wells.
3. 10 µl of buffy coat cells (experimental and controls) were added to appropriate wells and mixed gently.
2. 50 µL/well of Antibody Cocktail mix (detection antibody and HRP conjugated antibody) was added to all the assay test wells. The plate was incubated for 1 hr at room temperature on a microplate shaker (~300 rpm).
3. Wells were washed with 300 µL/well 1X Wash Buffer 4 times.
4. 100 µL of Detection Reagent (TMB) was added to each well and the wells were incubated for 10-30 minutes.
5. After color development, 100 µL of Stop Solution was added to each well.
6. Absorbance was measured using a colorimetric (spectrophotometric) plate reader (BioRad) set at 450 nm.

To ensure reproducibility of results, samples were run in duplicate and reported concentrations were the result of the average of at least two separate assays.

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Plasma and buffy coat (white blood cells)

Plasma and buffy coat cells, obtained from the patients at the Health Research Institute, were treated in an identical fashion - frozen at -70C immediately after collection and cell/serum separation, then stored at -70C until thawed for use in ELISAs.

Results

We used ELISAs to measure plasma OXT, as well as other biomarkers, in individuals with autism (N=85) and age/gender similar controls (N=55),

We found that mean OXT levels of individuals with autism (N=85) were significantly lower than OXT levels of controls (N=55) ($p = 2.19E-6$) (Figure 1).

We also found that phosphorylated mTOR was significantly higher in individuals with autism, compared to neurotypical, age and gender similar controls (p=0.0006) (Figure 2).

We also found that low OXT correlated significantly with high phosphorylated mTOR (r=-0.5; p=0.04).

Discussion

The PI3K/AKT/mTOR signaling pathway plays an important role in the regulation of cell growth, proliferation, differentiation, motility, survival, metabolism and protein synthesis. AKT, a serine/threonine-specific protein kinase, plays a fundamental role in cell survival and apoptosis (19). Activated AKT can phosphorylate a series of downstream signaling molecules, including mammalian target of rapamycin (mTOR, which participates in various aspects of signaling, including nutrition (such as amino acids), growth factors (such as insulin), energy levels and environmental pressures (such as hypoxia) (20).

Recent research demonstrates that oxytocin modulates mTOR activity in the gut by slowing protein translation in the gut. The abundance and phosphorylation of mTORC1 substrates is also reduced with OXT, and
(21).

OXT can reduce inflammatory and cellular oxidative stress (22,23) and, more specifically, when combined with secretin, OXT reduced rat colonic inflammation (24). This suggests that, in addition to its role in the brain, OXT may also have an important neuromodulatory role in the gut. Specifically, in the gut, OXT activates the phosphoinositide 3-kinase (PI3K)/Akt pathway in a dose and time-dependent manner in Caco2BB cells (*in vitro* model of enterocytes) (25).

Our data shows that OXT levels are significantly reduced in autistic children compared to neurotypical age and gender similar non-autistic siblings and that these levels correlate significantly with high mTOR levels. This supports the possibility that reduced OXT levels in individuals with autism results in high mTOR. High mTOR, in turn, may result in high protein translation in these patients which is associated with changes in behavior.

This data suggests that OXT therapy may be most affective in the population of individuals who have high mTOR. It also suggests that in individuals with autism who don't respond to OXT therapy, alternative mTOR inhibitors might be affective.

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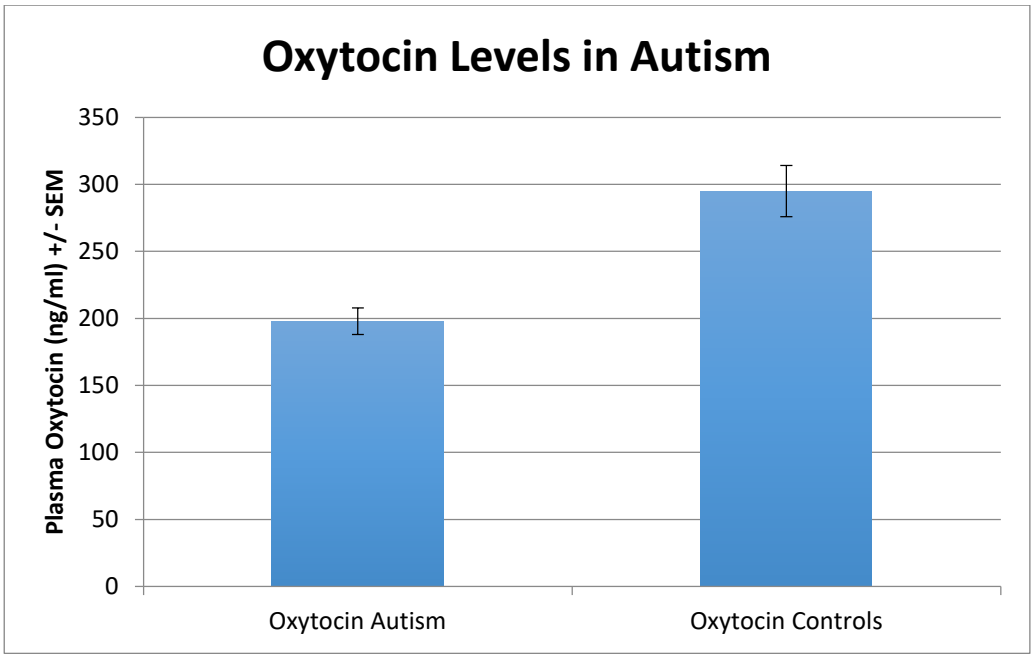

Figure 1. OXT levels of individuals with autism were significantly lower than OXT levels of controls (p = 2.19E-6).

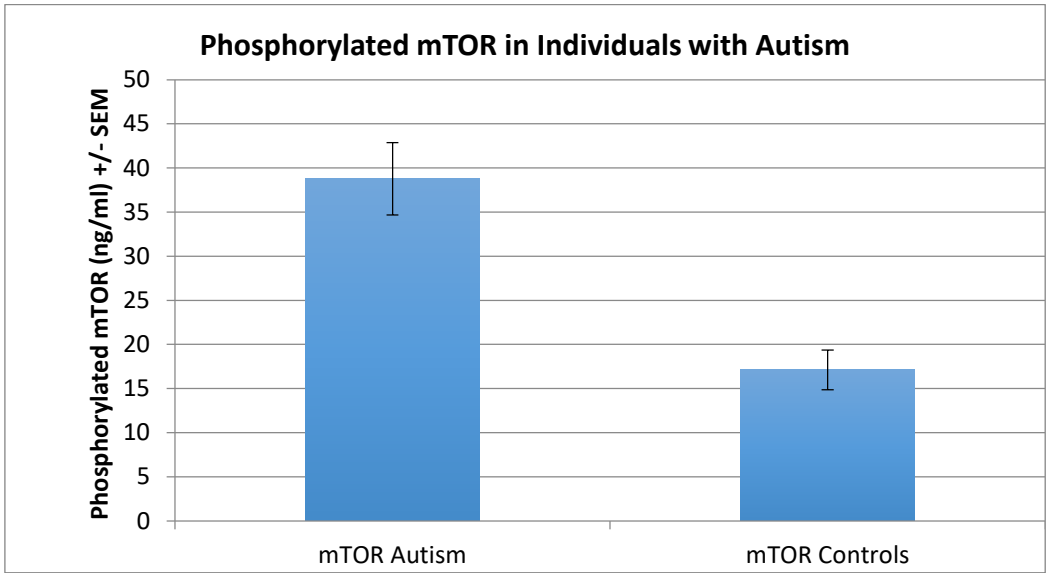

Figure 2. Phosphorylated mTOR was significantly higher in individuals with autism, compared to neurotypical, age and gender similar controls (p=0.0006).