PRELIMINARY INSIGHTS IN OXYTOCIN ASSOCIATION WITH THE ONSET OF DIABETIC NEUROPATHY

L. Kobylinska^{1,2}, M.A. Ghita³, C. Caruntu^{3,6}, G. Gabreanu⁷, C.P. Tataru^{4,*}, S.V. Badescu², O. Geicu⁸, M. Neagu⁷, C. Constantin⁷, I. Dobrescu^{1,5}, L. Zagrean²

¹“Prof. Dr. Al. Obregia” Clinical Psychiatry Hospital - Child and Adolescent Psychiatry Department, “Carol Davila” University of Medicine and Pharmacy - ²Physiology and Fundamental Neuroscience Department - ³Dermatology Research Laboratory - ⁴Division of Ophthalmology - ⁵Child and Adolescent Psychiatry Department, ⁶“Prof. N. Paulescu” National Institute of Diabetes, Nutrition and Metabolic Diseases - Dermatology, ⁷“Victor Babes” National Institute of Pathology - Immunology Department, ⁸University of Bucharest, Faculty of Biology - Biochemistry and Molecular Biology Department, Bucharest, Romania

Abstract

Diabetes is one of the most prevalent chronic disorders, associating numerous somatic and behavioral modifications. Oxytocin has been widely studied for its involvement in social behavior and psychiatric disorders.

This pilot study presents a series of 3 patients with type 1 diabetes and diabetic neuropathy in which the values of plasma oxytocin, neurotensin, β -endorphins, α -MSH, substance P and orexin A were measured in comparison to 3 healthy controls with matching ages.

In the diabetic patients group, there was a strong negative correlation between the value of plasma glucose and oxytocin ($r=-0.99$, $p=0.04$), respectively neurotensin ($r=-0.99$, $p=0.03$). These values did not correlate in the control group.

The results suggest that oxytocin, in conjunction with neurotensin, could be investigated as a potential early detection marker of diabetic neuropathy and, to our knowledge, this is the first report focusing on plasma oxytocin levels in patients with diabetic neuropathy.

Key words: oxytocin, diabetic neuropathy, type 1 diabetes.

INTRODUCTION

Diabetes is one of the most prevalent chronic disorders, with European incidence ranging from 2.4% to 14.9%, as reported in 2014 (1). The associated complications, such as diabetic neuropathy, nephropathy, retinopathy and vasculopathy are causes of significant disability and subsequent individual and healthcare system burdens. The higher the degree of invalidity, the higher the ratio of associated

psychiatric diagnoses from the depression spectrum, which further increase the functional impairments and worsen the prognosis (2, 3). Moreover, the comorbid depression and anxiety tend to be overlooked by the primary care physician in the case of patients with diabetes, with the severity of the psychiatric condition determining the referral towards a mental health professional (4). However, the psychiatric pathologies are long-term complications of diabetes, and it is still questionable whether the etiology resides in the endothelial dysfunction (5) associated with the long-term evolution of the disorder, or there may be some other underlying cause. Given the observation that sub-clinical behavioral changes are associated with diabetic neuropathy, raises the question whether there could be early-on plasma markers that might hint towards the development of diabetic neuropathy.

One of the most studied substances in the past decade for its known involvement in modulating behavior, particularly social behavior, is oxytocin. This neuropeptide decreases amygdala activation and the stress response associated with the perception of social threatening (6-9). Furthermore, it enhances the exactness in reading other people's emotions (10) and it increases eye contact (11). Some of the pro-social effects might be due to a decrease in social anxiety, secondary to an increment of peer trust and cooperating behavior (12-14). Several psychiatric disorders - such as schizophrenia (15), autism spectrum disorders (16), anxiety (17) post-partum depression (18) and obsessive-compulsive disorder (19) – can be associated with deregulations in the oxytocinergic system. Except for the central effects, oxytocin has various peripheral

*Correspondence to: Calin Petru Tataru MD, “Carol Davila” University of Medicine and Pharmacy, Division of Ophthalmology, Alexandru Lahovari Square no 1, Bucharest, 010464, Romania, E-mail: calinpetrutataru@yahoo.com

actions, among which a strong involvement in glucose metabolism (20). It has an insulin-like activity in the adipose tissue, increasing glucose oxidation and lipogenesis (21). Along with its structural analogue, vasopressin, it increases insulin release from the pancreas (22). The plasma level of oxytocin increases in hypoglycemia (23). This phenomenon is also observed in patients with type 1 diabetes without diabetic neuropathy, following insulin-induced hypoglycemia (24). Patients with type 2 diabetes have been reported to have lower oxytocin levels than patients with no alterations in glucose tolerance (25). Recent results have shown that chronic oxytocin administration increases the plasma glucose level in obese diabetic mice, while inducing weight-loss (26). Even if animal studies in the field have been conducted in several species (27), human studies are still scarce (28).

OBJECTIVE

In this context, the aim of the current pilot study to measure the values of plasma oxytocin, in young patients with type 1 diabetes, complicated with diabetic neuropathy in order to verify the potential of oxytocin as a biomarker in diagnosing diabetic neuropathy. The hypothesis of testing possible plasma markers for early diagnosis of diabetic complications was confirmed by recent results showing that plasma gangliosides could be an early-detection marker for diabetic nephropathy (29).

Besides oxytocin, β -Endorphin and substance P have also been determined, given their involvement in neuro-inflammation and pain perception (30, 31). α -MSH, in conjunction with β -Endorphin gives a glimpse into the proopiomelanochortin production; whereas orexin-A is well known for its involvement in appetite regulation, circadian entrainment and behavior (32). Neurotensin was also checked because of its involvement in central thermoregulation (33). Diabetic neuropathy associates sensory and motor deficits; therefore it is probable to have alterations in the levels of these neuropeptides. Orexin A was also dosed because of the relative cachexia induced by long-term fluctuating insulin-deficit.

The goal of the study was to verify potential trends of statistical significance in terms of correlations between the plasma levels of the measured neuropeptides and those of plasma glucose, as well as to check whether there are any trends of difference between the patients with diabetic neuropathy and those without in the values of the measured plasma neuropeptides.

MATERIALS AND METHODS

Subjects and Sample collection

Blood was drawn from 3 patients (2 females, both 28 years old, and one male, 30 years old) with type 1 diabetes and diabetic neuropathy and 3 healthy controls (2 females, both 28 years old, and one male, 33 years old). The blood was collected on EDTA vacutainers from an anterior cubital vein. The study was approved by the local ethics committee and written informed consent was obtained from all participants after receiving complete information about the study. All the patients had a diagnosis of type I diabetes and were under chronic insulin treatment. The presence of diabetic neuropathy was assessed through clinical and electrophysiological examination.

Sample processing

EMD Millipore's MILLIPLEX MAP Human Neuropeptide Magnetic Bead Panel was used to determine the plasma levels of α -Melanocyte Stimulating Hormone, β -Endorphin, Neurotensin, Orexin A, Oxytocin and Substance P.

Serum samples were treated with acetonitrile in proportion of 1:1.5 and the supernatant obtained after centrifugation was dried over-night using a High Speed Vacuum. Dried samples were stored at -20°C . Prior to beginning the immunoassay, samples were reconstituted with the Assay Buffer provided by the kit and pre-incubated in 96-well plate for 10 minutes. Samples were labeled with the primary antibodies and incubated on a plate shaker for 2 hours at room temperature. Mixed beads (anti- α -MSH; anti- β -Endorphin; anti-neurotensin; anti-Orexin A; anti-Oxytocin; anti-Substance P) were added and then incubated over night at $2-8^{\circ}\text{C}$. The beads capture the neuropeptide of interest and the complexes are recognized by a biotinylated detection antibody. Streptavidin-Phycoerythrin was used for the completion of reaction. The data was acquired and analyzed using the LUMINEX xMap technology MAGPIX.

Standard biochemical analysis of the serum was performed (using colorimetric, enzymatic and ion-selective electric potentiometry) from the fresh, serum in order to determine glycemia, serum cholesterol, liver enzymes (ALT, AST), triglycerides, creatinine, urea, uric acid, albumin and total proteins.

Statistical analysis

Microsoft Office Excel® and SPSS 22.0 were employed for the analysis. Data were compared using

unpaired t-test and p-values <0.05 were considered to display a trend of statistical significance. All the p-values are two-tailed. For Levene's test p values >0.05, equal variances were assumed for the t-test. Pearson's two-way correlation test was used in order to check for possible associations between the data. The results are presented as Mean±standard deviation.

RESULTS

The glucose levels of diabetic patients (299.76±49.7 mg/dL) were increased compared to controls (95.3±4.03 mg/dL) (p=0.01). There were no differences between the values of the measured neuropeptides between the diabetic patients and healthy controls (Table 1).

However, the male subjects had higher values for most of the measured neuropeptides than the females (Table 2).

In the diabetic patients group, there was a

strong negative correlation between the value of plasma glucose and oxytocin (r=-0.99, p=0.04) (Fig. 1), respectively neurotensin (r=-0.99, p=0.03) (Fig. 2). These values did not correlate in the group of non-diabetic patients.

DISCUSSION

Recently, the attention in the field of type 1 diabetes research has started to focus on pre-autoimmune seroconversion biomarkers as potential target for a "preventive treatment" approach (34). This idea could be translated into searching for appropriate predictors of diabetic complications. This is a pilot study, the results suggest that oxytocin, in conjunction with neurotensin, could be investigated as a potential early detection marker of diabetic neuropathy. As the study group is small, we cautiously regard the results as trends of potential significance. By this means, we propose the continuation of the investigations, in order

Table 1. The Mean values of the serum neuropeptides and t-test results. st. dev= standard deviation of the mean, df=degrees of freedom

	Group	Serum values (pg/mL)		Levene's Test for Equality of variances		t-test for Equality of means			95% Confidence Interval of the Difference	
		Average	St. dev	F	p	t	df	P	Lower	Upper
α -MSH	control	70.6	27.3	0.1	0.69	-0.31	4	0.76	-77.3	61.4
	diabetes	78.6	33.5							
β -endorphins	control	342.6	110.6	12.9	0.03	-0.13	1.18	0.91	-732.4	710.1
	diabetes	353.8	41							
Neurotensin	control	93.7	21.6	0	0.99	-0.46	4	0.66	-58.4	41.5
	diabetes	102.1	22.4							
Orexin A	control	819.6	88.5	1.2	0.33	0.15	4	0.88	-287.9	322.1
	diabetes	802.5	168.4							
Oxytocin	control	239.5	42.4	0.1	0.75	-0.31	4	0.76	-124.9	99.5
	diabetes	252.2	55.6							
Substance P	control	23.1	4.9	0.01	0.89	0.08	4	0.93	-11.2	11.8
	diabetes	22.7	5.2							

Table 2. The Mean values of the serum neuropeptides in males and females and the results of the t-test. st. dev= standard deviation of the mean, df=degrees of freedom

	Sex	Serum values (pg/mL)		Levene's Test for Equality of variances		t-test for Equality of means			95% Confidence Interval of the Difference	
		Average	St. dev	F	p	t	df	p	Lower	Upper
α -MSH	female	60.2	10.3	9.1	0.03	-3.6	3.9	0.02	-76.1	-10.3
	male	103.4	5.7							
β -endorphins	female	308.9	23.3	2	0.25	-3.2	3	0.04	-200.3	-1.6
	male	409.9	10.8							
Neurotensin	female	87.5	7.3	0.8	0.4	-2.5	4	0.06	-64.4	2.4
	male	118.5	7.9							
Orexin A	female	740.5	60.4	0.2	0.6	-4.2	4	0.01	-349.3	-74.1
	male	952.2	46							
Oxytocin	female	219.9	11.9	1.5	0.2	-4	4	0.01	-131.3	-24.2
	male	297.7	11.5							
Substance P	female	20.4	1.5	16.8	.01	-3.1	4	0.03	-14	-0.9
	male	27.9	0.3

Figure 1. Correlation trend between the levels of plasma oxytocin and plasma glucose in the patients with diabetic neuropathy ($r=-0.99$, $p=0.04$).

Figure 2. Correlation trend between the levels of plasma neurotensin and plasma glucose in the patients with diabetic neuropathy ($r=-0.99$, $p=0.03$).

to verify these trends. The difference in results between men and women raises the need to divide the study cohort according to the gender of the patients, in order to normalize for the potential differences induced by the physiological variations in neuropeptides levels.

Given the direct relationship between oxytocin level and plasma glucose level and the fact that oxytocin specifically increases in hypoglycemic conditions, the decrease in oxytocin level according to the high plasma glucose levels could represent an early sign of defectively managed glycemic values in diabetic patients. Our results corroborate with the ones previously reported (23, 24, 27), with negative correlates between the plasma level of oxytocin and that of the glycemia.

To our knowledge, based on an extensive literature review, this is the first paper to report plasma oxytocin levels in patients with diabetic neuropathy. Moreover, this is one of the first studies in our country dealing with plasma oxytocin levels in humans. Most of the studies conducted so far in Romania have dealt either with the oxytocin administration in human subjects (35), or with the study of its implications in animal behaviour (17,36) and physiology (37).

The observation that there seems to be a trend of reverse correlation between the levels of oxytocin in the plasma and the values of glycemia substantiates the mood changes that occur in long-evolving diabetes with comorbid vascular and neuronal. In this sense, the question remains: could this be one of the underlying mechanisms of the observed behavioral and mood disorders in diabetic patients?

A recent study has shown a similar trend of correlation of plasma oxytocin levels and plasma

glucose levels in diabetic (type 2) patients with metabolic syndrome (38). This was observed in both diabetic (type 2) as well as non-diabetic patients with metabolic syndrome.

In conclusion, based on the trends revealed by our pilot study, oxytocin could be investigated as a potential early marker for the onset of diabetic neuropathy.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

Conflict of interest

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