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PERIPHERAL NEURAL RESPONSE AND SEX HORMONES IN TYPE 1 GAUCHER DISEASE

PERIFERNI NEURALNI ODGOVOR I POLNI HORMONI KOD GOŠEOVE BOLESTI TIPA 1

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Summary

Background: In a rare Gaucher disease, reduced activity of lysosomal β-glucocerebrosidase incompletely blocks glucosphingolipid catabolism. Accumulation of the unhydrolyzed substrate glucosylceramide within lysosomes results in progressive, multisystem Gaucher disease, classified into three types. Both parkinsonism and peripheral neuropathy are observed in cases of putative non-neuronopathic type 1 disease. In the current study we investigated whether the peripheral neural response in type 1 Gaucher disease patients, with no neural manifestations is conditioned by the influence of sex hormones.
Methods: The catalytic activity of β-glucocerebrosidase in peripheral blood leukocytes was determined spectrofluorometrically. Direct sequencing of the *GBA1* gene was performed. Somatosensory evoked potentials were recorded after electrical stimulation of the median nerve of both arms. Stimuli of 0.2 ms duration at a frequency of 5 Hz were used. Sex hormones were determined by radioimmunoassay using a gamma scintillation counter.
Results: Analysis of the somatosensory evoked potentials revealed significant differences in peak latencies on periphery between men and women in both control and type 1 Gaucher disease groups. Analysis by gender showed significant associations between latencies and sex hormones

Kratak sadržaj

Uvod: Kod retke Gošeove bolesti, snižena aktivnost lizozomske β-glikocerebrozidaze uzrokuje nepotpuni blok katabolizma glikosfingolipida. Nagomilavanje nehidrolizovanog supstrata glukozilceramida u lizozomima dovodi do progresivne, multisistemske Gošeove bolesti klasifikovane u tri tipa. Ispostavilo se da se kod obavezno ne-neuronopatskog tipa 1 ipak javlja neuralna patologija – parkinsonizam i periferna neuropatija. U aktuelnoj studiji, istraživali smo da li je kod pacijenata sa Gošeovom bolešću tipa 1 koji su bez ikakvih neuroloških manifestacija periferni neuralni odgovor uslovljen uticajem polnih hormona.
Metode: Katalitička aktivnost β-glikocerebrozidaze u leukocitima periferne krvi utvrđena je spektrofluorometrijskim metodom. Analiza gena *GBA1* izvedena je direktnim sekvenciranjem. Snimljeni su somatosenzorni potencijali evocirani električnom stimulacijom nervusa medijanusa obe ruke. Nadražajima trajanja 0,2 ms delovalo se frekvecijom od 5 Hz. Polni hormoni su mereni radioimunološkim metodom pomoću gama scintilacionog brojača.
Rezultati: Metodom somatosenzornih evociranih potencijala otkrili smo značajnu razliku u latencama kod muškaraca i žena na periferiji, kako u kontrolnoj grupi tako i u grupi Gošeove bolesti tipa 1. Analiza po polu pokazuje značajne korelacije između latenci i polnih hormona samo kod

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only in female patients: negative correlation between oestradiol concentration and N9 peak latency, and a strong negative correlation of testosterone levels with all peak latencies on the periphery (N9-N13).

Conclusions: A relationship between testosterone concentrations and the latencies of potentials evoked on peripheral nerves exists only in females with type 1 Gaucher disease. We point out sexual dimorphism in the development of this entity.

Keywords: Type 1 Gaucher disease, somatosensory evoked potentials, latencies, oestradiol, testosterone

ženskih pacijenata: značajno negativnu korelaciju između koncentracije estradiola i latence N9, i jaku negativnu korelaciju nivoa testosterona sa svim latencama na periferiji (N9-N13).

Zaključak: Dokazom da povezanost koncentracija testosterona i latenci potencijala izazvanih na perifernim živcima postoji samo kod žena sa tipom 1 Gošeove bolesti ukazujemo na seksualni dimorfizam u razvoju toga entiteta.

Ključne reči: Gošeova bolest tip 1, somatosenzorni evocirani potencijali, latencije, estradiol, testosteron

Introduction

Gaucher disease (GD) is a rare lysosomal storage sphingolipidosis caused by compound heterozygous or homozygous mutations in the *GBA1* gene (Online Mendelian Inheritance in Man (OMIM) #606463) (1). Consecutive unnatural configuration and insufficient activity of lysosomal β-glucocerebrosidase leads to incomplete block in glucosphingolipid catabolism (2, 3). Since sphingolipids and their metabolites, as structural elements of membranes and signaling molecules, play a vital role in cell physiology, accumulation of the unhydrolyzed substrate glucosylceramide within lysosomes results in progressive, multisystem GD with a wide phenotypic spectrum (4). GD is classified into mandatory non-neuronopathic type 1 (OMIM #230800), acute neuronopathic type 2 (OMIM #230900) and chronic neuronopathic type 3 (OMIM #231000) disease. However, the occurrence of parkinsonism and peripheral neuropathy different from the specific characteristics defined by type 2 and 3 has been observed in a number of patients with type 1 GD (5, 6).

The aim of this study was to determine whether the peripheral neural response in type 1 GD is under the influence of sex hormones.

Materials and Methods

Patients

In this cross-sectional study, 20 type 1 GD patients with no clinical neurological manifestations (10 women and 10 men) aged 19 to 61 years were compared with healthy controls matched for gender and age. Among the patients, three were treatment-naïve, fourteen had received enzyme replacement therapy and three substrate-reductive therapy for more than 5 years.

Compliance with ethical standards

Approval for the study was obtained from the Ethics Committee of the Medical Faculty, University of Belgrade (decision number 29/III-12 from 27.03. 2015.).

Data aquisition methods

The algorithm for setting the GD diagnosis included clinical findings, assessment of biomarker activity followed by determination of specific enzyme activity and was confirmed by identification of *GBA1* gene mutations.

Biochemistry

The activity of the biomarker chitotriosidase in serum was measured spectrofluorometrically using the fluorogenic substrate 4-methyl-umbelliferyl-β-D-N-N'-N"-triacetylchitotrioside (Sigma Chemical Co, USA) on an SPF-500™C spectrofluorometer (SLM Instruments Inc, USA). The fluorogenic substrate 4-methylumbelliferyl-β-D-glucoside (Sigma Chemical Co, USA) was used for determining the catalytic activity of β-glucocerebrosidase in peripheral blood leukocytes spectrofluorometrically using the same spectrofluorometer.

The following ranges of control values (as 2.5 and 97.5 percentiles) for a healthy population were established at the Clinical Center of Serbia: for chitotriosidase activity 1.80–146.56 nmol/mL/h; for β-glucocerebrosidase activity 6.65–15.90 nmol/mg/h.

Molecular genetics

The *GBA1* gene was analyzed by direct sequencing (Applied Biosystems 3500, Hitachi, USA).

Neurophysiology

Somatosensory evoked potentials (SSEP) were recorded after electrical stimulation of the right and left median nerve at the wrist (MedelecSynergy, Viasys Healthcare, UK). Stimuli of 0.2 ms duration were given at a frequency of 5 Hz.

Hormones

Oestradiol (E2) (ESTR-US- CT, Cisbio Bioassays, France, coefficient of variance,CV, 2.8%) and testos-

Table I Anthropological data and sex hormones levels.

	Control (N = 20)			Gaucher (N = 20)			
	Women	Men	*p*	Women	Men	p	*p* (Con vs. GD)
Age (years)	36.5 ± 4.4	44.4 ± 3.9	0.19	38.2 ± 4.1	43.8 ± 4.2	0.52	0.93
Height (cm)	171.5 ± 1.3	180.2 ± 1.8	0.01	164 ± 2.7	180.0 ± 2.6	0.01	0.24
Testosterone (nmol/L)	1.07 ± 0.12	21 ± 2	0.01	0.89 ± 0.17	19.4 ± 1.3	0.01	0.63
Oestradiol (pmol/L)	252 ± 60	129 ± 10	0.06	274 ± 76	115 ± 13	0.07	0.88

Data are presented as mean values ± standard error.

terone (TESTO- CT2, Cisbio Bioassays, France, CV 3.1%) were measured by radioimmunoassay using a gamma scintillation counter (CliniGamma 1272, LKB-Wallac, USA).

Statistics

Normal distribution of the data was examined by the Shapiro-Wilk test. We used two way ANOVA (group, gender) to assess differences between the control and type 1 GD groups. Pearson's correlation coefficient was employed to estimate linear relationships between the variables. Data are presented as means ± standard error. Differences were considered statistically significant at $p < 0.05$. We used SPSS 19 (IBM) software for statistical analysis.

Results

Biochemistry

Pretreatment chitotriosidase activity levels ranged from 2404 to 26930 nmol/mL/h. Residual levels of β-glucocerebrosidase activity were between 0.50 to 3.14 nmol/mg/h.

Molecular genetics

Two patients were homozygous for the *GBA1*mutation c.1226A>G, while all the others had compound heterozygous mutations. In each control participant, the wild type *GBA1* gene was confirmed in both alleles.

Anthropology

There was no age difference between the groups (*Table I*). In terms of height, significant differences existed in each group: women were shorter than men in both groups; women with type 1 GD were shorter than the control women ($p = 0.03$) (*Table I*).

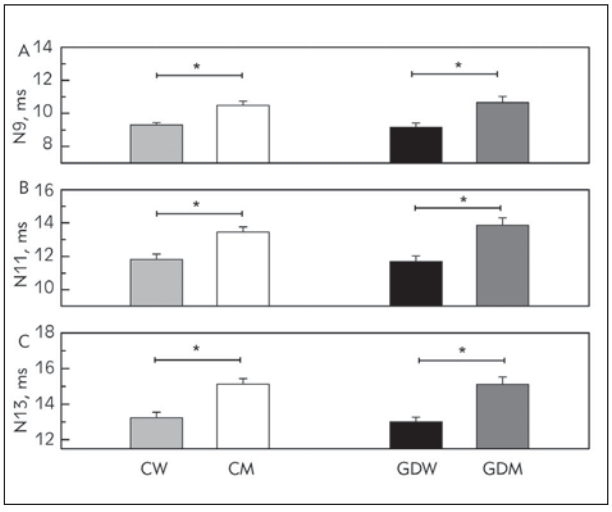

Figure 1 Peak latencies plus standard error in control women (CW), control men (CM), type 1 GD female patients (GDW), type 1 GD male patients (GDM), * $p < 0.01$.

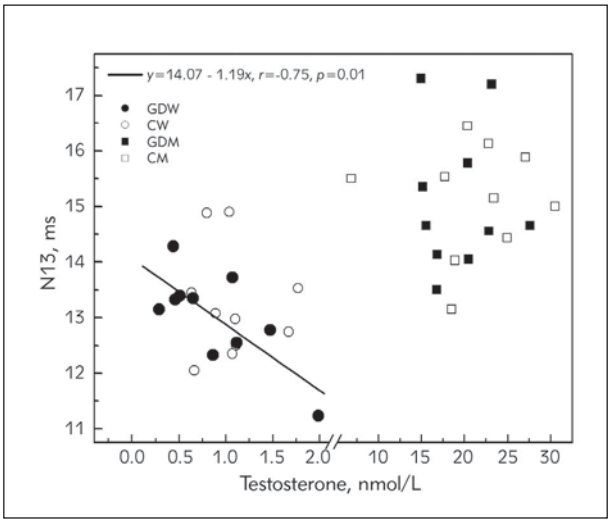

Figure 2 Relationship between peak latency N13 and plasma testosterone levels in GDW (solid circle), CW (open circle), GDM (solid square) and CM (open square).

SSEP

Regarding the average latency values there were no difference for all parameters between the left and right median nerves (latency of the N9, N11 and N13 waves), no when the control and GD groups were compared in total and for each gender separately. However, within each groups, a significant differences in peak latencies between men and women were identified from N9 to N13 ($p \leq 0.01$) (*Figure 1*).

Sex hormones

As expected both groups showed statistically significant differences in testosterone concentrations between women and men (*Table I*). There was a tendency for the difference between the sexes for oestradiol concentration to approach statistical significance both groups (*Table I*).

Correlations

Statistically significant latency correlations with the height of subjects were present only in women with type 1 GD: N9 ($r = 0.78$, $p < 0.01$), N11 ($r = 0.58$, $p = 0.08$, near), N13 ($r = 0.70$, $p = 0.03$).

Considering men and women together, there were significant positive correlations between all latencies and testosterone concentrations in both the control and type 1 GD groups: N9 ($r = 0.57$ vs. $r = 0.58$, $p < 0.01$, respectively), N11 ($r = 0.60$ vs. $r = 0.62$, $p < 0.01$, respectively), and N13 ($r = 0.65$ vs. $r = 0.67$, $p < 0.01$, respectively) (*Figure 2*).

However, when the analysis was performed for each gender, significant latency correlations with sex hormones were identified only in female patients with type 1 GD: negative correlation between estradiol concentration and N9 peak latency ($r = -0.63$, $p = 0.05$) and negative correlations of testosterone levels with all peak latencies on the periphery N9 ($r = -0.58$, $p = 0.08$, tendency), N11 ($r = -0.76$, $p = 0.01$), N13 ($r = -0.75$, $p = 0.01$).

Discussion

A crucial large-scale prospective observational cohort study, employing strictly defined criteria, provided evidence for polyneuropathy as part of the natural course of type 1 GD (6). In 103 patients enrolled at 18 to 75 years old, 13.6% were untreated and 86.4% received enzyme replacement therapy. Among them, 10.7% were diagnosed with sensory motor axonal polyneuropathy at baseline using standardized electrophysiological assessment. Six new cases of polyneuropathy were revealed during two-years monitoring (2.9 per 100 person-years). The same diagnostic procedure was used for the 25 healthy subjects. Since prevalence and incidence of polyneuropathy in the general population were estimated to be between 0.09 and 1.3% and 0.0046 and 0.015 per 100 person-years, respectively, it was concluded that both prevalence and incidence of polyneuropathy in type 1 GD patients are greater than for the general population. Therefore, for this study we enrolled only patients with no peripheral neurological manifestations.

Until now, only one multimodal neurophysiological investigation (including SSEP) has been performed in adult subjects with type 1GD (7). It involved eight female and four male adult patients aged 17 to 48 years. Findings were obtained from the right median nerve. In all subjects normal recordings from the periphery (N9, N11, N13) were read out. We have confirmed such normal findings but have gone further. Namely the present cross-sectional SSEP study on the median nerve periphery revealed gender differences – shorter latency peaks in women from both groups (healthy, diseased) which has not been noticed, so far.

Neuroactive steroids include those produced by the nervous system and hormones originating from the gonads and adrenal glands. The peripheral nervous system (PNS) not only synthesizes and metabolizes neuroactive steroids, but peripheral nerves also express receptors for neuroactive steroids and, therefore, are a target for their activity (8, 9). Steroids acting in the nervous system realize their effects via classical intracellular androgen, progesterone, oestrogen, glucocorticoid and mineralocorticoid receptors, as well as via non-classical steroid receptors expressed by different cellular components of the PNS (10, 11). Therefore, regulating PNS physiology over various signaling pathways, neuroactive steroids, including testosterone, can influence different peripheral nerves functions, among which Schwann cells proliferation and myelination have been studied in particular (8, 11).

Striking sexual dimorphism of white matter growth in adolescent brains has already been observed, but not explained (12, 13). In order to assess the role of the androgen receptor (AR) in mediating the effect of testosterone on white matter growth, 204 male and 204 female adolescents were studied (14). Functional polymorphism in the AR gene (number of CAG repeats in exon 1) was genotyped, togather with measurement of plasma testosterone concentration, computational analysis of magnetic resonance images and calculation of the magnetization transfer ratio (MTR) for white matter throughout the brain as an indirect index of myelination. Evidence emerged that a genetic variation in the AR gene moderates the effect of testosterone on white matter volume during male adolescence. Namely, the testosterone-related increase of white matter volume was stronger in male adolescents with lower versus higher numbers of CAG repeats in exon 1 of the AR gene. The MTR results indicated age-related growth in volume, but this could not be

explained by an increase in myelination. It was assumed that testosterone effected axonal diameter, rather than myelin sheath thickness. The direct consequence of increased axonal diameter under the influence of testosterone is a decrease in the number of fibers per unit volume, which is manifested as a lower index of myelination and lower MTR values (14). Likewise, the latter might explain the peripheral nerve response data obtained in the current study. Within the total testosterone concentration range (males plus females), the positive correlation between plasma testosterone levels and all peak latencies in all participants in each group is very significant. Since the number of nerve fibers is a key factors determining the efficiency of peripheral nerve transmission (15), the smaller number of myelinated fibers in the median nerve, due to their large testosterone-induced caliber in men, could be the reason for longer latencies.

When females were analyzed *per se*, using the lower range of testosterone values in women (up to 1.9 nmol/L), only female patients with type 1 GD showed a statistically significant negative correlations of plasma testosterone levels with latencies from N9 to N13. This association indicates that a normal SSEP finding alone is not sufficient to draw conclusions about undisturbed morphological and functional nerve integrity on the periphery in type 1 GD females. Why small changes in testosterone concentrations in women with type 1 GD affect the duration of impulse propagation should be clarified. Experimental models have indicated that pathology affects the concentration of neuroactive steroids present in peripheral nerves in a sex-dimorphic way (16, 17).

We have entered a serious area that has yet to be examined in detail due to insufficient available data on this matter.

Conflict of interest statement

The authors state that they have no conflicts of interest regarding the publication of this article.

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