Molecular Genetics and Metabolism xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Cerebrospinal fluid synaptic proteins as useful biomarkers in tyrosine hydroxylase deficiency

C. Ortez [a,c], S.T. Duarte [h,i], A. Ormazábal [b,c], M. Serrano [c], A. Pérez [a], R. Pons [d], M. Pineda [a,c], Z. Yapici [e], E. Fernández-Álvarez [a], R. Domingo-Jiménez [f], P. De Castro [g], R. Artuch [b,c], A. García-Cazorla [a,b,*]

[a] Department of Neurology, CIBER-ER Biomedical Network Research Centre on Rare Diseases, Instituto de Salud Carlos III, Barcelona, Spain
[b] Department of Biochemistry, CIBER-ER Biomedical Network Research Centre on Rare Diseases, Instituto de Salud Carlos III, Barcelona, Spain
[c] Hospital Sant Joan de Déu (HSJD), CIBER-ER Biomedical Network Research Centre on Rare Diseases, Instituto de Salud Carlos III, Barcelona, Spain
[d] Department of Pediatrics, University of Athens, Aghia Sofia Hospital, Athens, Greece
[e] Istanbul Faculty of Medicine, Department of Child Neurology, Istanbul, Turkey
[f] Departament of Pediatric Neurology, Hospital V. Arrixaca, Murcia, Spain
[g] Department of Pediatric Neurology, Hospital Gregorio Marañón, Madrid, Spain
[h] Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal
[i] Hospital Dona Estefânia, CHLC, Lisbon, Portugal

ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form 27 October 2014
Accepted 28 October 2014
Available online xxxx

Keywords:
Tyrosine hydroxylase deficiency
Cerebrospinal fluid
Dopaminergic receptor type 2
Infantile parkinsonism
Synaptic proteins

ABSTRACT

Tyrosine hydroxylase (TH) deficiency is an inborn error of dopamine biosynthesis and a cause of early parkinsonism. Two clinical phenotypes have been described. Type "B": early onset severe encephalopathy; type "A": later onset, less severe and better response to L-dopa. We aimed to study the expression of several key dopaminergic and gabaergic synaptic proteins in the cerebrospinal fluid (CSF) of a series of patients with TH deficiency and their possible relation with the clinical phenotype and response to L-DOPA.

Dopamine transporter (DAT), D2-receptor and vesicular monoamine transporter (VMAT2) were measured in the CSF of 10 subjects with TH deficiency by Western blot analysis. In 3 patients, data of pre- and post-treatment with L-DOPA were available, and in one of them, GABA vesicular transporter was determined. Results were compared to an age-matched control population.

The concentration of D2-receptors in CSF was significantly higher in patients with TH deficiency than in controls. Similarly, DAT and vesicular monoamine transporter type 2 were up-regulated. Studies performed before L-DOPA, and on L-DOPA therapy showed a paradoxical response with D2 receptor expression increase as L-Dopa doses and homovanillic concentration gradually raised in a B phenotype patient. The opposite results were found in two patients with A phenotype. However, this is a very small sample, and further studies are needed to conclude robust differences between phenotypes.

Synaptic proteins are detectable in the CSF and their quantification can be useful for understanding the pathophysiology of neurotransmitter defects and potentially to adjust and personalize treatments in the future.

1. Introduction

Tyrosine hydroxylase (TH) deficiency (OMIM 191290) is a rare autosomal recessive inborn error of dopamine transmission. TH converts tyrosine into L-DOPA, the direct precursor of catecholamine biosynthesis (Fig. 1). This enzymatic conversion is a rate-limiting step in the biosynthesis of catecholamines. Around sixty patients with TH deficiency have been reported worldwide [1–11]. TH deficiency causes a neurological disease with predominant extrapyramidal signs and a variable response to L-DOPA. Although different neurological manifestations have been described (recessive form of Segawa disease, infantile parkinsonism with dystonia, early-onset progressive encephalopathy), a comprehensive review[7] has divided them into two main forms: type A, a progressive hypokinetic-rigid syndrome plus dystonia, with onset in infancy or childhood; and type B, a complex encephalopathy with neonatal or early infancy onset (hypokinetic-rigid syndrome plus developmental delay, a variety of movement disorders and occasionally epilepsy). Generally, motor and cognitive prognosis is worse in type B. However, the pathophysiological aspects that may underlie these differences have been poorly described. Although TH deficiency is a disease of neuronal communication, the role of key synaptic proteins responsible for dopaminergic transmission has not been reported so far. Synaptic transmission depends on neurotransmitter pools stored within vesicles that undergo regulated exocytosis. In the case of dopaminergic transmission, the vesicular monoamine transporter-2 (VMAT2) is responsible for the loading of dopamine (DA) and other monoamines into synaptic

* Corresponding author at: Neurology Department, Hospital Sant Joan de Deu, Passeig Sant Joan de Deu, 2, 08950 Esplugues, Barcelona, Spain. Fax: +34 93 203 3959.
E-mail address: agarcia@hsjdbcn.org (A. García-Cazorla).

http://dx.doi.org/10.1016/j.ymgme.2014.10.014
1096-7192/

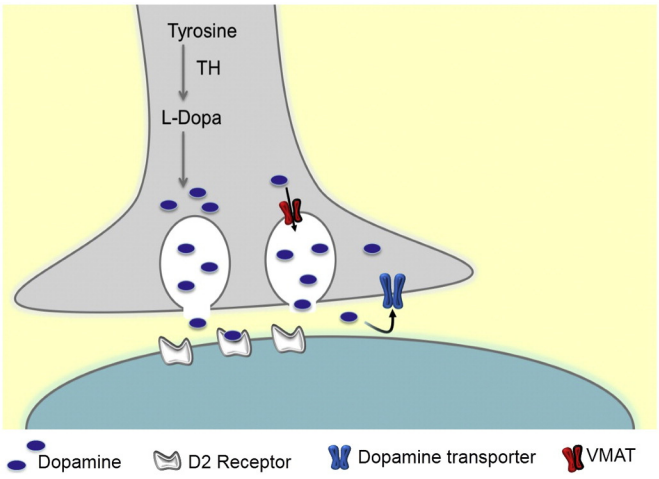

Fig. 1. Schematic representation of dopaminergic synapse and synaptic proteins studied. VMAT2 (vesicular monoamine transporter 2).

vesicles [12]. The DA transporter (DAT) carries DA across the plasmalemmal membrane from the synaptic cleft into the cytoplasm [13]. The central actions of DA are mediated by five distinct receptors that belong to the G-protein receptor family (Fig. 1). They are classified as D1-like (D1 and D5) and D2-like (D2, D3 and D4), and their interaction with dopamine translates into activation/inhibition of specific neurons and circuitries [14]. D2-receptor (D2 R) genetic mutations are related to postural abnormalities, bradykinesia, impaired coordination and prolonged periods of immobility [15]. These symptoms are also characteristic of patients suffering from other dopaminergic neurotransmission disorders. Following recent reports on the co-release of classical neurotransmitters and on interactions between different neurotransmission pathways, we wanted to explore the role of the GABA vesicular transporter (GABAVT), which gives an estimation of GABA release, after L-DOPA treatment.

The detection and quantification of dopaminergic and GABAergic synaptic proteins in the CSF of a control population have been previously reported by our group [16]. We aimed to study synaptic proteins involved in dopaminergic and GABAergic transmission in the CSF of patients with TH deficiency, in order to relate their expression to the different clinical phenotypes and L-DOPA response.

2. Methods

2.1. Patients

Ten patients with diverse pathogenic mutations in tyrosine hydroxylase gene were diagnosed at Hospital Sant Joan de Déu, Barcelona, from 2002 to 2010. Some of them have been already published as individual case reports [5,6,8,17]. Clinical, biochemical and molecular data of the whole series are described in Table 1. Five patients were classified as "B" phenotype and five as "A". Follow-up and detailed response to L-dopa treatment are also reported in Table 1.

2.2. CSF studies

CSF samples from patients were collected by lumbar puncture as previously described [18,19]. After lumbar puncture, the first ten drops were used for routine cytochemical/microbiological studies and then CSF was immediately stored in 4 aliquots at −80 °C until the moment of analysis. Biogenic amines metabolites and synaptic proteins were analyzed in the next 20 drops.

Biogenic amines metabolites were analyzed by high performance liquid chromatography (HPLC) with electrochemical and fluorescence detection, respectively. Results were compared to our reference values that were established in a control population from our geographical area. Details of reference interval establishment were reported elsewhere [19].

The synaptic proteins, VMAT2, DAT and D2R, involved in dopaminergic transmission, were analyzed by Western blot. In 3 patients (5, 6, 7 in Table 1), these proteins were studied before and after L-dopa treatment, and included GABA vesicular transporter (GABA VT), which gives an estimation of GABA release, in patient 5. Furthermore, GFAP (glial fibrillar acidic protein) was also analyzed as loading and protein concentration inner control of the technique. A total of 40 μl of CSF sample was loaded into the gel and proteins were separated on 10% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Amersham™ Hybond™ –ECL, GE Healthcare). Membranes were blocked in TBST buffer (0.02 M Tris-base, pH7.6, 0.8% NaCl, 0.1% Tween 20) with 5% dried skimmed milk for 60 min. Anti-DAT extracellular loop (1:1000; Sigma®), anti-D2 R (1:1000; Millipore®), anti-VMAT 2 (1:1000; Santa Cruz Biotechnology®), anti-GABA VT (1/500; Millipore®) and anti-GFAP (1:1000; Santa Cruz Biotechnology®) antibodies were added, and incubated at 4 °C overnight. Membranes were washed three times with TBST buffer and then incubated with appropriate anti-rabbit (1:3000, Promega®) or anti-mouse (1:5000, Promega®) IgG secondary antibodies at room temperature for 1 h. The blot was then washed six times with TBST and signal was revealed with ECL (Pierce® ECL Western Blotting Substract, Thermo Scientific). Relative levels of each protein were quantified by measuring optical densities (OD) of the corresponding bands with Quantity One ® V 4.3.1. software. CSF total protein was measured by standard automated procedures in an Architect ci8200 analyzer (Abbott, USA). Results of synaptic proteins were compared to a control population whose CSF samples were submitted to our laboratory for analysis under suspicion of nervous system infection.. Exclusion criteria were diagnosis of viral or bacterial meningitis, a chronic neurological condition, and hematic or xanthochromic CSF (blood contamination). We studied 3 to 5 age-matched controls for every patient with the exception of the oldest patients, aged 17 and 30 years. Since HSJD is a pediatric hospital, we could only recruit two and one controls respectively.

Samples from patients were obtained in accordance with the Helsinki Declaration of 1964, as revised in 2000. Written informed consent was obtained from legal guardians of all patients included. The ethical committee of the Hospital Sant Joan de Déu approved the study.

2.3. Statistical analysis

All analyses were performed by using SPSS 20.0. Student's *t*-test was used to find differences in synaptic proteins between patients and controls. A P value <0.05 was considered significant.

3. Results

3.1. Differences in synaptic proteins between patients (before treatment) and controls

Total protein concentration in the CSF samples from patients and controls was within normal limits according to different age ranges (data not shown). DAT, D2R, VMAT2 and GABA vesicular transporter were clearly detectable at the expected molecular weight using conventional western blot analysis in all the CSF samples studied (Fig. 2). The concentration of dopaminergic proteins D2R, DAT and VMAT2 in CSF measured by the average optical density (AOD) was significantly higher in pre-treated patients than in controls ($p < 0.0001$) (Fig. 3A).

Please cite this article as: C. Ortez, et al., Cerebrospinal fluid synaptic proteins as useful biomarkers in tyrosine hydroxylase deficiency, Mol. Genet. Metab. (2014), http://dx.doi.org/10.1016/j.ymgme.2014.10.014