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Short Communication

Infantile parkinsonism and gabaergic hypotransmission in a patient with pyruvate carboxylase deficiency

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ABSTRACT

Pyruvate carboxylase deficiency is a rare metabolic disorder, with three different phenotypes. We aim to report the case of a newborn presenting the severe neonatal form of this deficiency (the B or "French" phenotype, hypokinesia and rigidity being the main features) and the results of the study of classic neurotransmitters involved in movement control. Hyperdopaminergic transmission (both in the cerebrospinal fluid and in the substantia nigra) and hypoGABAergic transmission (in the substantia nigra) were found. Both gamma-aminobutyric acid and dopamine markers were found coexisting in individual neurons of the substantia nigra. This is the first time this phenomenon has been reported in the literature. We discuss the possible role of GABAergic deficiency, its interaction with other neurotransmitters and its implication in neurotransmitter homeostasis. A better comprehension of that field would increase understanding of the pathophysiology of neurological symptoms and neurotransmitter plasticity.

1. Introduction

Pyruvate carboxylase (PC) deficiency is an inborn error of metabolism with different clinical presentations. The B or "French" phenotype is a neonatal form with severe lactic acidosis and fatal outcome. Patients manifest severe axial hypotonic, pyramidal tract signs, an initially preserved level of consciousness with rapid deterioration, and an abnormal pattern of movements that includes Parkinsonism signs (rigidity, tremor and hypokinesia) and bizarre ocular movements (García-Cazorla et al., 2006). Parkinsonism in a severely ill newborn is difficult to assess, and its identification strongly depends on the neuropediatrician's expertise. Rigidity, tremor and hypokinesia are common signs of the hypokinetic-rigid syndrome or "Parkinsonism". This neurological syndrome is very rare in infants and in the pediatric population. Actually, studies assessing the global incidence and prevalence of pediatric Parkinsonism are lacking. In a long series study regarding movement disorders in children carried out in Hospital Sant Joan de Déu, a 2% prevalence was reported from a total of 673 patients (Fernádez-Álvarez and Aicardi, 2001). PC deficiency in particular has a reported incidence in most populations of 1:250,000 (Wang and De Vivo, 1993–2009). This specific disorder has been poorly studied from a pathophysiological point of view and we aim to bring new insights into neurotransmitter balance and functioning.

With respect to the hypokinetic-rigid syndrome in the pediatric population, it's not only explained by a presynaptic dopaminergic neuronal loss, as in Parkinson's disease, but also by other factors. These include: 1) secondary Parkinsonism due to different exogenous agents (pharmacological treatment, other drugs, intoxication, infections…), 2) genetically determined causes (inborn errors of metabolism, such as PC deficiency and tyrosine hydroxylase deficiency, and genetic causes other than metabolic causes) and 3) unknown origin (García-Cazorla et al., 2011). In general, dopamine deficiency seems to be the main biochemical finding, whatever the origin and the onset age are. However, chemical neurotransmission is complex and co-release of classical transmitters may appear in certain neuron populations (González-Hernández et al., 2001).

Abbreviations: CSF, cerebrospinal fluid; GABA, gamma-aminobutyric acid; GAD, glutamate decarboxylase; MRI, magnetic resonance image; MRS, magnetic resonance spectroscopy; PC, pyruvate carboxylase; SN, substantia nigra; SPNs, spiny projection neurons; STN, subthalamic nucleus.

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In order to delve deep into the pathophysiology of these abnormal movements, we studied neurotransmitters in the cerebrospinal fluid (CSF) and the substantia nigra (SN) of a newborn with PC deficiency.

2. Case report

The patient was a female born at term (41 weeks gestation) after a normal delivery, Apgar 9/10 and no need for resuscitation. At 27 weeks gestation, a brain ventriculomegaly was detected by a regular ultrasonography and confirmed by fetal magnetic resonance image (MRI) (although this finding was not confirmed in the post-natal MRI). The gestational anamnesis was otherwise negative, without any relevant event. She was the first child of a nonconsanguineous healthy Caucasian couple with a family history negative for any relevant pathology. She had a low birth-weight of 2115 g (−3.1SD), was 46 cm in length (−2.6SD) and had a normal (for her gestational age) head circumference of 34 cm.

At 15 h of life, after an initial free symptom period, lethargy, polypnea and truncal hypotonia appeared. Due to this sudden clinical presentation, a neonatal sepsis was suspected and antibiotic therapy with cefotaxime and ampicillin was initiated; this was changed to ceftazidime and vancomycin on her 3rd day of life. She also received sedoanalgesic treatment with midazolam and fentanyl.

The initial blood analysis disclosed a severe metabolic acidosis (pH 6.94, pCO_2 11.6 mm Hg, pO_2 87 mm Hg, bicarbonate 2.4 mmol/l and base excess −27.7 mmol/l) with lactate 18 mmol/l (normal: 1.33–2.33) and ammonia 179 μmol/l (normal in neonatal period: <110), glucose 40 mg/dl (normal: 45–88), normal renal function, sodium 143 mmol/l, potassium 4.40 mmol/l (normal: 3.70–5.50), alanine aminotransferase 13 UI/l (normal: 3–33), aspartate aminotransferase 146 UI/l (normal: 3–95), total bilirubin 6 mg/dl (normal: 2–5.97), conjugated bilirubin 0.6 mg/dl (normal: <0.2), lactate dehydrogenase 1904 UI/l (normal: <776), creatine kinase 1433 UI/l (normal: 128–1540), and reactive C protein <5 mg/l (normal: <15). Further metabolic investigations were carried and revealed a high pyruvate level of 0.557 (normal: 0.06–012), lactate level of 21.17 mmol/l and an elevated lactate to pyruvate ratio of 38.01 (normal: 10.1–35.5). The plasmatic amino acids showed a barely detected level of aspartate, low glutamine (298 μmol/l (normal: 420–750)) and alanine (152 μmol/l (normal: 190–337)) levels, and high citrulline (187 μmol/l (normal: 8–27)), proline (1312 μmol/l (normal: 90–270)) and lysine (1222 μmol/l) levels. The rest of the amino acid profile was normal. A study of urine organic acids disclosed elevated lactate and 3-OH-butyrate. An investigation of urine amino acids showed high lysine (12,849 μmol/g creat. (normal: 400–2300)), citrulline (2572 μmol/g creat. (normal: 0–50)) and glutamine (67 μmol/g creat. (normal: 110–1380)) levels, in accordance with the plasmatic profile. The highest lactate values were 22 μmol/l and the highest ammonia levels were the abovementioned values.

Neurological examination showed generalized hypokinesia, with almost absent spontaneous movements, poor blinking and limb rigidity. Some dysmorphic features such as thin upper lip, wide philtrum and small palpebral fissures were observed. An abnormal ocular behavior was also observed, which consisted of a horizontal nystagmus alternating with a pendular movement. The electroencephalogram showed a brain suppression pattern. Brain MRI disclosed periventricular white matter cysts with a global abnormal white matter signal, normal ventricular size, and two juxtacortical frontal and parietal hemorrhagic focuses (Fig. 1). Brain MRS showed an abnormal lactate peak.

These results were consistent with a pyruvate carboxylase deficiency type B, and thus anaplerotic treatment with oral citrate was initiated on the 2nd day of life.

In spite of symptomatic and anaplerotic treatment, persistent lactic acidosis led to progressive deterioration. Due to this clinical evolution and the devastating prognostic with fatal outcome, and always with a debated consensus with her parents, no further extraordinary medical measures were taken. She died of cardiorespiratory arrest at 5 days of life.

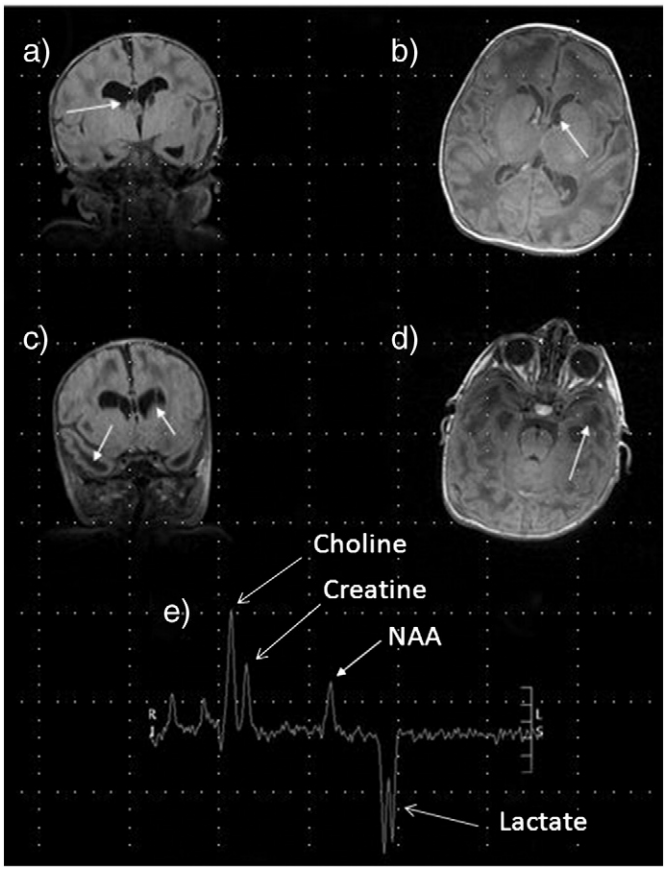

Fig. 1. (a, b, c, d) MRI images showing a T2 weighted image with periventricular white matter cysts and a global affection of the white matter signal. Remarkable bilateral implication of temporal lobes and periventricular zones. Spectroscopy (e) shows a high peak of lactate. The NAA (N-acetylaspartate) peak is low (revealing neuronal loss) and the choline peak is high (due to white matter damage) compared to age-matched controls.

PC activity was absent in fibroblasts (less than 5% compared to normal subjects). Mutation analysis revealed two novel changes [c.616G>T; c.827A>C] in the same allele in the homozygous state. The *in silico* analysis of the predicted missense changes performed with the bioinformatics application PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) indicates that the variant change p.V206L (c.616G>T) is benign while p.E276A (c.827A>C) is pathogenic, according to the severely diminished PC activity found in the patient's fibroblasts. The disease has a pattern of recessive inheritance, as confirmed by the presence of these changes in parental DNA in heterozygous fashion.

3. Cerebrospinal fluid (CSF) and brain tissue studies

CSF samples were collected to rule out an infectious disease, and allowed us biogenic amine studies as described (Ormazábal et al., 2006). Homovanillic acid concentration was high (1782 nmol/l; normal: 658–1434), whereas other dopaminergic and serotoninergic metabolites were normal. Gamma-aminobutyric acid (GABA) could not be measured due to CSF volume limitations. Parents authorized a cerebral histopathological examination, disclosing periventricular cysts, gemistocytic astrocytes in white matter with focal spongiosis and neurons with central chromatolysis in substantia nigra (Fig. 2). Immunofluorescence studies were performed in paraffin embedded samples of SN pars compacta, in our patient and in three age-matched controls without neurological disease. Dopaminergic staining was obtained using a monoclonal antibody against tyrosine hydroxylase (TH) (Millipore, 1/500), and GABAergic