Mental retardation and hypotonia seen in the knock out mouse for Canavan disease is not due to succinate semialdehyde dehydrogenase deficiency

S. Surendra, E.L. Ezell, M.J. Quast, J. Wei, S.K. Tyring, K. Michals-Matalon, R. Matalon,

Department of Pediatrics, Children’s Hospital, The University of Texas Medical Branch, Galveston, TX 77555-0359, USA
Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston, TX 77555-0359, USA
Sealy Center for Structural Biology, The University of Texas Medical Branch, Galveston, TX 77555-0359, USA
Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, TX 77555-0359, USA

Received 4 September 2003; received in revised form 26 November 2003; accepted 18 December 2003

Abstract

Canavan disease (CD) is an autosomal recessive disorder caused by aspartoacylase deficiency leading to accumulation of N-acetylaspartic acid (NAA) in the brain. Whether the low levels of glutamate and GABA observed in the CD mouse brain lead to abnormal production of glutamate-GABA associated enzymes and resulting succinate production is not obvious. While glutamate dehydrogenase and α-ketoglutarate dehydrogenase complex activities are lower in the cerebellum and brain stem of the CD mouse, alanine aminotransferase and succinate semialdehyde dehydrogenase (SSADH) activities and succinate level are similar to the levels observed in the wild type. Deficiency of SSADH has been suggested to be associated with mental retardation and hypotonia, similar to the clinical features of CD. The normal SSADH activity in the CD mouse brain suggests that mental retardation and hypotonia seen in the CD mouse is not due to SSADH activity and if documented also in patients with CD.

Keywords: Succinate semialdehyde dehydrogenase; α-Ketoglutarate dehydrogenase complex; Glutamate dehydrogenase; Canavan disease; Alanine aminotransferase; Succinate

Canavan disease (CD) is an autosomal recessive leukodystrophy caused by aspartoacylase deficiency leading to accumulation of N-acetylaspartic acid (NAA) in the brain [13]. The clinical features of the disease include psychomotor retardation, megalencephaly and hypotonia [10]. Seizures often develop in the second year of life [11,12]. Abnormalities in the brain of patients with CD include spongy degeneration with swollen astrocytes and elongated mitochondria [3,10]. Although CD is observed in various ethnic groups, the disease is most prevalent in Ashkenazi Jewish population [12].

Murine knockout models are important to characterize pathophysiology associated with the corresponding human disorder. The knockout mouse for CD showed abnormalities similar observed in patients with CD include aspartoacylase (ASPA) deficiency, NAA accumulation and spongy degeneration of the brain and excessive urinary NAA excretion [14]. The homozygous mouse failed to thrive, gained low weight compared to the wild type [14]. The knockout mouse showed clinical features such as macrocephaly, hypotonia and ataxia as observed in children with CD [14,19–21]. The homozygous mouse had tremors, walked with splayed legs and stood with a wide base. Their pace was slower, shaky and less mobile compared to the wild type [14]. The homozygous mouse developed seizures at about 6 months age with clonic jerks and a post ictal state after the seizure that lasted for several minutes. Homozygous mice were able to sustain their balance on the rotorod for a very short period of time due to Ataxia [14]. Histology of the brain showed vacuolation of the white
Glutamate and γ-aminobutyric acid (GABA) levels were lower in the CD mouse brain suggesting that these neurotransmitters likely to lead abnormal cortical excitability [21]. A low level of aspartate aminotransferase (AST) may be one of the possible factors affecting glutamate level in the CD mouse brain [19]. Since alanine aminotransferase (ALT) also produces glutamate, by converting alanine to glutamate [1], the level of ALT in the CD mouse brain was determined. Whether the down-regulation of glutamate and GABA leads to abnormal activity of glutamate-GABA associated enzymes and resulting succinate production are being investigated.

Glutamate dehydrogenase (GDH) converts glutamate into α-ketoglutarate and α-ketoglutarate dehydrogenase (KGDH) converts α-ketoglutarate into succinyl coA to form succinate [1]. In addition, succinate semialdehyde dehydrogenase (SSADH) is the last enzyme of producing succinate from GABA [1] and therefore levels of these enzymes and succinate are to be investigated.

Six weeks old wild type and Canavan mice were sacrificed and brain parts were separated into cerebrum, hypothalamus, cerebellum and brainstem as followed earlier [21]. All protocols and procedures were approved by the Institution’s Animal Care and Use Committee. Tissues were homogenized and homogenates were used for the following enzyme assays. All biochemicals for enzyme activities were purchased from Sigma (St. Louis, MO). Enzyme activities were measured using the methods followed earlier for ALT [6], GDH [9], KGDHC [17] and SSADH [16] and measurements were read using Shimadzu UV-VIS spectrophotometer (Torrance, CA). The reaction mixture for ALT contained 100 mM/l Tris–HCl, 10 mM/l L-leucine, 0.3 mM/l NADH, 1 mM/l EDTA, 125 mM/l ammonium acetate, 1 mM/l adenosine 5′-diphosphate and 2-oxoglutarate. Activity of GDH was measured at 340 nm. To assay KGDH, the reaction mixture contained 63 mM/l Tris, 0.63 mM/l EDTA, 1.25 mM/l magnesium chloride, 1.25 mM/l calcium chloride, 0.63 mM/l DTT, 0.19 mM/l thiamine pyrophosphate, 3.1 mM/l nicotinamide adenine dinucleotide (NAD), 0.13 mM/l coenzyme A, 0.13% Triton X100, 0.63 mM/l 2-mercaptoethanol and 0.1 mol/l 2-ketoglutarate. KGDHC activity was measured at 460 nm. The SSADH reaction mixture contained 100 mM sodium pyrophosphate, 5 mM EDTA, 500 μM NAD and 10 μM succinic semialdehyde. SSADH activity was measured at 340 nm. Data were calculated using ANOVA.

Brain content of succinate was measured using nuclear magnetic resonance (NMR) spectra analysis as performed earlier [21]. Crushed brain samples were placed in 12% perchloric acid for overnight extraction at 4 °C and centrifuged at 10,000 g for 10 min at 4 °C. Supernatants were neutralized, lyophilized and redissolved in D2O. High resolution, 400 or 750 MHz protein NMR spectra were run on the supernatants. NMR measurements were performed on a Varian Unity-plus spectrometers using water-suppressed proton NMR spectroscopy. The NMR parameters for the single pulse experiments were: TR = 10 s, acquisition time = 3 s, saturation delay = 2 s, signal averages = 128. The lactate doublet was centered at 1.32 ppm. Peak integrals for the succinate (singlet, at 2.39 ppm) was compared with the creatine (singlet, at 3.02 ppm).

### Table 1

Levels of alanine aminotransferase, succinic semialdehyde dehydrogenase, glutamate dehydrogenase and α-ketoglutarate dehydrogenase complex activity in the brain of CD and wild type mice

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Alanine aminotransferase (ALT) activity (mU/mg protein)</th>
<th>NAD+ dependent succinic semialdehyde dehydrogenase (SSADH) activity (mU/mg protein)</th>
<th>Glutamate dehydrogenase (GDH) activity (mU/mg protein)</th>
<th>α-ketoglutarate dehydrogenase complex (KGDHC) activity (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrum</td>
<td>0.73 ± 0.02</td>
<td>0.27 ± 0.02</td>
<td>3.81 ± 0.64</td>
<td>0.14 ± 0.005</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>6.01 ± 1.32</td>
<td>0.64 ± 0.02</td>
<td>8.29 ± 1.04</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>3.26 ± 0.72</td>
<td>0.47 ± 0.05</td>
<td>8.94 ± 0.74</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>Brainstem</td>
<td>1.80 ± 0.16</td>
<td>0.38 ± 0.02</td>
<td>6.9 ± 0.45</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>CD mouse:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrum</td>
<td>0.69 ± 0.03</td>
<td>0.27 ± 0.01</td>
<td>2.7 ± 0.45</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>4.93 ± 0.41</td>
<td>0.76 ± 0.04</td>
<td>7.25 ± 1.67</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>2.66 ± 0.45</td>
<td>0.36 ± 0.03</td>
<td>3.9 ± 0.42</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Brainstem</td>
<td>1.35 ± 0.14</td>
<td>0.30 ± 0.03</td>
<td>3.53 ± 0.31</td>
<td>0.06 ± 0.005</td>
</tr>
</tbody>
</table>

The activity of ALT and SSADH were normal in the CD mouse brain as observed in the wild type. While cerebrum and hypothalamus of CD mouse brain had normal GDH activity, cerebellum (P < 0.001) and brainstem (P < 0.05) showed reduced activity. KGDHC activity in the cerebellum (P < 0.001) and brainstem (P = 0.001) was reduced in the CD mouse brain compared to the wild type (n = 7 ± SE).
Alanine aminotransferase activity in the CD mouse brain was normal as observed in the wild type (Table 1). Glutamate dehydrogenase activity was lower in the cerebellum \( (P < 0.001) \) and brainstem \( (P < 0.05) \) of the CD mouse brain while cerebrum and hypothalamus had normal levels as observed in wild type (Table 1). The KGDHC activity was also lower in the cerebellum \( (P < 0.001) \) and brainstem of the CD mouse brain (Table 1). Two-tailed \( P \) value for brainstem KGDHC activity was 0.0001. Succinic semialdehyde dehydrogenase activity was normal in the CD mouse brain as observed in the wild type (Table 1). NMR spectral analysis in the CD mouse brain suggested a normal succinate level. The ratio of succinate/creatinine in the CD mouse brain was 0.0592 ± 0.0082 \((n = 11)\), while in wild type the level was, 0.0500 ± 0.0065 \((n = 7 ± SE)\).

Glutamate carboxypeptidase II or N-acetylated \( \alpha \)-linked acidic dipeptidase or NAAG peptidase hydrolyzes NAAG to NAA and glutamate [2,18]. The NAA is hydrolyzed to aspartate and acetate by ASPA [16]. The ASPA deficiency in the patients with CD leads to accumulation of NAA in the brain and NAA aciduria [13]. Asparatoacylase gene mutation, resulting in an enzyme deficiency in the CD mouse brain, leads to low level of glutamate and GABA levels [21]. Glutamate is reversibly converted from aspartate and alanine by AST and ALT, respectively [1,6]. The low level of AST in the CD mouse brain may be one of the possible agents of inducing low glutamate level [19]. Normal activity of ALT in the CD mouse brain suggests that glutamate production from alanine is not affected. Alanine links glucose, tricarboxylic acid (TCA) cycle and aminoacid metabolism via its reaction with \( \alpha \)-ketoglutarate to form pyruvate and glutamate, when catalyzed by ALT.

GDH (EC 1.4.1.3) serves as a link between the TCA and glutamine cycles by converting glutamate into 2-oxoglutarate or reverse [15]. The enzyme, GDH links glutamate with the Kreb’s cycle. The low level of GDH is associated with vitamin B6 deficiency [4] and the low level of GDH may lead to reduced amplitude and abnormal motor nerve conduction [8]. These studies suggest that low level of GDH in the CD mouse cerebellum and brainstem may impair the normal sensorimotor function. These studies suggest that if a GDH abnormality is documented in patients with CD, treatment with vitamin B6 may improve the low level of GDH activity seen in CD.

Subsequently, \( \alpha \)-ketoglutarate is converted into succinyl CoA by KGDH. The low level of KGDHC is associated with thiamin deficiency [7]. The KGDHC loss resulting reduction of carbohydrate oxidation, impairs normal acetylcholine synthesis, leads to motor disorders [7]. The low level of KGDHC in the cerebellum and brainstem of the CD mouse brain, if documented in patients with CD, may possibly be responsible for motor abnormalities seen in CD. Since thiamin treatment improves KGDHC activity [7], efficacy of thiamin on low level of KGDHC seen in CD is to be studied.

Succinic semialdehyde dehydrogenase is the last enzyme converting GABA to succinate, prior to entering the TCA cycle. Deficiency of SSADH in the brain leads to \( \gamma \)-hydroxybutyric aciduria, mental retardation, hypotonia and seizures [7]. Mental retardation, hypotonia and seizure were also observed in patients with CD [13]. Since SSADH activity was not affected in the CD mouse brain, which catalyzes the production of succinate from GABA, it is likely that succinate level is not affected. So far no SSADH abnormality in the brain or \( \gamma \)-hydroxybutyric aciduria has been reported in patients with CD. The findings in our report suggest that patient with CD most likely have normal SSADH activity in the brain similar to the CD mouse counterpart. Therefore a different mechanism is responsible for hypotonia, mental retardation and seizure seen in CD.

References


