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Evidence that glutaric acid reduces glutamate uptake by cerebral cortex of infant rats

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Abstract

The role of excitotoxicity in the cerebral damage of glutaryl-CoA dehydrogenase deficiency (GDD) is under intense debate. We therefore investigated the in vitro effect of glutaric (GA) and 3-hydroxyglutaric (3-OHGA) acids, which accumulate in GDD, on [^{3}H]glutamate uptake by slices and synaptosomal preparations from cerebral cortex and striatum of rats aged 7, 15 and 30 days. Glutamate uptake was significantly decreased by high concentrations of GA in cortical slices of 7-day-old rats, but not in cerebral cortex from 15- and 30-day-old rats and in striatum from all studied ages. Furthermore, this effect was not due to cellular death and was prevented by *N*-acetylcysteine preadministration, suggesting the involvement of oxidative damage. In contrast, glutamate uptake by brain slices was not affected by 3-OHGA exposure. Immunoblot analysis revealed that GLAST transporters were more abundant in the cerebral cortex compared to the striatum of 7-day-old rats. Moreover, the simultaneous addition of GA and dihydrokainate (DHK), a specific inhibitor of GLT1, resulted in a significantly higher inhibition of [^{3}H] glutamate uptake by cortical slices of 7-day-old rats than that induced by the sole presence of DHK. We also observed that both GA and 3-OHGA exposure did not alter the incorporation of glutamate into synaptosomal preparations from cerebral cortex and striatum of rats aged 7, 15 and 30 days. Finally, GA in vivo administration did not alter glutamate uptake into cortical slices from 7-day-old rats. Our findings may explain at least in part why cortical neurons are more vulnerable to damage at birth as evidenced by the frontotemporal cortical atrophy observed in newborns affected by GDD.

Keywords: Glutaryl-CoA dehydrogenase deficiency; Glutamate transporters; Excitotoxicity

Introduction

Glutaryl-CoA dehydrogenase deficiency (GDD, McKusick 23167; OMIM # 231670) is an inherited metabolic disorder biochemically characterized by increased concentrations of glutaric (GA) and 3-hydroxyglutaric (3-OHGA) acids in the body fluids and in the brain (GA, 500–5000 μmol/L; 3-OHGA, 40–200 μmol/L) of affected individuals (Goodman et al., 1975; Goodman and Frerman, 2001; Kölker et al., 2004a; Sauer et al., 2006).

Neuropathology of this disease is characterized by frontotemporal cortical atrophy at birth, progressive spongy formation and attenuation of the white matter signal (leukoencephalopathy), as well as by acute bilateral destruction of caudate and putamen after encephalophatic crises precipitated by infections or vaccination in the first 36 months of age (Amir et al., 1987; Chow et al., 1988; Brismar and Ozand, 1995; Hoffmann and Zschocke, 1999). Thereafter, patients present severe dystonia–dyskinesia, sometimes associated with extreme hypotonia, rigidity and spasticity (Hoffmann and Zschocke, 1999; Strauss et al., 2003; Kölker et al., 2004a).

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Although GDD is considered a "cerebral" organic acidemia because affected individuals present essentially neurological symptoms, the underlying mechanisms of brain damage in this disorder are only partly understood. In this scenario, considerable body of evidence have indicated that excitotoxicity, oxidative stress and energy impairment caused by the metabolites accumulating in GDD may be involved in the cerebral injury (Flott-Rahmel et al., 1997; Ullrich et al., 1999; Kölker et al., 1999, 2004a,b; Latini et al., 2002, 2005a,b; Sauer et al., 2005).

With regard to excitotoxicity, postmortem examination of the basal ganglia and cerebral cortex of patients with GDD revealed postsynaptic vacuolization characteristic of glutamate-mediated brain damage indicating that this process may represent an important mechanism underlying the pathophysiology of this disorder (Goodman et al., 1977; Forstner et al., 1999; Hoffmann and Zschocke, 1999). This is in accordance with in vivo and in vitro studies demonstrating that GA and particularly 3-OHGA are excitotoxic to cultured neurons and may interact with glutamate receptors or transporters (Flott-Rahmel et al., 1997; Kölker et al., 1999, 2002a,b, 2004a; Rosa et al., 2004; Wajner et al., 2004). However, recent works did not find excitotoxic actions for 3-OHGA (Lund et al., 2004; Freudenberg et al., 2004), so that the role of excitotoxicity in GDD pathophysiology is still under intense debate.

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (Fonnum, 1984; Danbolt, 2001). Glutamate receptors are located on the surface of the neural cells. Since excessive stimulation of glutamate receptors may lead to neuronal damage, it is essential to keep the extracellular concentrations of glutamate low (Choi, 1992; Danbolt, 1994, 2001; Lipton and Rosenberg, 1994; Maragakis and Rothstein, 2001). Glutamate is removed from the synaptic cleft mainly by a glial high-affinity sodium-dependent excitatory amino acid transporter (EAAT) system (Danbolt, 2001; Schlüter et al., 2002). To date, five subtypes of glutamate transporters have been cloned: GLAST (EAAT1), GLT1 (EAAT2), EAAC1 (EAAT3), EAAT4 and EAAT5 (Danbolt, 2001). GLAST and GLT1 are predominantly localized in astrocytes and mainly responsible for the clearance of glutamate from the synaptic cleft in rat brain, while EAAC1, EAAT4 and EAAT5 appear to be mostly expressed in neurons (Rothstein et al., 1996; Berger and Hediger, 1998; Kugler and Schmitt, 1999; Danbolt, 2001; Amara and Fontana, 2002). The expression of these transporter subtypes changes along rat brain development and differs among cerebral structures (Ullensvang et al., 1997; Furuta et al., 1997; Schlüter et al., 2002). Thus, GLAST is well expressed at birth, in contrast to GLT1 whose content increases from the second to the third postnatal week in the rat brain. Both transporters are fully expressed at postnatal week 5, while GLT1 is the predominant glutamate astroglial transporter in the adult brain (Ullensvang et al., 1997).

The objective of the present investigation was to study the effects of GA and 3-OHGA, at the concentrations usually found in GDD, on glutamate transport in the CNS during rat brain development. We therefore evaluated the role of these metabolites on glutamate uptake by slices and synaptosomal preparations from cerebral cortex and striatum of rats aged 7 to 30 days. We choose these rat ages because children with GDD are more vulnerable to metabolic stress during the first 3 years (Bjugstad et al., 2000), which correspond approximately to 7–15 days of life in the rat, whereas 30-day-old rats match to young adult humans (Haberny et al., 2002). We also examined the effects of GA on the viability of cortical slices and the density of GLAST transporters in cerebral cortex and striatum at an early age.

Materials and methods

Animals and reagents

Wistar rats of 7, 15 and 30 days of life from our breeding colony were used. They were maintained at 25 °C, on a 12:12 h light/dark cycle, with free access to food and water. The "Principles of laboratory animal care" (NIH publications No. 80-23, revised 1996) were followed in all experiments and the protocols approved by the Ethics Committee for Animal Research of the Federal University of Rio Grande do Sul. All efforts were made to minimize the number of animals used and their suffering. All chemicals, including glutaric acid free acid (GA, 99% pure), were purchased from Sigma (St Louis, MO, USA), whereas 3-hydroxyglutaric acid (3-OHGA free acid, 99% pure) was prepared by Dr Ernesto Brunet (Universidad Autonoma de Madrid, Spain) and L-[^{3}H]glutamate (52 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA, USA). GA and 3-OHGA solutions were prepared on the day of the experiments. The acids were first dissolved in the buffer solutions utilized in each assay and the pH was adjusted to 7.4 with 0.5 M NaOH when necessary. For the nonspecific uptake assays, these organic acid solutions were neutralized to pH 7.4 with *N*-methyl-D-glucamine (glutamate uptake by slices) or Trizma base solution (synaptosomal glutamate uptake). Control and experimental groups (GA or 3-OHGA) contained the same concentrations of the counter ion in the incubation medium.

Glutamate uptake by cerebral cortex and striatum slices

The animals were decapitated, the brain was immediately removed and submerged in Hank's balanced salt solution (HBSS), containing 137 mM NaCl, 0.63 mM Na_2HPO_4, 4.17 mM $NaHCO_3$, 5.36 mM KCl, 0.44 mM KH_2PO_4, 1.26 mM $CaCl_2$, 0.41 mM $MgSO_4$, 0.49 mM $MgCl_2$ and 1.11 mM glucose, adjusted to pH 7.2. Cerebral cortex and striatum were dissected and tissue slices (400 μm) were obtained using a McIlwain chopper. The slices were washed with HBSS.

Glutamate uptake was performed according to Frizzo et al. (2002). Slices from 7-, 15- and 30-day-old rats were preincubated at 35 °C for 23 min in the presence or absence of GA (1–50 mM) or 3-OHGA (0.1–1 mM). Some experiments were performed in the presence of 0.1 mM or 1 mM dihydrokainic acid (DHK), which is a specific inhibitor of GLT1 transporters (Moussa et al., 2007). Incubation was carried out at 35 °C by adding 100 μM [^{3}H]glutamate (0.1 μCi) in HBSS to the assays. The reaction was stopped after 7 min by two ice-cold washing with 1 mL HBSS, immediately followed by addition of 0.5 M NaOH. Sodium