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TOXIC SYNERGISM BETWEEN QUINOLINIC ACID AND ORGANIC ACIDS ACCUMULATING IN GLUTARIC ACIDEMIA TYPE I AND IN DISORDERS OF PROPIONATE METABOLISM IN RAT BRAIN SYNAPTOSOMES: RELEVANCE FOR METABOLIC ACIDEMIAS

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Abstract—The brain of children affected by organic acidemias develop acute neurodegeneration linked to accumulation of endogenous toxic metabolites like glutaric (GA), 3-hydroxyglutaric (3-OHGA), methylmalonic (MMA) and propionic (PA) acids. Excitotoxic and oxidative events are involved in the toxic patterns elicited by these organic acids, although their single actions cannot explain the extent of brain damage observed in organic acidemias. The characterization of co-adjuvant factors involved in the magnification of early toxic processes evoked by these metabolites is essential to infer their actions in the human brain. Alterations in the kynurenine pathway (KP) – a metabolic route devoted to degrade tryptophan to form NAD^+ – produce increased levels of the excitotoxic metabolite quinolinic acid (QUIN), which has been involved in neurodegenerative disorders. Herein we investigated the effects of subtoxic concentrations of GA, 3-OHGA, MMA and PA, either alone or in combination with QUIN, on early toxic endpoints in rat brain synaptosomes. To establish specific mechanisms, we pre-incubated synaptosomes with different protective agents, including the endogenous N-methyl-D-aspartate (NMDA) receptor antagonist kynurenic acid (KA), the antioxidant S-allylcysteine (SAC) and the nitric oxide synthase (NOS) inhibitor nitro-L-arginine methyl ester (L-NAME). While the incubation of synaptosomes with toxic metabolites at subtoxic concentrations produced no effects, their co-incubation (QUIN + GA, +3-OHGA, +MMA or +PA) decreased the mitochondrial function and increased reactive oxygen species (ROS) formation and lipid peroxidation. For all cases, this effect was partially prevented by KA and L-NAME, and completely avoided by SAC. These findings suggest that early damaging events elicited by organic acids involved in metabolic acidemias can be magnified by toxic synergism with QUIN, and this process is mostly mediated by oxidative stress, and in a lesser extent by excitotoxicity and nitrosative stress. Therefore, QUIN can be hypothesized to contribute to the pathophysiology of brain degeneration in children with metabolic acidemias.

Key words: organic acidemias, excitotoxicity, oxidative stress, mitochondrial dysfunction, cell damage, toxic synergism.

INTRODUCTION

The hereditary metabolic disorders known as organic acidemias (OA) are characterized by a blockage of the aberrant catabolism of amino acids and lipids due to a deficient activity of specific enzymes. These alterations are responsible for the accumulation and high urinary excretion of potentially toxic organic acids (Bodamer et al., 2006). Neurological symptoms and brain abnormalities are seen in patients suffering from OA. Glutaric acidemia type I (GA I), methylmalonic acidemia (MMAcidemia) and propionic acidemia (PAcidemia) have a relatively high prevalence in the population, all with a severe clinical presentation in the neonatal period.

GA I is known to be caused by a deficiency of glutaryl-CoA dehydrogenase (GDD, McKusick 23167; OMIM # 231670) activity, resulting in the accumulation of glutaric (GA, 500–5000 μmol/L) and 3-hydroxyglutaric (3-OHGA, 40–200 μmol/L) acids in the CNS (Kölker et al., 2004; Sauer et al., 2006). Among its pathological features are

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Abbreviations: GA, glutaric acid; GA I, glutaric acidemia type I; GDD, glutaryl-CoA dehydrogenase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; KA, kynurenic acid; KP, kynurenine pathway; L-NAME, L-nitro-L-arginine methyl ester; MDA, Malondialdehyde; MMA, methylmalonic acid; MMAcidemia, methylmalonic acidemia; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMDA, N-methyl-D-aspartate; NMDAr, NMDA receptors; NOS, nitric oxide synthase; OA, organic acidemias; PA, propionic acid; PAcidemia, propionic acidemia; QUIN, quinolinic acid; RNS, reactive nitrogen species; ROS, reactive oxygen species; SAC, S-allylcysteine; TBA, thiobarbituric acid; 3-OHGA, 3-hydroxyglutaric acid.

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a fronto-temporal cortical atrophy at birth, progressive spongy formation, leukoencephalopathy and acute damage of the caudate/putamen occurring between 6 months and 4 years of age (Amir et al., 1987; Hoffmann and Zschocke, 1999). Experimental evidence suggests that accumulating organic acids induce excitotoxicity, oxidative stress and energy metabolism impairment (Flott-Rahmel et al., 1997; Latini et al., 2002; de Oliveira Marques et al., 2003; Kölker et al., 2004; Wajner et al., 2004; Sauer et al., 2005; Latini et al., 2005a,b; Ferreira et al., 2007; Rosa et al., 2007), although the precise pathogenic mechanisms occurring in GA I have not been fully described.

In turn, MMAcidemia and PAcidemia are caused by severe deficiencies of methylmalonyl-CoA mutase (EC 5.4.99.2) and propionyl-CoA carboxylase (EC 6.4.1.3) activities, respectively. MMAcidemia is biochemically characterized by accumulation of methylmalonic acid (MMA) (1-2.5 mmol/L), whereas PAcidemia by propionic acid (PA) (5 mmol/L) in blood. Clinical manifestations of these two OA comprise lethargy, psychomotor delay/mental retardation, focal and generalized convulsions, vomiting, dehydration, hepatomegaly, hypotonia, and encephalopathy further leading to coma and death (Deodato et al., 2006; Hauser et al., 2011). Disrupted myelination revealing progressive cortical atrophy, as well as histopathological injury of the basal ganglia can be observed (Brismar and Ozand, 1994; Chemelli et al., 2000; Harting et al., 2008). For both acidemias, brain damage has been related to the toxic actions produced by their corresponding accumulating metabolites. This suggestion is based on experimental evidence demonstrating that MMA can cause brain mitochondrial energy metabolism disruption, as well as redox status and glutamatergic transmission alterations (Kölker et al., 2006; Sauer et al., 2006, 2010; Stellmer et al., 2007), whereas PA has also been shown to exert toxic effects in the rat brain (Wyse et al., 1998; Brusque et al., 1999; de Mattos-Dutra et al., 2000; Fontella et al., 2000; Pettenuzzo et al., 2002; Trindade et al., 2002; Rigo et al., 2006; Ribas et al., 2010a,b).

Tryptophan catabolism and NAD^+ synthesis occur in cells from different tissues through the kynurenine pathway (KP). This metabolic route is relevant for biomedical research as neuroactive intermediary metabolites are synthesized throughout (reviewed by Pérez-De La Cruz et al. (2007)), some of which are involved in pathogenic processes of neurological disorders, including Huntington's disease (HD) (reviewed by Schwarcz et al., 2010, 2012). One of these KP metabolites, quinolinic acid (QUIN or 2,3-pyridine dicarboxylic acid) is an endogenous N-methyl-D-aspartate receptor (NMDAr) agonist (Stone et al., 2003). QUIN induces excitotoxicity in animal models and cell cultures, provoking enhanced intracellular $[Ca^{2+}]$, augmented levels of extracellular glutamate, increased reactive oxygen species (ROS) and reactive nitrogen species (RNS) formation, decreased activity and expression of antioxidant systems, oxidative stress, stimulated protease activity and cell death (Rios and Santamaría, 1991; Rodríguez-Martínez et al., 2000; Tavares et al., 2000; Braidy et al., 2009, 2010; Pérez-De La Cruz et al., 2010). Moreover, QUIN could exert a pathogenic role in different neurodegenerative disorders since increased levels of this metabolite have been described in these pathological conditions (Schwarcz et al., 2010).

When considered separately, the toxic profiles characterized at the experimental level for the organic acids accumulating in OA and for QUIN in human neurological disorders could be not sufficient to explain the extent of cell and tissue damage produced by them *per se*, yielding the assumption that additional and additive mechanisms could account for the toxic profiles of these metabolites. Therefore, the aim of this work was to investigate whether GA, 3-OHGA, MMA or PA can exert synergic toxic effects with QUIN when tested in rat brain synaptosomes at subtoxic concentrations, upon the hypothesis that QUIN might eventually contribute to neurodegenerative processes in OA.

EXPERIMENTAL PROCEDURES

Reagents

GA, MMA, PA, QUIN, HEPES, thiobarbituric acid (TBA), kynurenic acid (KA), L-nitro-L-arginine methyl ester (L-NAME), malondialdehyde (MDA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and other reagents were obtained from Sigma–Aldrich Chemical Co. (St Louis, MO, USA). Dr. Ernesto Brunet (Universidad Autónoma de Madrid, Spain) kindly supplied 3-OHGA. Other reagents were obtained from other well-known commercial sources. S-allylcysteine (SAC) was synthesized according to previous reports (García et al., 2008, 2014).

Animals

Male Wistar adult (250–300 g) rats were used throughout the study. Animals ($N = 40$) were obtained from the vivarium of the Universidad Nacional Autónoma de México. All rats were housed five per cage and provided with food and water *ad libitum* under constant conditions of temperature (25 ± 3 °C), humidity and light (12:12-h light:dark schedule). All animal manipulations were carried out following the "Guidelines for the Use of Animals in Neuroscience Research" from the Society of Neuroscience, the local Ethics Committees, and in compliance with the ARRIVE guidelines.

Isolation of brain synaptosomal P2 fractions and treatments

Isolation of synaptosomal P2 fractions from rat brains was carried out according to Lopachin et al. (2009), with modifications (Rangel-López et al., 2015). All brains (without cerebellum) were surgically removed, weighted, transferred to ice-cooled PBS (pH 7.4), and homogenized in 10 volumes (g/ml) of sucrose (0.32 M). The cerebellum was excluded because this brain region is generally not altered in GA I, MMAcidemia and PAcidemia, whose accumulating metabolites were tested in our work. Homogenates were centrifuged for 10 min at 1073 × *g* (4 °C) and