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BRAIN RESEARCH

Research Report

Creatine administration prevents Na^+,K^+-ATPase inhibition induced by intracerebroventricular administration of isovaleric acid in cerebral cortex of young rats

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ARTICLE INFO

Article history:
Accepted 6 January 2009
Available online 13 January 2009

Keywords:
Isovaleric acidemia
Energy metabolism
Organic acidemia
Na^+
K^+-ATPase
Cerebral cortex
Creatine

ABSTRACT

Isovaleric acidemia (IVAcidemia) is an inborn error of metabolism due to deficiency of isovaleryl-CoA dehydrogenase activity, leading to predominant accumulation of isovaleric acid (IVA). Patients affected by IVAcidemia suffer from acute episodes of encephalopathy, whose underlying mechanisms are poorly known. In the present study we investigated whether an intracerebroventricular injection of IVA could compromise energy metabolism in cerebral cortex of young rats. IVA administration significantly inhibited $^{14}CO_2$ production from acetate (22%) and citrate synthase activity (20%) in cerebral cortex homogenates prepared 24 h after injection. However, no alterations of these parameters were observed 2 h after injection. In contrast, no significant differences were found in the activities of succinate dehydrogenase, isocitrate dehydrogenase, electron transfer chain complexes or creatine kinase in rats sacrificed 2 or 24 h after IVA administration. Moreover, IVA injection significantly inhibited Na^+,K^+-ATPase activity (25%) in cerebral cortex of rats 2 or 24 h after IVA administration, while pre-treatment of rats with creatine completely prevented the inhibitory effects of IVA on Na^+,K^+-ATPase. In conclusion, *in vivo* administration of IVA inhibits the citric acid cycle probably through the enzyme citrate synthase, as well as Na^+,K^+-ATPase, a crucial enzyme responsible for maintaining the basal potential membrane necessary for a normal neurotransmission. It is presumed that inhibition of these activities may be involved in the pathophysiology of the neurological dysfunction of isovaleric academic patients. The present findings are of particular interest because treatment with creatine supplementation may represent a potential novel adjuvant therapeutic strategy in IVAcidemia.

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Abbreviations: IVAcidemia, Isovaleric acidemia; IVD, Isovaleryl-CoA dehydrogenase; IVA, Isovaleric acid; IVG, Isovalerylglycine; icv, Intracerebroventricular; CAC, Citric acid cycle; CS, Citrate synthase; SDH, Succinate dehydrogenase; IDH, Isocitrate dehydrogenase; ETC, Electron transfer chain; CNS, Central nervous system; CK, Creatine kinase; SPSS, Statistical Package for the Social Sciences

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doi:10.1016/j.brainres.2009.01.005

1. Introduction

Isovaleric acidemia (IVAcidemia, MIM 243500) is an autosomal recessive inherited metabolic disorder of leucine metabolism caused by a deficiency of isovaleryl-CoA dehydrogenase (IVD; E.C. 1.3.99.10), a homotetrameric mitochondrial flavoenzyme of the family of acyl-CoA dehydrogenases (Tanaka et al., 1966). IVD catalyses the conversion of isovaleryl-CoA to 3-methylcrotonyl-CoA, and its deficiency leads to accumulation of the isovaleryl-CoA derivatives isovaleric acid (IVA) and their carnitine (isovalerylcarnitine) and glycine (isovalerylglycine, IVG) derivatives, as well as 3-hydroxyisovaleric acid (3-OHIVA). The amount of free IVA during episodes of acute decompensation can achieve several hundred times the normal values (up to 5 mM) (Sweetman and Williams, 2001) in the blood, while isovalerylcarnitine and IVG are the hallmark of this disorder in plasma and urine, respectively. IVAcidemia is considered a severe, potentially life-threatening disorder that manifests with acute neonatal encephalopathy with recurrent episodes of vomiting, lethargy, coma in about half of the affected individuals, and with poor feeding, tachypnea, dehydration and varying degrees of developmental delay in the other half of the patients (Lee et al., 2007; Lin et al., 2007; Sweetman and Williams, 2001). Neuropathological abnormalities include alterations in the globi pallidi and corticospinal tracts of the mesencephalon on MRI (Sogut et al., 2004), as well as diffuse cerebral edema, massive cerebellar hemorrhage, upward transtentorial herniation, and focal degeneration of clusters of glial cells in white and gray matter (Fischer et al., 1981).

The incidence of IVAcidemia varies from 1/62,500 live births in Germany (Schulze et al., 2003) to 1/250,000 in the United States (Chace et al., 2003; Zytkovicz et al., 2001). The diagnosis of IVAcidemia is based on the presence of high amounts of predominantly IVG and 3-OHIVA in urine, with other metabolites appearing at lower amounts (Tanaka and Isselbacher, 1967; Tanaka et al., 1980).

Despite the large number of accumulating and excreted metabolites in this disorder, their occurrence do not explain the clinical symptoms of the affected patients, so that investigation on the role of these metabolites on the central nervous system (CNS) function and development will eventually lead to a better understanding of the relationship between the clinical conditions and their biochemical abnormalities, as well as the pathophysiology of the cerebral damage in this disorder. Furthermore, although neurological alterations are pronounced in IVAcidemia, the exact mechanisms of brain damage in this disease are poorly understood. It was recently reported that IVG, but not IVA, induces oxidative stress in cerebral cortex of rats (Solano et al., 2008). Furthermore, we previously described that *in vitro* exposure of cortical homogenates to IVA provoked a significant reduction of Na^+, K^+-ATPase activity (Ribeiro et al., 2007).

Since IVA is the major accumulating metabolite in IVAcidemia and is produced inside the mitochondria, it seems appropriate to evaluate its role on cellular energy metabolism, which basically occurs in this organelle. Thus, the present study was undertaken to investigate the influence of *in vivo* intracerebroventricular (icv) administration of IVA to rats on important parameters of energy production, transfer and utilization in order to clarify the mechanisms involved in the neuropathology of IVAcidemia. We evaluated the activity of the citric acid cycle (by measuring $^{14}CO_2$ production from [1-^{14}C]acetate and the activities of citrate synthase, isocitrate dehydrogenase and succinate dehydrogenase), as well as the activities of the electron transfer chain complexes I–IV, creatine kinase and Na^+,K^+-ATPase and respiratory parameters in cerebral cortex of young rats at distinct periods after IVA injection. We also examined the role of creatine on the bioenergetic alterations caused by IVA to clarify the involved mechanisms of its effects.

2. Results

We first examined the influence of IVA treatment on citric acid cycle (CAC) rate by measuring $^{14}CO_2$ production from [1-^{14}C]acetate in total homogenates prepared from cerebral cortex of rats that received IVA or NaCl 2 or 24 h after injection. $^{14}CO_2$ production from acetate was significantly reduced by about 22% in cerebral cortex of IVA-injected animals sacrificed 24 h after IVA administration ($P<0.01$). However, no differences in $^{14}CO_2$ formation were found 2 h after IVA injection (Fig. 1).

The activities of some CAC enzymes were determined in cerebral cortex homogenates prepared 2 or 24 h after IVA or NaCl injection to clarify the reduction of CAC functioning. The activity of citrate synthase (CS) remained unmodified in cerebral cortex of IVA-injected rats sacrificed 2 h after IVA administration but was significantly reduced (up to 20%,

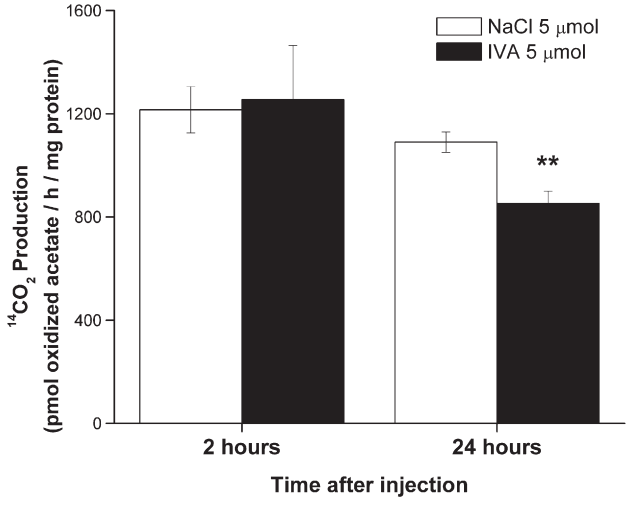

Fig. 1 – $^{14}CO_2$ production from [1-^{14}C]acetate in cerebral cortex obtained at different periods (2–24 h) after icv administration of 5 μmol isovaleric acid (IVA) or NaCl. Animals received NaCl or IVA into each the lateral ventricule as described in Experimental procedures. Data are represented as mean ± SEM for 4–5 independent experiments performed in triplicate and are expressed as pmol oxidized acetate/h/mg protein. $^{**}P<0.01$ compared to NaCl (Student's *t* test for unpaired samples).