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Inhibition of brain energy metabolism by the α -keto acids accumulating in maple syrup urine disease

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Abstract

Neurological dysfunction is a common finding in patients with maple syrup urine disease (MSUD). However, the mechanisms underlying the neuropathology of brain damage in this disorder are poorly known. In the present study, we investigated the effect of the *in vitro* effect of the branched chain α -keto acids (BCKA) accumulating in MSUD on some parameters of energy metabolism in cerebral cortex of rats. [^{14}C] production from [^{14}C] acetate, glucose uptake and lactate release from glucose were evaluated by incubating cortical prisms from 30-day-old rats in Krebs–Ringer bicarbonate buffer, pH 7.4, in the absence (controls) or presence of 1–5 mM of α -ketoisocaproic acid (KIC), α -keto- β -methylvaleric acid (KMV) or α -ketoisovaleric acid (KIV). All keto acids significantly reduced $^{14}\text{CO}_2$ production by around 40%, in contrast to lactate release and glucose utilization, which were significantly increased by the metabolites by around 42% in cortical prisms. Furthermore, the activity of the respiratory chain complex I–III was significantly inhibited by 60%, whereas the other activities of the electron transport chain, namely complexes II, II–III, III and IV, as well as succinate dehydrogenase were not affected by the keto acids. The results indicate that the major metabolites accumulating in MSUD compromise brain energy metabolism by blocking the respiratory chain. We presume that these findings may be of relevance to the understanding of the pathophysiology of the neurological dysfunction of MSUD patients.

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1. Introduction

Maple syrup urine disease (MSUD), or branched chain keto aciduria (BCKA), is an inborn error of metabolism caused by severe deficiency of the branched chain α -ketoacid dehydrogenase complex (BCKAD, E.C. 1.2.4.4) activity [1]. The inability of this enzyme complex to oxidize α -ketoiso-

caproic acid (KIC), α -keto- β -methylvaleric acid (KMV) and α -ketoisovaleric acid (KIV) leads to tissue accumulation of these metabolites and their precursor amino acids leucine, isoleucine and valine, respectively, in the affected individuals. Patients with MSUD present poor feeding, apnoea, ketoacidosis, convulsions, coma and psychomotor delay. Central nervous system (CNS) imaging reveals low density of white matter corresponding to hypomyelination/demyelination and cerebral atrophy. The disease is severe enough to cause a fatal outcome in a significant number of patients if not diagnosed and treated promptly. Those who survive present a variable degree of mental retardation [1].

The reduction of branched chain amino acids (BCAA) has been the main target for treating MSUD patients and diet has been the mainstay of treatment [2], whenever the disease is not responsive to thiamine [1]. Although this approach has contributed decisively to the survival of the

Abbreviations: MSUD, maple syrup urine disease; BCAA, branched chain amino acids; BCKA, branched chain keto acids; BCKAD, branched chain L- α -keto acid dehydrogenase; KIC, α -ketoisocaproic acid; KMV, α -keto- β -methylvaleric acid; KIV, α -ketoisovaleric acid; CNS, central nervous system; GABA, gamma-aminobutyric acid

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affected individuals, a considerable number of the “well-treated” patients present a variable degree of developmental delay/mental retardation accompanied by chronic brain structural changes. This may possibly be because the pathophysiology of the neurological dysfunction of MSUD is poorly known. However, there is a large body of evidence associating defective leucine metabolism and the neurological symptoms of these patients. In fact, leucine and/or its keto acid, α -ketoisocaproate, have been considered to be the main neurotoxic metabolites in MSUD [1,3,4]. Accordingly, we have previously shown that early chronic subcutaneous administration of high doses of leucine to young rats induces learning/memory deficits verified in the open field and in the shuttle avoidance tasks during adult age [5], indicating that high leucine levels during brain development may significantly contribute to the learning and memory deficits. Furthermore, convulsive properties for KIV have been also demonstrated, suggesting that this metabolite is probably involved in the genesis of the convulsions characteristic of MSUD patients [6]. In addition, it has been postulated that brain energy deficit provoked by the metabolites accumulating in MSUD [2,7–10], competition of KIV, KIC and their hydroxyderivatives with L-glutamate for decarboxylation and the consequent reduction of γ -aminobutyric acid (GABA) concentration [11], impairment of myelin development [12–15], low plasma and brain levels of essential amino acids [16,17] and reduced brain uptake of essential amino acids leading to decreased neurotransmitter synthesis [18] may contribute to brain injury. A recent study observed that the BCKA that accumulate in MSUD trigger apoptosis in glial and neuronal cells, being more toxic than the corresponding BCAA [19]. These investigators also found a reduction in cell respiration, as measured by cellular oxygen consumption. Although some of these results indicate that deficit of energy metabolism is involved in neural cell damage, the exact mechanisms underlying impairment of energy metabolism in brain cells are not yet established.

Therefore, in the present study, we investigated the *in vitro* effect of the α -keto acids, which primarily accumulate in MSUD on some parameters of energy metabolism in cerebral cortex of rats. We evaluated CO_2 production, from [U- ^{14}C] acetate, lactate release and glucose uptake, as well as the activities of the respiratory chain complexes, in the hope to contribute to the understanding of the mechanisms underlying the neurological damage present in MSUD patients.

2. Material and methods

2.1. Reagents

All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA, except for the radiolabeled compound [U- ^{14}C] acetate, which was purchased from Amersham International plc, UK.

2.2. Animals

Wistar rats obtained from the Central Animal House of the Departamento de Bioquímica, ICBS, UFRGS were used. Rats were kept with dams until weaning at 21 days of age. The animals had free access to water and to a standard commercial chow and were maintained on a 12:12 h light/dark cycle in an air-conditioned constant temperature ($22 \pm 1^\circ\text{C}$) colony room. The “Principles of Laboratory Animal Care” (NIH publication 85-23, revised 1985) were followed in all the experiments and the experimental protocol was approved by the Ethics Committee for Animal Research of the Federal University of Rio Grande do Sul, Porto Alegre.

2.3. Tissue and homogenate preparation

Thirty-day-old rats were sacrificed by decapitation, the brain was rapidly removed and the cerebral cortex was isolated. Cerebral cortex was cut into two perpendicular directions to produce 400- μm -wide prisms using a McIlwain chopper. Prisms were pooled, weighed and used for $^{14}\text{CO}_2$ production, lactate release and glucose uptake assays. For the respiratory chain enzyme activities determination, cerebral cortex was homogenized (1:10, w/v) in SETH buffer, pH 7.4 (250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, 50 UI ml^{-1} heparin). The homogenates were centrifuged at $800 \times g$ for 10 min and the supernatants kept at -70°C until used for enzyme activity determination. The period between homogenate preparation and enzyme analysis was always less than 5 days. The various parameters of energy metabolism were determined in the presence of various concentrations (1.0–5.0 mM) of KIC, KMV and KIV according to standard methods. Control groups did not contain any acid in the incubation medium.

2.4. $^{14}\text{CO}_2$ production

Cortical prisms (50 mg) were added to small flasks (11 cm^3) containing 0.5 ml Krebs–Ringer bicarbonate buffer, pH 7.4. Flasks were pre-incubated in a metabolic shaker at 37°C for 15 min (90 oscillations min^{-1}). After pre-incubation, 0.2 μCi [U- ^{14}C] acetate and 0.5 mM of the unlabeled acetate were added to the incubation medium. In some experiments, we measured CO_2 production from [1,5- ^{14}C] citrate (0.2 μCi) in the presence of 0.5 mM unlabeled citrate. KIC, KMV or KIV were added to the incubation medium at final concentrations of 1.0 or 5.0 mM. The controls did not contain the α -keto acids. The flasks were gassed with a O_2/CO_2 (95:5) mixture and sealed with rubber stoppers and Parafilm M. Glass center wells containing a folded 65 mm/5 mm piece of Whatman 3 filter paper were hung from the stoppers. After 60 min of incubation at 37°C in a metabolic shaker (90 oscillations min^{-1}), 0.1 ml of 50% trichloroacetic acid was added to the medium and 0.1 ml of benzethonium hydroxide was added to the center

of the wells with needles introduced through the rubber stopper. The flasks were left to stand for 30 min to complete $^{14}\text{CO}_2$ trapping and then opened. The filter papers were removed and added to vials containing scintillation fluid, and radioactivity was counted [20].

2.5. Lactate release and glucose uptake

Cortical prisms (100 mg) were incubated under an O_2/CO_2 (19:1) mixture at 37 °C for 60 min in Krebs–Ringer bicarbonate buffer, pH 7.0 containing 5.0 mM glucose (in a total volume of 1 ml) in a metabolic shaker (90 oscillations min^{-1}) [21]. After incubation, two volumes of 0.6 N perchloric acid were immediately added to the prisms and the excess of perchloric acid was precipitated as a potassium salt by the addition of one volume of a solution containing 0.5 N KOH, 0.1 M imidazol, and 0.1 KCl. The solution was then centrifuged for 5 min at $800 \times g$. Glucose and lactate were measured in the medium before and after incubation by the glucose oxidase method [22] and by the lactase–peroxidase method [23], respectively. Glucose uptake was determined by subtracting the amount after incubation from the total amount measured before incubation, whereas lactate release was calculated by subtracting lactate content found after incubation from the amount found before incubation. Lactate concentrations in the medium at the beginning of the incubation were practically nilled.

2.6. Respiratory chain enzyme activities

The activities of succinate-DCIP-oxireductase (complex II) and succinate/cytochrome *c* oxireductase (complex II + CoQ + complex III) were determined according to the method of Fisher et al. [24] and the activity of succinate/phenazine oxireductase (soluble succinate dehydrogenase—SDH) according to Sorensen and Mahler [25]. The activity of cytochrome *c* oxidase (complex IV) was measured by the method of Rustin et al. [26], whereas the activity of NADH/cytochrome *c* oxireductase (complex I + CoQ + complex III) was assayed according to the method described by Schapira et al. [27]. The activity of ubiquinol/cytochrome *c* oxireductase (complex III) was determined according to Birch-Machin et al. [28].

2.7. Protein determination

Protein was measured by the method of Lowry et al. [29] using bovine serum albumin as standard.

2.8. Statistical analysis

Unless otherwise stated, results are presented as means \pm standard deviation. All assays were performed in duplicate and the mean was used for statistical analysis. Data were analyzed using the one-way analysis of variance (ANOVA) followed by the post hoc Duncan multiple range test when *F*

was significant. For analysis of dose-dependent effect, linear regression was used. The Student's *t*-test for paired samples was also used for comparison of two means in some experiments. Differences between the groups were rated significant at $P < 0.05$.

3. Results

First we investigated the in vitro effect of KIC, KMV and KIV on CO_2 production from $[\text{U-}^{14}\text{C}]$ acetate in the cerebral cortex from 30-day-old rats. It can be seen in Fig. 1 that all keto acids significantly inhibited CO_2 production at doses of

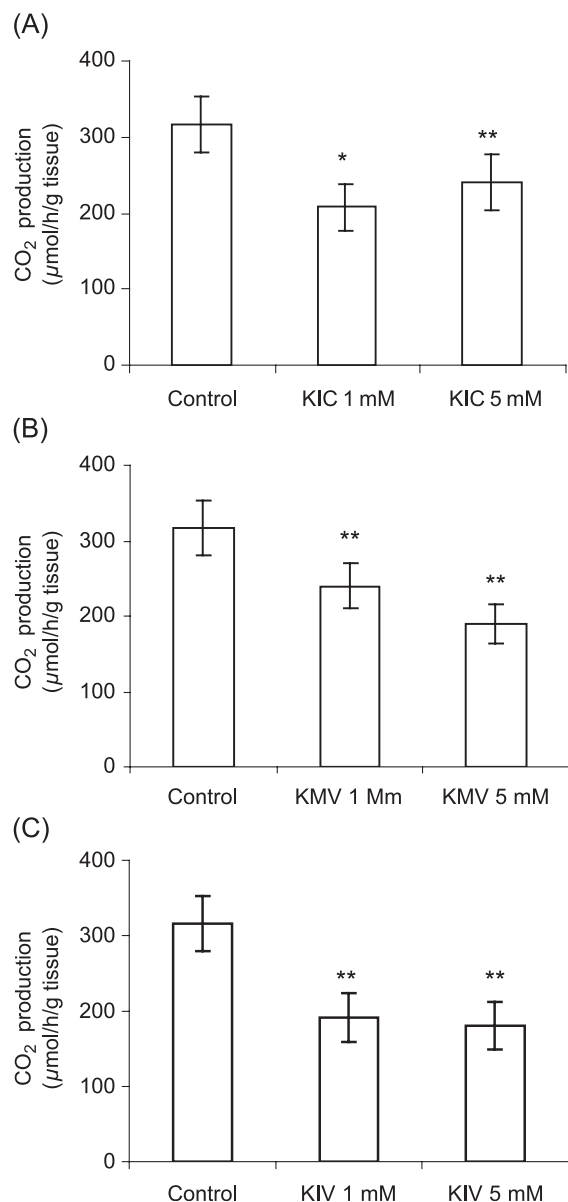


Fig. 1. In vitro effect of KIC, KMV and KIV on CO_2 production from acetate in cerebral cortex from young rats. Data are expressed as means \pm S.D. for four independent experiments performed in duplicate. * $P < 0.05$, ** $P < 0.01$ compared to control (Duncan multiple range test).

1 mM and higher with maximal inhibition around 42% (KIC: [$F(2,9)=10.349$; $P<0.01$]; KMV: [$F(2,9)=16.781$; $P<0.01$]; KIV: [$F(2,9)=20.146$; $P<0.01$]). The effect was concentration-dependent for KMV ($\beta = -0.7996$; $P<0.01$) and KIV ($\beta = -0.6408$; $P<0.05$). We also tested the effect of 5.0 mM KIC on CO_2 production from [$1,5\text{-}^{14}\text{C}$] citrate. We verified that the acid significantly inhibited CO_2 formation [$t(17)=21.41$; $P<0.001$] (means: control = 1388.94; KIC = 1161.47).

Next we investigated whether the keto acids could affect glycolysis, by assessing the effect of these compounds on glucose uptake and lactate release in the brain tissue. As can be observed in Fig. 2, KIC [$F(2,12)=17.652$; $P<0.01$],

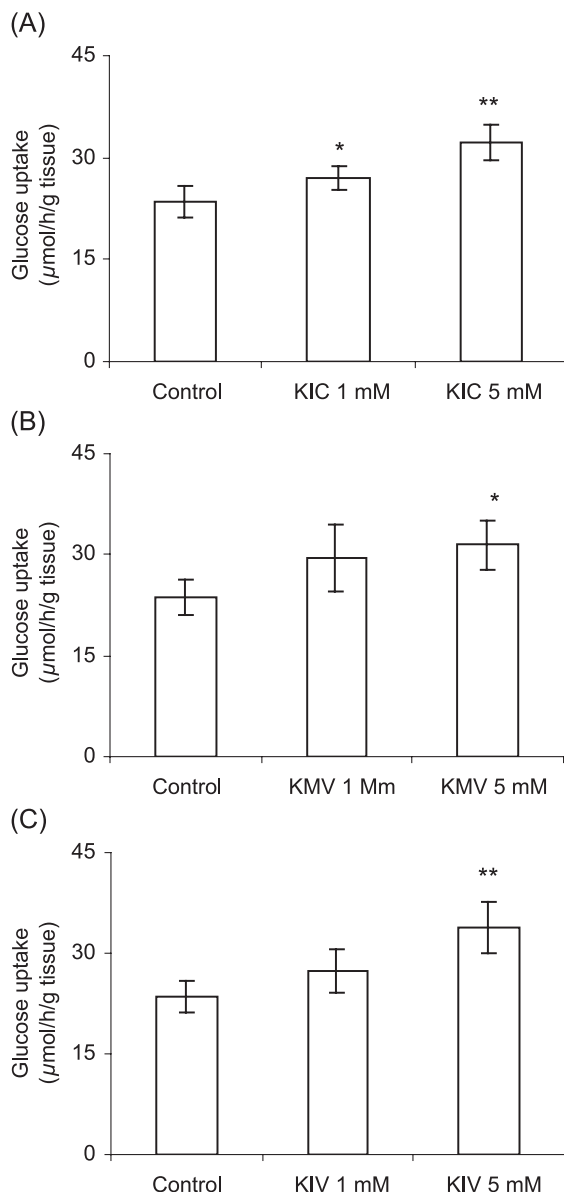


Fig. 2. In vitro effect of KIC, KMV and KIV on glucose utilization by cerebral cortex from young rats. Data are expressed as means \pm S.D. for four or five independent experiments performed in duplicate. * $P<0.05$, ** $P<0.01$ compared to control (Duncan multiple range test).

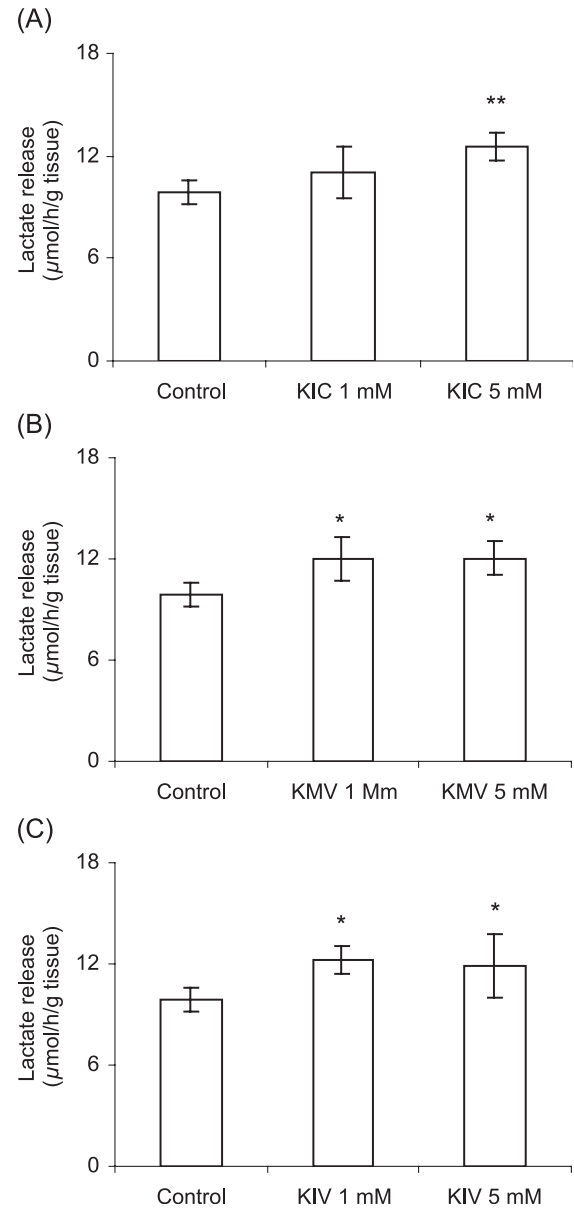


Fig. 3. In vitro effect of KIC, KMV and KIV on lactate release from glucose in cerebral cortex from young rats. Data are expressed as means \pm S.D. for four or five independent experiments performed in duplicate. * $P<0.05$, ** $P<0.01$ compared to control (Duncan multiple range test).

KMV [$F(2,9)=4.326$; $P<0.05$] and KIV [$F(2,12)=12.631$; $P<0.01$] significantly increased glucose uptake by cortical prisms with maximal stimulation around 45%. The effect occurred at doses of 1.0 mM and higher and in a dose-dependent manner for KIC ($\beta = 0.8446$; $P<0.0001$) and KIV ($\beta = 0.8082$; $P<0.001$).

Fig. 3 shows the in vitro effect of KIC, KMV and KIV on lactate release by cerebral cortex of rats. It can be observed that lactate release was significantly stimulated by KIC [$F(2,12)=7.873$; $P<0.01$], KMV [$F(2,10)=7.700$; $P<0.01$] and KIV [$F(2,12)=5.253$; $P<0.05$] up to 27% at concentrations as low as 1 mM for KMV and KIV, being the effect dose-dependent only for KIC ($\beta = 0.7246$; $P<0.01$).

Table 1

In vitro effect of KIC, KMV and KIV on the activities of the mitochondrial respiratory chain complexes in cerebral cortex from young rats

Respiratory chain complexes	Control	KIC (1 mM)	KMV (1 mM)	KIV (1 mM)
Complex I+CoQ+III	20 ± 6.3	9.9 ± 2.1*	8.1 ± 1.7*	9.9 ± 4.7*
Succinate dehydrogenase	25 ± 3.0	25 ± 2.8	24 ± 2.1	23 ± 1.1
Succinate DCIP oxidoreductase (II)	12 ± 0.7	12 ± 1.8	13 ± 2.1	13 ± 2.4
Complex II+CoQ+III	52 ± 1.7	52 ± 2.4	53 ± 3.2	53 ± 2.4
Complex III	92 ± 19	80 ± 16	73 ± 5.1	83 ± 3.7
Cytochrome <i>c</i> oxidase (IV)	132 ± 13	148 ± 16	145 ± 15	139 ± 5.0

Data are expressed as means ± S.D. for three or five independent experiments performed in triplicate.

* $P < 0.05$ compared to control (Duncan multiple range test).

We also assessed the effect of KIC, KIV and KMV on the respiratory chain enzyme activities in an attempt to elucidate the biochemical defect responsible for the inhibition of aerobic glycolysis (lower CO₂ formation) and activation of anaerobic glycolysis (increased lactate release) by the α -keto acids accumulating in MSUD. It can be seen in Table 1 that all α -keto acids significantly inhibited complex I–III [$F(3,16) = 8.492$, $P < 0.001$] at 1 mM concentration with maximal inhibition around 60%, without affecting the activity of the other respiratory chain complexes (complex II: [$F(3,8) = 0.225$; $P > 0.05$], SDH: [$F(3,8) = 0.253$; $P > 0.05$], complex II–III: [$F(3,8) = 0.190$, $P > 0.05$], complex III: [$F(3,8) = 1.105$, $P > 0.05$], complex IV: [$F(3,8) = 0.925$, $P > 0.05$]).

4. Discussion

Tissue accumulation and high urinary excretion of KIC, KMV and KIV occurs in MSUD, an inherited metabolic disorder caused by deficiency of the BCKAD activity. Although neurological symptoms are predominant in this disease, the mechanisms responsible for the brain damage in the affected individuals are poorly established. The understanding of the exact biochemical alterations in brain may possibly contribute to a better therapeutic management of MSUD patients.

Since the BCKA, which are converted by transamination to their respective BCAA leucine, valine and isoleucine, are the major metabolites accumulating in MSUD, in the present study we studied the in vitro effect of KIC, KIV and KMV on various biochemical parameters of energy metabolism in cerebral cortex of rats. We investigated the activity of the Krebs cycle by measuring the CO₂ generated from acetate and the anaerobic metabolism by measuring lactate release from glucose. We initially verified a significant reduction of CO₂ formation from acetate by over 40% in cortical prisms incubated in the presence of each α -keto acid. Since the BCKA are monocarboxylic acids and therefore use the same membrane transporter as acetate (monocarboxylic transporter), the inhibition observed could be due

to a competition between the BCKA and acetate [30]. However, we also verified that CO₂ formation from citrate was also inhibited by KIC, the BCKA found in greater concentrations and that easily cross cell membranes. Since citrate uses the tricarboxylic transporter, it is feasible that the reduction of CO₂ caused by the BCKA reflects a true inhibition of the Krebs cycle or the respiratory chain. It is interesting to observe that all keto acids significantly inhibited CO₂ production at 1 mM concentration.

Lactate is produced in considerable amounts by the brain, and more specifically by glial cells. Lactate release, which reflects lactate production, was significantly stimulated in the presence of all metabolites by around 20–30%. Therefore, it may be concluded that the α -keto acids accumulating in MSUD reduce the Krebs cycle activity and increase anaerobic glycolysis, indicating that they may alter the energy metabolism in brain cortex of rats.

We have also verified that the BCKA provoked a significant increase of glucose uptake by around 40% in brain cortical prisms, and this is expected once anaerobic metabolism, which was activated by these compounds, uses more glucose because less ATP is produced. It should be stressed that glucose is the major substrate utilized for neural cell metabolism.

The next experiments were performed in order to evaluate the effect of the BCKA on the respiratory chain function by measuring the activities of complexes I–IV in cerebral cortex of rats. We verified that all BCKA significantly inhibited complex I–III by around 50–60%, without altering the activities of complexes II, II–III, III, IV and succinate dehydrogenase. Since complex III was not affected by the BCKA, it may be presumed that complex I activity was blocked by the BCKA. In summary, our findings indicate that the BCKA accumulating in MSUD impair brain energy metabolism, possibly due to inhibition of the respiratory chain complex I. These results confirm other reports showing that energy metabolism is compromised by the metabolites accumulating in MSUD [2,7–10,19]. They are also in agreement with a recent report showing that the BCKA markedly reduced cell respiration but did not impair the rate of mitochondrial succinate oxidation in neuronal and glial cells [19]. Since succinate enters the respiratory chain via complex II, these investigators conclude that the BCKA did not affect the function of respiratory chain complexes II, III and IV. Taken together, these observations and our present investigation, it is feasible that cell respiration is inhibited by BCKA at complex I, and this is possibly the mechanism through which the Krebs cycle is blocked and the anaerobic glycolysis is stimulated in the presence of these metabolites.

Interestingly, isolated human complex I defects have been identified in a number of neurodegenerative diseases, including Parkinson's disease, focal dystonia and Leber's hereditary optic neuropathy [31], a fact that suggests that the activity of this respiratory chain complex is important for normal CNS function. Another interesting observation was that the administration of 1-methyl-4-phenyl 1,2,3,6 tetra-

hydropyridine (MPTP), which is an inductor of Parkinsonism in animals, inhibits complex I activity probably by oxidative damage of complex I since the inhibition is prevented by free radical scavengers [32,33]. Furthermore, complex I inhibition induces free radical generation from the respiratory chain, suggesting a self-amplifying cycle of complex I deficiency that may result in progressive cell damage [34]. In this context, we have recently demonstrated that the BCKA elicits oxidative stress in brain [35]. Therefore, it is possible that the inhibition of complex I by these compounds may have occurred via oxidation of essential subunits of this complex. The observation that the activity of NADH-CoQ oxidoreductase (complex I) is very sensitive to reactive oxygen species [36–38] corroborates with this hypothesis. It seems that the reduction of complex I activity depends either on a reversible oxidation of sulfhydryl groups or on an irreversible oxidative modification of [4Fe–4S] clusters of the enzyme [38].

In conclusion, we present evidence of an electron transport chain inhibition, probably at complex I in the brain caused by the BCKA accumulating in MSUD at the concentrations usually found in the affected individuals [39]. We observed that 1 mM concentration of the various BCKA compromised brain energy metabolism. Although lower concentrations of these compounds were not used in our assays, it is feasible that doses less than 1 mM might also be inhibitory, and this may possibly have pathophysiologic relevance for MSUD patients with moderate increases in these keto acids. This probably explains previous reports of impaired energy production caused by these metabolites, as identified by lower CO₂ production or increased lactate release [9,40–44]. It is difficult to extrapolate our findings to the human condition. However, if the *in vitro* inhibition of brain energy metabolism caused by the metabolites which most accumulate in MSUD also occurs under *in vivo* conditions, it is conceivable that lack of energy may be involved in the neurological symptoms present in MSUD patients. An interesting observation is that these patients present hypoglycemia and cerebral edema, particularly during metabolic decompensation, when the levels of the BCKA and BCAA dramatically increase [1], reflecting a failure of the active ionic transport necessary to maintain the normal volume of neural cells. Whether energy deficit or other abnormalities, such as oxidative stress or excitotoxicity, is mainly responsible for brain damage in MSUD patients is a matter of future investigation.

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References

- [1] D.T. Chuang, V.E. Shih, Maple syrup urine disease (branched-chain ketoaciduria), in: C.R. Scriver, A.L. Beaudet, W.L. Sly, D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, 8th ed., McGraw-Hill, New York, 2001, pp. 1971–2005.
- [2] D.J. Danner, L.J. Elsas II, Disorders of branched chain amino acid and keto acid metabolism, in: C.R. Scriver, A.L. Beaudet, W.L. Sly, D. Valle (Eds.), *The Metabolic Basis of Inherited Disease*, McGraw-Hill, New York, 1989, pp. 671–692.
- [3] M.L. Efron, Aminoaciduria, *N. Engl. J. Med.* 272 (1965) 1058–1067.
- [4] S.E. Snyderman, P.M. Norton, E. Roitman, Maple syrup urine disease with particular reference to diet therapy, *Pediatrics* 34 (1964) 454–472.
- [5] C.F. Mello, L. Feksa, A.M. Brusque, C.M. Wannmacher, M. Wajner, Chronic early leucine administration induces behavioral deficits in rats, *Life Sci.* 8 (1999) 747–755.
- [6] A.S. Coitinho, C.F. de Mello, T.T. Lima, J. de Bastiani, M.R. Figuera, M. Wajner, Pharmacological evidence that alpha-ketoisovaleric acid induces convulsions through GABAergic and glutamatergic mechanisms in rats, *Brain Res.* 894 (2001) 68–73.
- [7] R.K. Howell, M. Lee, Influence of α -keto acids on the respiration of brain *in vitro*, *Proc. Soc. Exp. Biol. Med.* 113 (1963) 660–663.
- [8] A.P. Halestrap, M.D. Brand, R.M. Denton, Inhibition of mitochondrial pyruvate transport by phenylpyruvate and α -ketoisocaproate, *Biochem. Biophys. Acta* 367 (1974) 102–108.
- [9] J.M. Land, J. Mowbray, J.B. Clark, Control of pyruvate and β -hydroxybutyrate utilization in rat brain mitochondria and its relevance to phenylketonuria and maple syrup urine disease, *J. Neurochem.* 26 (1976) 823–830.
- [10] C. Pilla, R.F.D. Cardozo, C.S. Dutra, A.T.S. Wyze, M. Wajner, C.M.D. Wannmacher, Effect of leucine administration on creatine kinase activity in rat brain, *Metab. Brain Dis.* 18 (2003) 17–25.
- [11] R.E. Tashian, Inhibition of brain glutamic acid decarboxylase by phenylalanine, valine, and leucine derivatives: a suggestion concerning the etiology of the neurological defect in phenylketonuria and branched-chain ketonuria, *Metabolism* 10 (1961) 393–402.
- [12] S.H. Appel, Inhibition of brain protein synthesis: an approach to a biochemical basis of neurological dysfunction in the amino-acidurias, *Trans. N. Y. Acad. Sci.* 29 (1966) 63–70.
- [13] T. Taketomi, T. Kunishita, A. Hara, S. Mizushima, Abnormal protein and lipid compositions of the cerebral myelin of a patient with maple syrup urine disease, *Jpn. J. Exp. Med.* 53 (1983) 109–116.
- [14] D. Tribble, R. Shapira, Myelin proteins: degradation in rat brain initiated by metabolites causative of maple syrup urine disease, *Biochem. Biophys. Res. Commun.* 114 (1983) 440–446.
- [15] E. Treacy, C.L. Clow, T.R. Reade, D. Chitayat, O.A. Mamer, C.R. Scriver, Maple syrup urine disease: interrelationship between branched chain amino-, oxo-, and hydroxyacids; implications for treatment; association with CNS dysmyelination, *J. Inher. Metab. Dis.* 15 (1992) 121–135.
- [16] M. Wajner, C.R. Vargas, Reduction of plasma concentrations of large neutral amino acids in patients with maple urine disease during crises, *Arch. Dis. Child.* 80 (1999) 579.
- [17] M. Wajner, D.M. Coelho, A.G. Barschak, P.R. Araújo, R.F. Pires, F.L. Lulhier, C.R. Vargas, Reduction of large neutral amino acid concentration in plasma and CSF of patients with maple syrup urine disease during crises, *J. Inher. Metab. Dis.* 23 (2000) 505–512.
- [18] P. Araujo, G.F. Wassermann, K. Tallini, V. Fumaleto, C.R. Vargas, C.M. Wannmacher, C.S. Dutra-Filho, A.T. Wyse, M. Wajner, Reduction of large neutral amino acid levels in plasma and brain of hyperleucinemic rats, *Neurochem. Int.* 38 (2001) 529–537.
- [19] P. Jouvret, P. Rustin, D.L. Taylor, J.M. Pocock, U. Felderhoff-Mueser, N.D. Mazarakis, C. Sarraf, U. Joashi, M. Koszma, K. Greenwood, A.D. Mehmet, H. Mehmet, Branched chain amino acids induce apoptosis in neural cells without mitochondrial membrane depolarization or cy-

- tochrome *c* release: implications for neurological impairment associated with maple syrup urine disease, *Mol. Biol. Cell.* 11 (2000) 1919–1932.
- [20] C.S. Dutra-Filho, M. Wajner, E. Gassen, R. Candiago, A. Wihlems, H. Malfussi, C.M.D. Wannmacher, Effect of organic acids on in vitro glucose oxidation by cerebral cortex of young rats, *Med. Sci. Res.* 23 (1995) 25–26.
- [21] J.C. Dutra, M. Wajner, C.F. Wannmacher, C.S. Dutra-Filho, C.M.D. Wannmacher, Effects of methylmalonate and propionate on glucose and ketone bodies uptake in vitro by brain of developing rats, *Biochem. Med. Metab. Res.* 45 (1991) 56–64.
- [22] P.A. Trinder, Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen, *J. Clin. Pathol.* 22 (1969) 158–161.
- [23] N. Shimajo, K. Naka, C. Nakajima, C. Yoshikawa, K. Okuda, K. Okada, Test-strip method for measuring lactate in whole blood, *Clin. Chem.* 35 (1989) 1992–1994.
- [24] J.C. Fisher, W. Ruitenbeek, J.Á. Berden, J.M. Trijbels, J.H. Veerkamp, M. Stadhouders, R.C. Sengers, A.J. Janssen, Differential investigation of the capacity of succinate oxidation in human skeletal muscle, *Clin. Chim. Acta* 153 (1985) 23–36.
- [25] R.G. Sorensen, H.R. Mahler, Localization of endogenous ATPases at the nerve terminal, *J. Bioenerg. Biomembranes* 14 (1982) 527–547.
- [26] P. Rustin, D. Chretien, T. Bourgeron, B. Gérard, A. Rötig, J.M. Saudubray, A. Munnich, Biochemical and molecular investigations in respiratory chain deficiencies, *Clin. Chim. Acta* 228 (1994) 35–51.
- [27] A.H. Schapira, V.M. Mann, J.M. Cooper, D. Dexter, S.E. Daniel, P. Jenner, J.B. Clark, C.D. Marsden, Anatomic and disease specificity of NADH CoQ1 reductase (complex I) deficiency in Parkinson's disease, *J. Neurochem.* 55 (1990) 2142–2145.
- [28] M.A. Birch-Machin, H.L. Briggs, A.A. Saborido, L.A. Bindoff, D.M. Turnbull, An evaluation of the measurement of the activities of complexes I–IV in the respiratory chain of human skeletal muscle mitochondria, *Biochem. Med. Metabol. Biol.* 51 (1994) 35–42.
- [29] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [30] G.F. Oldendorf, Carrier-mediated blood–brain barrier transport of short-chain monocarboxylic organic acids, *Am. J. Physiol.* 224 (1973) 1450–1453.
- [31] A.H.V. Schapira, Human complex I defects in neurodegenerative diseases, *Biochem. Biophys. Acta* 1364 (1998) 261–270.
- [32] M.W. Cleeter, J.M. Cooper, A.H. Schapira, Irreversible inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium: evidence for free radical involvement, *J. Neurochem.* 58 (1992) 786–789.
- [33] K.F. Tipton, T.P. Singer, Advances in our understanding of the mechanisms of neurotoxicity of MPTP and related compounds, *J. Neurochem.* 61 (1993) 1191–1206.
- [34] M.L. Genova, B. Ventura, G. Giuliano, C. Bovina, G. Formiggini, G.P. Castelli, G. Lenaz, The site of production of superoxide radical in mitochondrial complex I is not a bound ubiquinone but presumably iron–sulfur cluster N2, *FEBS Lett.* 505 (2001) 364–368.
- [35] F.U. Fontella, E. Gassen, V. Pulrolnik, C.M.D. Wannmacher, A.B. Klein, M. Wajner, C.S. Dutra, Stimulation of lipid peroxidation in vitro in rat brain by metabolites accumulating in maple syrup urine disease, *Metab. Brain Dis.* 17 (2002) 47–54.
- [36] A. Dupius, J.M. Skehel, J.M. Walker, NADH: ubiquinone oxidoreductase from bovine mitochondria, *Biochem. J.* 277 (1991) 11–15.
- [37] Y. Zhang, O. Marcillat, C. Giulivi, L. Ernster, K.J. Davies, The oxidative inactivation of mitochondrial electron transport chain components and ATPase, *J. Biol. Chem.* 265 (1990) 16330–16336.
- [38] A.P. Kudin, T.A. Kudina, J. Seyfried, S. Vielhaber, H. Beck, C.E. Elger, W.S. Kunz, Seizure-dependent modulation of mitochondrial oxidative phosphorylation in rat hippocampus, *Eur. J. Neurosci.* 15 (2002) 1105–1114.
- [39] Y. Shigematsu, K. Kikuchi, T. Momoi, Organic acids and branched-chain amino acids in body fluids before and after multiple exchange transfusions in maple syrup urine disease, *J. Inher. Metab. Dis.* 6 (1983) 183–189.
- [40] R.H. Jackson, T.P. Singer, Inactivation of the 2-ketoglutarate and pyruvate dehydrogenase complexes of beef heart by branched chain keto acids, *J. Biol. Chem.* 258 (1983) 1857–1865.
- [41] G.E. Gibson, J.P. Blass, Inhibition of acetylcholine synthesis and of carbohydrate utilization by maple syrup urine disease metabolites, *J. Neurochem.* 26 (1976) 1073–1078.
- [42] E. Walajtys-Rode, J.R. Williamson, Effects of branched chain α -ketoacids on the metabolism of isolated rat liver cells: III. Interactions with pyruvate dehydrogenase, *J. Biol. Chem.* 255 (1980) 413–418.
- [43] H.R. Zielke, Y. Huang, P.J. Baab, R.M. Collins, C.L. Zielke, J.T. Tildon, Effect of alpha-ketoisocaproate and leucine on the in vivo oxidation of glutamate and glutamine in the rat brain, *Neurochem. Res.* 22 (1997) 1159–1164.
- [44] M.C. McKenna, U. Sonnewald, X. Huang, J. Stevenson, S.F. Johnsen, L.M. Sande, H.R. Zielke, Alpha-ketoisocaproate alters the production of both lactate and aspartate from [^{13}C] glutamate in astrocytes: a ^{13}C NMR study, *J. Neurochem.* 70 (1998) 1001–1008.