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Mitochondrial energy metabolism in neurodegeneration associated with methylmalonic acidemia

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Abstract Methylmalonic acidemia is one of the most prevalent inherited metabolic disorders involving neurological deficits. *In vitro* experiments, animal model studies and tissue analyses from human patients suggest extensive impairment of mitochondrial energy metabolism in this disease. This review summarizes changes in mitochondrial energy metabolism occurring in methylmalonic acidemia, focusing mainly on the effects of accumulated methylmalonic acid, and gives an overview of the results found in different experimental models. Overall, experiments to date suggest that mitochondrial impairment in this disease occurs through a combination of the inhibition of specific enzymes and transporters, limitation in the availability of substrates for mitochondrial metabolic pathways and oxidative damage.

Keywords Central nervous system · Methylmalonic acid · Mitochondria · Neurodegeneration · Organic acidemias · Oxidative metabolism

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Introduction

Methylmalonic acidemia consists of a group of autosomal recessive genetic disorders affecting catabolic pathways of the branched chain aminoacids isoleucine, valine, methionine and threonine, as well as thymine, odd carbon number fatty acids and the side chain of cholesterol. Methylmalonic acidemia is usually caused by mutations in the *MUT* gene (609058) that lead to partial (*mut*$^{-}$) or complete (*mut*°) deficiency in the activity of methylmalonyl-CoA mutase (MCM, EC 5.4.99.2), a mitochondrial matrix enzyme that catalyses the conversion of L-methylmalonyl-CoA to succinyl-CoA (Fenton et al. 2001; Tanpaiboon 2005; Venditti 2005). In addition, defects in the synthesis of 5′-deoxyadenosyl cobalamin (AdoCbl), a vitamin B_{12}-derived cofactor required for MCM activity, or failure in the metabolism of cobalamin itself can promote a functional deficiency of this enzyme, also resulting in methylmalonic acidemia. Methylmalonyl-CoA, among other metabolites, accumulates in the mitochondrial matrix as a result of MCM deficiency. Methylmalonyl-CoA is subsequently hydrolyzed to CoA and methylmalonic acid (MMA) (Kovachy et al. 1983; Fenton et al. 2001; Chandler and Venditti 2005).

One in 48,000 newborns in the United States is estimated to be affected by methylmalonic acidemia (Coulombe et al. 1981), while incidences range from 1:115,000 in Italy to 1:169,000 in Germany (Deodato et al. 2006). Generally, *mut*$^{-}$ patients and those with AdoCbl synthesis defects present good clinical responses when supplemented with vitamin B_{12}, while *mut*° patients are not responsive, constituting the most severe phenotype of the disease (Tanpaiboon 2005; Deodato et al. 2006).

MMA is produced within tissues and transported to the plasma and cerebrospinal fluid, where it can reach high

levels during periods of metabolic decompensation (Fenton et al. 2001). The upper reference values for MMA are 4 mmol/mol creatine in the urine, 0.27 μmol/L in the blood and 0.59 μmol/L in the cerebrospinal fluid. In severely affected individuals, MMA concentrations lie in the range of 1,000–10,000 mmol/mol creatine in the urine and 220–2,900 μmol/L in the blood (Fenton et al. 2001; Venditti 2005). MMA varies from tens to hundreds mmol/mol creatine in the urine and 5 to 100 μmol/L in the blood for B_{12}-responsive patients (Venditti 2005). MMA concentrations in the cerebrospinal fluid of patients are similar to those found in blood but can reach even higher levels (Rosenberg 1978; Hoffmann et al. 1993).

MMA is predominantly produced in the liver and kidneys because of the high methylmalonyl-CoA mutase activity in these organs (Fenton et al. 1982; Kennedy et al. 1990). MMA may also be produced in the skeletal muscle and brain (Chandler et al. 2007). Although low amounts of MMA are generated in the brain, the neurodegeneration in methylmalonic acidemia possibly results from intracerebral accumulation of this metabolite (Kölker et al. 2006). The blood–brain-barrier is virtually impermeable to dicarboxylic acids such as MMA (Hoffmann et al. 1993), trapping these compounds within the central nervous system (Kölker et al. 2006). Many transport systems have been reported for monocarboxylic acids and aminoacids (Tamai and Tsuji 2000), whereas dicarboxylic acids have a limited flux in and out of the central nervous system (Hassel et al. 2002; Sauer et al. 2006). In fact, only a weak expression of the organic anion transporters 1 and 3 (OAT1 and OAT3) for dicarboxylic acids was identified in brain capillary endothelial cells (Sauer et al. 2010).

A substantial body of evidence suggests that neurodegeneration in methylmalonic acidemia is associated with a failure of mitochondrial oxidative metabolism. In fact, disruption of mitochondrial homeostasis has been proposed to take part in the mechanism of tissue damage in several organic acidemias, as reviewed by Wajner and Goodman in this issue (Wajner and Goodman 2011).

Patients with methylmalonic acidemia usually present acute clinical features early in life resulting from metabolic decompensation, with recurrent vomiting, dehydration, respiratory distress and neurological symptoms, including psychomotor delay, irritability, lethargy, hypotonia, convulsions and coma. Without specific therapy, these episodes result in severe handicap or death. Most children survive the first acute metabolic crisis, but develop long-term complications including neurological deficits (Baumgarter and Viardot 1995; Leonard 1995; Horster et al. 2007). Although the brain neuropathological findings are nonspecific, patients often develop acute extrapyramidal signs due to bilateral destruction of the *globus pallidus*, which has been attributed to the accumulation of toxic metabolites (Heidenreich et al. 1988; Fenton et al. 2001; Harting et al. 2008). Remarkably, the *globus pallidus* is highly vulnerable to hypoxic or ischemic damage, also observed in carbon monoxide and cyanide intoxication and in some organic acidemias involving energy metabolism impairment such as propionic acidemia and mitochondrial encephalopathy (Burgeois et al. 1992; Brismar and Ozand 1994; Haas et al. 1995; Albin 2000). Elevation of lactic acid in the *globus pallidus* and in cerebrospinal fluid was observed in patients with methylmalonic acidemia during metabolic decompensation, indicating an impairment of mitochondrial pyruvate oxidation (Trinh et al. 2001). Therefore, it may be presumed that bioenergetic defects are associated with neurodegeneration in methylmalonic acidemia. Interestingly, a recent study showed a significantly and long-term improvement of optic neuropathy in a patient with methylmalonic acidemia under coenzyme Q10 and alpha-tocopherol therapy (Pinar-Sueiro et al. 2010).

Additional evidence that patients with methylmalonic acidemia present impairment of mitochondrial oxidative metabolism is the urinary excretion and accumulation of large amounts of lactic acid and citric acid cycle intermediates in the blood (Fenton et al. 2001). Findings of mitochondrial abnormalities, reduced oxygen uptake, low activities of cytochrome *c* oxidase and of other respiratory chain enzyme complexes were observed in various tissues from methylmalonic acidemic patients (Hayasaka et al. 1982; Ostergaard et al. 2005; Chandler et al. 2009; de Keyzer et al. 2009).

The next sections of this review will describe several toxic effects of MMA and related metabolites on mitochondrial functions and their relationship with the neurodegeneration found in different *in vitro* and *in vivo* experimental models for methylmalonic acidemia.

On the toxic role of accumulated metabolites in methylmalonic acidemia

Systemic and neurological manifestations in methylmalonic acidemia are thought to be associated with the accumulation of MMA in tissues and biological fluids and the consequent impairment of energy metabolism and redox imbalance (Wajner and Coelho 1997; Fenton et al. 2001; Morath et al. 2008; Chandler et al. 2009). The reported effects of MMA on mitochondrial function are primarily related to the inhibition of enzymatic activities and transport systems (Halperin et al. 1971; Dutra et al. 1993; Toyoshima et al. 1995; Wajner and Coelho 1997; Brusque et al. 2002; Maciel et al. 2004; Schuck et al. 2004; Pettenuzzo et al. 2006; Mirandola et al. 2008; Morath et al. 2008). Most MMA effects on these systems present competitive inhibitory characteristics. Interestingly, mito-

chondria have the property to take up and accumulate exogenous MMA, leading to 3–9 times higher MMA concentrations within the mitochondrial matrix than in the extramitochondrial environment (Toyoshima et al. 1995; D. R. Melo and R. F. Castilho, unpublished results). This may be a reason why these organelles are more prone to the toxic effects of MMA.

It has also been argued that MMA is not the major toxic metabolite in methylmalonic acidemia and that part of the pathophysiology of this disease can be ascribed to other accumulated metabolic intermediates (Okun et al. 2002; Kölker et al. 2003; Kölker and Okun 2005). In addition to MMA, patients with methylmalonic acidemia present elevated levels of propionic, 3-hydroxypropionic, malonic and 2-methylcitric acids, as well as propionylglycine and butanone (Fenton et al. 2001; Kölker and Okun 2005). Malonic acid is a competitive inhibitor of mitochondrial complex II and could explain tissue lesions observed in the disease, considering it is formed in reasonable amounts (Okun et al. 2002). 2-Methylcitric acid, propionic acid and propionyl-CoA have also been ascribed to take part in tissue degeneration, although the high [D-methylmalonyl-CoA]/[propionyl-CoA] thermodynamic equilibrium ratio precludes a significant production of these compounds (Reszko et al. 2003). For further discussions regarding the toxicity of 2-methylcitric, malonic and propionic acids and propionyl-CoA in methylmalonic acidemia, we recommend reviews by Kölker and Okun (2005) and Morath et al. (2008).

Metabolic derangement in methylmalonic acidemia was also suggested to occur due to trapping of CoA as methylmalonyl-CoA, producing a general inhibition of oxidative metabolism (Fenton et al. 2001). Methylmalonyl-CoA may also be toxic in itself: Utter et al. (1964) showed that this metabolite is a competitive inhibitor of pyruvate carboxylase. However, Sauer et al. (2008) did not observe inhibitory effects of methylmalonyl-CoA (up to 1 mM) on complexes I–V of the mitochondrial respiratory chain, and only a minor inhibition of pyruvate and α-ketoglutarate dehydrogenases activities was detected.

Impairment of mitochondrial energy metabolism in in vitro methylmalonic acidemia models

Oberholzer et al. (1967) first proposed that inhibition of pyruvate carboxylase by methylmalonyl-CoA could promote diminished gluconeogenesis and the inability to maintain blood glucose levels. Indeed, hypoglycemia is present in a considerable number of patients with methylmalonic acidemia. Shortly after, results by Halperin et al. (1971) highlighted an alternate site for gluconeogenesis inhibition in methylmalonic acidemia, which involves MMA-inhibited malate transport by the mitochondrial dicarboxylate carrier. In fact, MMA itself can be transported by all three mitochondrial carrier systems for malate: the dicarboxylate, tricarboxylate and α-ketoglutarate transport carriers (Halperin et al. 1971). In addition, the same work suggested an inhibitory effect of MMA on succinate dehydrogenase (SDH), a component of the mitochondrial respiratory chain and citric acid cycle.

Initial experiments suggesting MMA was a weak competitive SDH inhibitor were conducted using isolated brain and liver mitochondria (Dutra et al. 1993; Toyoshima et al. 1995). This effect was attributed to the structural similarity between this compound, malonate (a classical SDH inhibitor), and succinate. Nonetheless, the inhibitory effect of MMA on SDH activity was not detected in purified submitochondial particles (Okun et al. 2002; Kölker et al. 2003). Furthermore, we recently reported that MMA does not inhibit succinate-supported oxygen consumption in permeabilized mitochondria or inverted submitochondrial particles (Mirandola et al. 2008) and provided evidence that MMA is a potent inhibitor of mitochondrial succinate transport by the dicarboxylate carrier. Therefore, this explains the inhibitory effect of MMA on succinate-supported oxygen consumption in intact isolated mitochondria.

In liver and kidney, the mitochondrial dicarboxylate carrier is important for gluconeogenesis, promoting malate transport from mitochondria to the cytosol, while its role in brain mitochondria is less understood. The brain mitochondrial dicarboxylate carrier is probably associated with the supply of intermediates to the citric acid cycle expended during de novo glutamate synthesis (Hertz 2004). In addition, neurons are considered incapable of de novo synthesis of oxaloacetate, which requires pyruvate carboxylation, occurring only in astrocytes (Yu et al. 1983). In this regard, neuronal energy metabolism and glutamatergic neurotransmission may be compromised by high concentrations of MMA. In addition, mitochondrial dicarboxylate carrier inhibition by MMA may also inhibit glutathione transport into mitochondria (Lash 2006; Morath et al. 2008), leading to mitochondrial antioxidant defense depletion and redox imbalance, which may be related to the glutathione deficiency observed in methylmalonic acidemia (Treacy et al. 1996). Furthermore, several studies show that MMA elicits lipid peroxidation in cerebral tissues, suggesting that redox processes take part in the neurological dysfunction found in methylmalonic acidemia (Fontella et al. 2000; Fighera et al. 2003; Malfatti et al. 2003; Wajner et al. 2004).

When evaluating the effects of MMA on oxygen consumption in diced rat brains we observed a delayed inhibition of uncoupled respiration (Fig. 1), with no change in resting respiration. These results indicate either a

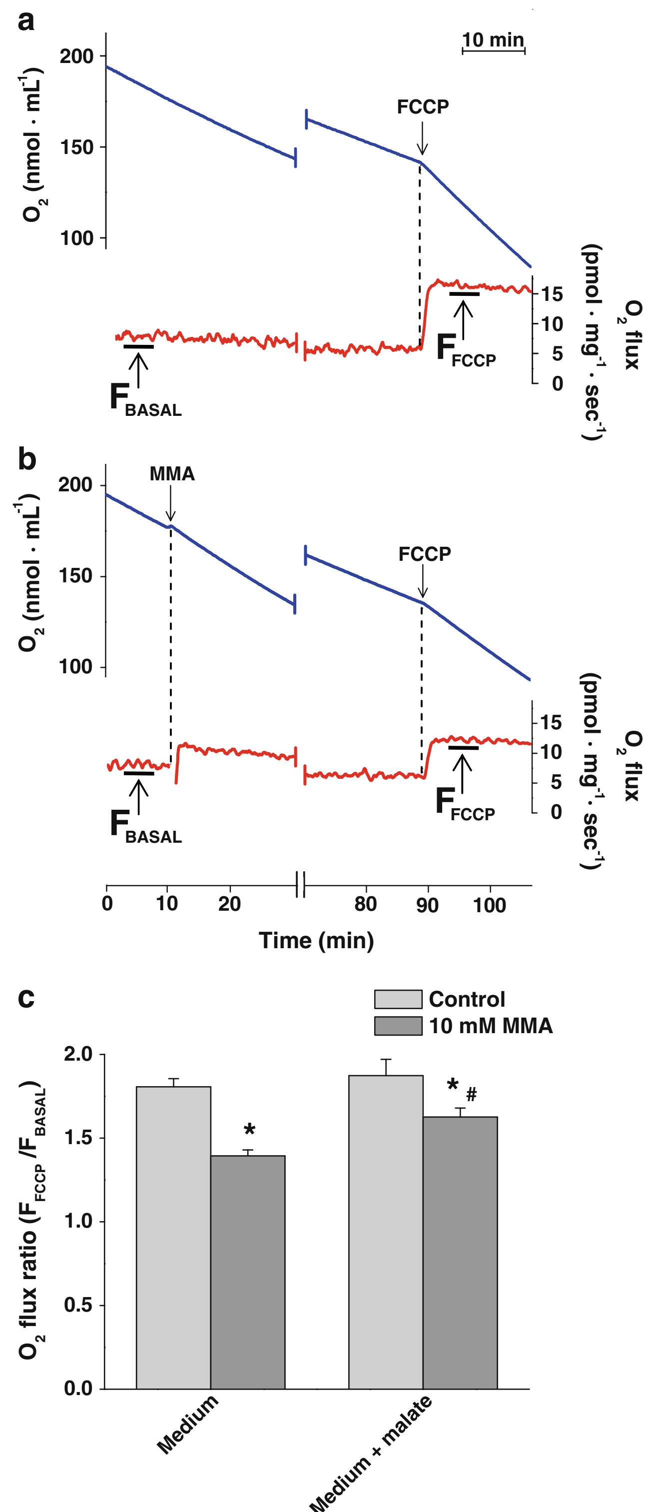

Fig. 1 Malate partially prevents the inhibitory effect of MMA on oxygen consumption in diced rat forebrain. Rat forebrains (brain minus cerebellum and brainstem) were diced into pieces of approximately 1 mm^3 using a tissue chopper (McIlwain Tissue Chopper), suspended in standard medium (Hank's balanced salt solution containing 1 g/L glucose and 20 mM HEPES, pH 7.2), kept over an ice bath and used within 4 h. For each experiment, four forebrain pieces weighing about 6 mg were incubated in standard medium at 37 °C under constant stirring (200 rpm) in a 2 mL sealed chamber of a high-resolution respirometer (OROBOROS Oxygraph-2k). The graphs in Panels A and B present a 40 min gap starting at minute 30 for clarity. To avoid anoxia, medium reoxygenation was promoted at 60 min by keeping the chamber open for 8 min. **Panel a**: Representative results for oxygen concentration (*blue*) and flux (respiration, *red*) measurements when brain pieces were incubated under control conditions, in the absence of malate. Where indicated by the arrow, the protonophore p-trifluoromethoxy carbonyl cyanide phenyl hydrazone (FCCP; 250 nM) was added to achieve the maximum respiratory rate. **Panel b**: 10 mM MMA was added where indicated by the arrow. **Panel c**: Oxygen flux ratios between FCCP-induced uncoupled (F_{FCCP}) and basal respiration (F_{BASAL}) under different experimental conditions. Respiratory assays were conducted in the presence or absence of 5 mM malate and/or 10 mM MMA, as indicated. Data represent the mean ± S.E.M. of at least 5 independent experiments done in duplicate and were analyzed by ANOVA followed by Tukey's post-hoc test. *Significantly different from the respective control, $p<0.05$. #Significantly different from MMA in the absence of malate, $p<0.05$

decreased activity of respiratory chain components or a diminished substrate supply for aerobic oxidation. Supporting the idea that substrate availability may be limiting, we found that MMA-induced inhibition of uncoupled respiration was partially prevented when the incubation medium was supplemented with malate. Indeed, MMA significantly reduced brain CO_2 production from [2-^{14}C]glucose and [U-^{14}C]acetate in a dose-dependent manner, and also increased lactate production in diced rat brains (Wajner et al. 1992). These MMA effects were followed by increased glucose uptake, indicating that aerobic glucose oxidation is blocked by this metabolite. Overall, these experiments support the concept that MMA may compromise citric acid cycle activity due to the lack of intermediates, including oxaloacetate (generated by pyruvate carboxylase), intramitochondrial succinate and malate. The absence of succinyl-CoA derived from methylmalonyl-CoA mutase may also contribute to the reduction of citric acid cycle activity in methylmalonic acidemia.

MMA also inhibits brain mitochondrial use of alternative substrates such as ketone bodies and lactate through competitive inhibition of β-hydroxybutyrate (Dutra et al. 1991, 1993) and lactate dehydrogenases (Saad et al. 2006). The inhibitory effect of MMA on β-hydroxybutyrate dehydrogenase in the liver would result in a decreased supply of ketone bodies to the brain, and a consequent impairment of their utilization in this organ.

Another possibility is that MMA can inhibit mitochondrial respiratory chain components. Although the evidence presented above indicate that MMA is not an inhibitor of SDH (respiratory complex II), this compound can lead to diverse alterations in respiratory chain complexes activities. Diminished activities of NADH:cytochrome *c* oxireductase

(complexes I + CoQ + III) and succinate:cytochrome *c* oxireductase (complexes II + CoQ + III) were found in brain, liver and kidney homogenates after MMA exposure (Brusque et al. 2002; Pettenuzzo et al. 2006). On the other hand, MMA did not show an inhibitory effect on complex IV activity in isolated rat liver mitochondria and homogenates from different rat tissues (Hayasaka et al. 1982; Brusque et al. 2002; Pettenuzzo et al. 2006). Importantly, respiratory complexes III and IV were found to be deficient in liver extracts from methylmalonic acidemia patients (Hayasaka et al. 1982; Chandler et al. 2009; de Keyzer et al. 2009; Valayannopoulos et al. 2009). In addition, diminished activities of respiratory chain complexes III and IV were also found in liver from two rodent models for methylmalonic acidemia (Krahenbuhl et al. 1991; Chandler et al. 2009), as will be described latter in this article. Because reduced electron flow in the mitochondrial respiratory chain is associated with an increased generation of reactive oxygen species (Kowaltowski et al. 2009), respiratory complex impairments associated with methylmalonic acidemia are likely to be responsible for increased oxidant production.

Furthermore, Schuck et al. (2004) observed that MMA inhibits mitochondrial creatine kinase in rat cerebral cortex. This enzyme is an important component of the cellular energy buffering and transport system, connecting oxidative phosphorylation to ATP consumption (Schlattner et al. 2006). The reduced activity of creatine kinase may lead to a decreased cellular ATP/ADP ratio, as observed in cultured striatal cells exposed to MMA (McLaughlin et al. 1998).

Toxic MMA effects on cultured neuronal cells

In cultured neurons, MMA decreases ATP/ADP ratio, collapses ion gradients, causes membrane depolarization and increases intracellular Ca^{2+} levels, leading to necrotic and apoptotic cell death (McLaughlin et al. 1998, Okun et al. 2002, Maciel et al. 2004, Kowaltowski et al. 2006). Cell death induced by MMA is prevented by ionotropic glutamate receptor antagonists, suggesting the involvement of excitotoxic mechanisms of cell damage (McLaughlin et al. 1998; Okun et al. 2002). Moreover, the involvement of excitotoxicity is also evidenced by the prevention promoted by glutamate receptor antagonist MK-801 on rotational behavior and convulsions induced by intrastriatal administration of MMA (de Mello et al. 1996).

Excitotoxicity is a central nervous system process that may be associated with increased glutamate release in response to impairment of brain energy metabolism (secondary excitotoxicity) (Albin and Greenamyre 1992). Glutamate toxicity is promoted mainly by activation of *N*-methyl-D-aspartate (NMDA) receptors with Ca^{2+} and Na^{+} influx (Choi 1987; Rothman and Olney 1987). Under excitotoxic conditions, mitochondria are the main organelle responsible for Ca^{2+} sequestration, an event associated with neuronal cell death (Nicholls and Budd 2000). Increased Ca^{2+} concentrations in the mitochondrial matrix may induce organelle dysfunction by promoting mitochondrial permeability transition (MPT), a non-selective permeabilization of the inner mitochondrial membrane (Kowaltowski et al. 2001). The involvement of MPT in MMA-mediated neurotoxicity was evidenced in experiments using cultured PC12 cells and rat brain slices, where an important inhibition of MMA-induced cell death was observed in the presence of MPT inhibitors (Maciel et al. 2004; Kowaltowski et al. 2006). Since redox imbalance is an important inducer of MPT (Castilho et al. 1995), increased mitochondrial reactive oxygen species generation in cells from methylmalonic acidemic patients, as observed in fibroblasts by Richard et al. (2007), may facilitate the occurrence of MPT.

We also observed that mitochondrial ATP-sensitive K^{+} channel (mitoK_{ATP}) opening by diazoxide protects against MMA-induced cell death (Kowaltowski et al. 2006). MitoK_{ATP} opening results in decreased mitochondrial reactive oxygen species production (Facundo et al. 2005). In addition, under energy deprivation conditions, mitoK_{ATP} opening inhibits mitochondrial ATP hydrolysis by ATP synthase (Belisle and Kowaltowski 2002; Kowaltowski et al. 2006), which helps to keep the cytosolic ATP/ADP ratio and also to limit mitochondrial Ca^{2+} uptake, indirectly preventing MPT.

Although the cytotoxic effects of MMA were documented in neuron-enriched primary cultures and in a neuronal cell line, the toxic effect of MMA on glial cells remains unexplored and deserves future investigation.

Mitochondrial oxidative metabolism in animal methylmalonic acidemia models

At least four in vivo rodent models have been used to study the pathogenesis of methylmalonic acidemia: young rats subcutaneously injected with MMA, adult rats intrastriatally infused with MMA, rats chronically treated with the inhibitory vitamin B_{12} analog hydroxycobalamin[c-lactam] (HCCL) and *MUT* knockout mice. Several evidences of mitochondrial oxidative metabolism impairment in these experimental models have been observed, as described below.

Acute or chronic chemically-induced methylmalonic acidemia promoted by subcutaneous treatment of rats with MMA was characterized in detail by Wajner and collaborators (Wajner et al. 1988; Dutra et al. 1991; de Mattos-Dutra et al. 1998; Wyse et al. 2000; Pettenuzzo et al. 2003).

This experimental model does not exactly mimic the human disease but reproduces the elevated tissue levels of MMA (Pettenuzzo et al. 2003). MMA administration twice a day to young rats from the first to the fourth week of life promoted plasma levels of 2–2.5 mM (Wajner et al. 1988; Dutra et al. 1991), near to those reported in patients with methylmalonic acidemia during crisis periods. A reduction in β-hydroxybutyrate metabolism was observed in brain tissue from MMA-treated rats, probably due to β-hydroxybutyrate dehydrogenase inhibition by MMA (Patel et al. 1976; Dutra et al. 1991). In addition, behavioral deficits presented by rats after chronic MMA treatment seems to be related to oxidative brain damage, since co-administration of the antioxidant ascorbic acid prevented such effects (Pettenuzzo et al. 2003).

By means of these well established chemically-induced models for methylmalonic acidemia, we recently evaluated ADP-stimulated oxygen consumption by isolated brain mitochondria. We did not observe significant differences between control and MMA-treated rats using different substrates specific for each complex of the mitochondrial respiratory chain (D.R. Melo, S.R. Mirandola and R.F. Castilho, unpublished results). These results indicate that MMA does not lead to permanent inhibition of mitochondrial oxidative phosphorylation, and corroborates with observations that impairment of mitochondrial function by MMA is mainly related to a decreased supply of substrates, as observed in *in vitro* experimental methylmalonic acidemia models.

An alternative *in vivo* methylmalonic acidemia model induced by MMA was achieved by infusion of this metabolite into adult rat striatum. Stereotaxic injections of MMA (2–10 μmol) lead to extensive striatal degeneration (Narasimhan et al. 1996), rotational behavior and convulsions (de Mello et al. 1996; Malfatti et al. 2003; Royes et al. 2003), in a mechanism involving excitotoxicity, oxidative damage and impairment of energy metabolism. With respect to energy metabolism, increased brain lactate production as well as rotational behavior and convulsions were prevented by intraperitoneal creatine or intrastriatal succinate pretreatments (de Mello et al. 1996; Malfatti et al. 2003; Royes et al. 2003).

Another way to mimic methylmalonic acidemia in rodents and in cultured cells is through chronical treatment with the vitamin B_{12} analog hydroxycobalamin[c-lactam] (HCCL) (Krahenbuhl et al. 1991; Sauer et al. 2009). Adult rats subcutaneously treated for 5–6 weeks with HCCL showed diminished activities for respiratory chain complexes III and IV related to decreased contents of cytochromes *b* and $a+a_3$ (Krahenbuhl et al. 1991).

The two engineered *MUT* knockout mice present severe symptoms immediately after birth and perish within 2 days of life (Peters et al. 2003; Chandler et al. 2007). A background-modified *MUT* knockout mouse that was capable of surviving beyond the neonatal period was developed (Chandler et al. 2009) and encouraged studies involving mitochondrial function. Changes in mitochondrial morphology, including megamitochondria, were observed in the liver, kidney and pancreas, but not in skeletal muscle and heart of this knockout mouse (Chandler et al. 2009; Murphy et al. 2010). Furthermore, respiratory chain complex III and IV activities as well as glutathione levels were decreased in liver extracts. On the other hand, the activity of citrate synthase was increased (Chandler et al. 2009). Further studies using these *MUT* knockout mice may help to improve the current knowledge about mitochondrial dysfunction in the pathogenesis of this disease. Interestingly, the presence of megamitochondria and respiratory chain complex IV inhibition was also identified in patients with methylmalonic acidemia (Hayasaka et al. 1982; Chandler et al. 2009), confirming that there are important parallels between the animal model and human disease.

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