Experimental Evidence that Mitochondrial Succinate and Malate Transport is Inhibited by Ethylmalonic Acid in Rat Brain

Amaral, A.U.; Cecatto, C.; Busanello, E.N.B.; Ribeiro, C.A.J.; Melo, D.R.; Leipnitz, G.; Castilho, R.F.; Wajner, M.

1Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre, RS, Brazil, 2Serviço de Genética Médica, HCPA, Porto Alegre, RS, Brazil; 3Departamento de Patologia Clínica, FCM, UNICAMP, Campinas, SP, Brazil.

Ethylmalonic acid (EMA) accumulates in short chain acyl-CoA dehydrogenase (SCAD) deficiency and ethylmalonic encephalopathy (EE), disorders in which the pathophysiological mechanisms of brain damage are poorly unknown. In the present study we investigated the in vitro effects of EMA on important parameters of mitochondrial homeostasis in isolated rat brain mitochondria supported by succinate, malate, or glutamate plus malate. The parameters evaluated were states 3 and 4 respiration, respiratory control ratio (RCR), uncoupled state, succinate dehydrogenase (SDH) activity, mitochondrial membrane potential ($\Delta \Psi_m$) and mitochondrial dicarboxylate transporter activity. EMA inhibited state 3 respiration, RCR and uncoupled state in succinate- and malate-., but not glutamate plus malate-supported isolated rat mitochondria. In addition, EMA mildly increased state 4 succinate-respiring mitochondria. Methylmalonic acid (MMA), malonic acid (MA) and butylmalonic acid (BtMA) had a similar effect on state 3 respiration. Furthermore, EMA-, MMA- and BtMA-, but not MA-induced inhibitory effect on succinate oxidation, was significantly minimized by nonselective permeabilization of mitochondrial membranes provoked by alamethicin. In addition, MA was the only tested compound that reduced SDH activity in an apparently competitive manner. We also observed that EMA markedly inhibited succinate and malate transport through the mitochondrial dicarboxylate transporter. $\Delta \Psi_m$ was also reduced by EMA and MA, but not by MMA, using succinate as electron donor, whereas none of these compounds was able to alter the $\Delta \Psi_m$ using glutamate plus malate as electron donors. Our results strongly indicate that EMA impairs succinate and malate uptake through the mitochondrial dicarboxylate carrier.

Keywords: short chain acyl-CoA dehydrogenase deficiency, ethylmalonic encephalopathy, ethylmalonic acid

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