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Neuropsychological functioning and brain energetics of drug resistant mesial temporal lobe epilepsy patients

Camila Moreira Osório[a], Alexandra Latini[a,b], Rodrigo Bainy Leal[a,c], Maria Emília Rodrigues de Oliveira Thais[a], Helena Dresch Vascouto[a], Aline Pertile Remor[b], Mark William Lopes[b], Marcelo Neves Linhares[a,d,e], Juliana Ben[a,c], Roberta de Paula Martins[a,b], Rui Daniel Prediger[f], Alexandre Ademar Hoeller[a], Hans Joachim Markowitsch[g], Peter Wolf[a,i,j], Kátia Lin[a,h,i], Roger Walz[a,h,i,*]

[a] Centro de Neurociências Aplicadas, Hospital Universitário (HU), Universidade Federal de Santa Catarina (UFSC), Florianópolis, SC, Brazil
[b] Laboratório de Bioenergética e Estresse Oxidativo, LABOX, Depar tamento de Bioquímica, UFSC, Florianópolis, SC, Brazil
[c] Laboratório de Transdução de Sinal no Sistema Nervoso Central, Departamento de Bioquímica, UFSC, Florianópolis, SC, Brazil
[d] Divisão de Neurocirurgia, Departamento de Cirurgia, HU, UFSC, Florianópolis, SC, Brazil
[e] Serviço de Neurocirurgia, Hospital governador Celso Ramos (HGCR), Florianópolis, SC, Brazil
[f] Departamento de Farmacologia, UFSC, Florianópolis, SC, Brazil
[g] Physiological Psychology, University of Bielefeld, Bielefeld, Germany
[h] Centro de Epilepsia do Estado de Santa Catarina, CEPESC, HU, UFSC, Florianópolis, SC, Brazil
[i] Serviço de Neurologia, Departamento de Clínica Médica, HU, UFSC, Florianópolis, SC, Brazil
[j] Danish Epilepsy Centre, Dianalund, Denmark

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ABSTRACT

Interictal hypometabolism is commonly measured by 18-fluoro-deoxyglucose Positron Emission Tomography (FDG-PET) in the temporal lobe of patients with mesial temporal lobe epilepsy (MTLE-HS). Left temporal lobe interictal FDG-PET hypometabolism has been associated with verbal memory impairment, while right temporal lobe FDG-PET hypometabolism is associated with nonverbal memory impairment. The biochemical mechanisms involved in these findings remain unknown. In comparison to healthy controls (n = 21), surgically treated patients with MTLE-HS (n = 32, left side = 17) had significant lower scores in the Rey Auditory Verbal Learning Test (RAVLT retention and delayed), Logical Memory II (LMII), Boston Naming test (BNT), Letter Fluency and Category Fluency. We investigated whether enzymatic activities of the mitochondrial enzymes Complex I (C I), Complex II (C II), Complex IV (C IV) and Succinate Dehydrogenase (SDH) from the resected samples of the middle temporal neocortex (mTCx), amygdala (AMY) and hippocampus (HIP) were associated with performance in the RAVLT, LMII, BNT and fluency tests of our patients. After controlling for the side of hippocampus sclerosis, years of education, disease duration, antiepileptic treatment and seizure outcome after surgery, no independent associations were observed between the cognitive test scores and the analyzed mitochondrial enzymatic activities ($p > 0.37$). Results indicate that memory and language impairment observed in MTLE-HS patients are not strongly associated with the levels of mitochondrial CI, CII, SDH and C IV enzymatic activities in the temporal lobe structures ipsilateral to the HS lesion.

1. Introduction

Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS) is the most frequent drug-resistant epilepsy referred for surgery (Fiest et al., 2014; Pauli et al., 2017a, 2017b, 2012; Wiebe et al., 2001). Epilepsy may affect different domains of cognitive functioning depending on the relationship between epileptogenic and symptomatogenic zone (Rosenow and Lüders, 2001). MTLE-HS is characterized by temporal lobe dysfunction, affecting particularly memory and language (Pauli et al., 2017a; Knopman et al., 2015), but impairments may extend beyond those functions thought to be mediated by the temporal lobe. These include attention, working memory, speed of processing,

* Corresponding author at: Departamento de Clínica Médica, Hospital Universitário, 3 andar, Universidade Federal de Santa Catarina (UFSC), Trindade, Florianópolis, SC CEP 88.040-970, Brazil.
E-mail address: rogerwalz@hotmail.com (R. Walz).

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visuospatial and executive deficits. (Bell et al., 2013; Oyegbile et al., 2004; Sherman et al., 2011) Several studies identified hypometabolism by 18-fluoro-deoxyglucose positron emission tomography (FDG-PET) in the typically epileptogenic areas of MTLE-HS (Akman et al., 2010; Chassoux et al., 2016; Knopman et al., 2015) The degree of hypometabolism in these regions was shown to correlate to some degree with neuropsychological test results (Nickel et al., 2003). Dominant temporal lobe hypometabolism has been associated with verbal memory impairment, while nondominant temporal lobe hypometabolism was associated with nonverbal memory performance (Knopman et al., 2015).

Mitochondria are critical modulators of cell function and are recognized as proximal metabolic sensors and effectors (Babcock and Wikström, 1992). The mitochondrial respiratory chain (RC) consists of five enzyme complexes that are distributed in a special way in the inner mitochondrial membrane. The electrons coming from the Krebs cycle and other reactions catalyzed by dehydrogenases are transferred to the RC with molecular oxygen as the final acceptor. Along with this process, there is translocation of protons across the inner mitochondrial membrane and ATP synthesis. (Babcock and Wikström, 1992). The RC occurs due to the presence of four enzymatic complexes. The electrons are transported through these four complexes and the last one reduces O_2 to H_2O. Respiration begins with the oxidation of fuels in metabolic pathways that transfer electrons to NAD^+ and FAD. These coenzymes can come from various metabolic processes including the tricarboxylic acid cycle (TCA). Energy from the reoxidation of NADH and FAD(2H) by O2 is converted to the high energy phosphate bonds of ATP via oxidative phosphorylation (Milane et al., 2015).

Epilepsy surgery offers an opportunity for association studies between brain mitochondrial metabolism and clinical variables of patients (Osório et al., 2017; Marcelo Fernando Ronsoni et al., 2016). We identified the neuropsychological tests significantly impaired in our MTLE-HS patients in comparison to healthy controls matched for age and sociocultural characteristics. Thereafter we investigated if the neuropsychological test results showing impairments in patients correlated with the mitochondrial respiratory chain complex enzyme activities analyzed in their brain samples from middle temporal neocortex (mTCx), amygdala (AMY) and hippocampus (HIP), resected during epilepsy surgery. We hypothesized the mitochondrial enzyme activities would be significantly associated with the cognitive performance of our patients. The results serve to understand the biochemical and metabolic mechanisms involved in patients with cognitive impairment after MTLE-HS.

2. Material and methods

2.1. Patients

Thirty two consecutive adult patients with drug-resistant MTLE-HS were included in the research protocol (265-FR304969) previously described by our group (Lopes et al., 2016; Ronsoni et al., 2016). All patients failed to respond to adequate treatment with at least two antiepileptic drugs in monotherapy and had seizures impairing awareness at least once a month. They were treated surgically between May 2009 and December 2012 at the Centro de Epilepsia de Santa Catarina (CEPESC).

All patients had complete medical history, seizure semiology, neurological examination, neuropsychological and psychiatric evaluation, interictal and ictal video-EEG analysis and MRI (1.5 T) findings consistent with unilateral MTLE-HS. We excluded patients with any diagnosis of an epilepsy syndrome other than unilateral MTLE-HS, focal motor or sensory abnormalities on physical examination, and generalized or extra-temporal interictal EEG spikes. (Araújo et al., 2006; de Lemos Zingano et al., 2015; Guarnieri et al., 2009; Pauli et al., 2017a, 2017b, 2012; Velasco et al., 2011). Patients with mental retardation determined by neuropsychological tests (Intelligence Quotient, IQ of 60 or less) and psychiatric diagnoses were excluded. This less rigid criterion for IQ is due to the high prevalence of low IQ in our patients. WADA or fMRI were not performed to asses the hemispheric dominance for language.

Clinical variables analyzed were age, years of education, gender, hand dominance, side of the hippocampus sclerosis (HS), duration of epilepsy (in years), age of epilepsy onset (recurrent seizure) and monthly frequency of seizures impairing awareness. Patients who used only one AED were rated as monotherapies. Patients using two or more AEDs, associated or not with benzodiazepines, were classified as being under polytherapy. The benzodiazepines were clobazam or clonazepam. The AEDs were carbamazepine, phenobarbital, diphenylehydatoin, valproic acid, lamotrigine or topiramate.

Control subjects matched for gender, hand dominance, age and education level and recruited during the same period of patients were companion persons of patients from other outpatient clinics and had no previous history of neurological or psychiatric disorders.

2.2. Anesthesia protocol, surgery and brain tissue sampling

The anesthesia protocol, surgical procedures and brain tissue sampling were performed by the same team of neurosurgery as described previously (Lopes et al., 2016; Ronsoni et al., 2016). Surgeries followed the standard procedure for MTLE-HS surgery were a maximum of 4 cm of the anterior lateral temporal lobe including the middle and inferior temporal gyrus. Was ressected (Pauli et al., 2017a, 2017b; Wiebe et al., 2001). The mesial resection included the amygdala and up to 3 cm of the anterior hippocampus. For further biochemical analysis, samples were frozen in liquid nitrogen immediately after collection and then transferred to a −80 °C freezer.

2.3. Tissue homogenization procedure

Brain samples were processed 24 (SD ± 9) months after the surgery. After being defrosted, samples were weighed and mechanically homogenized with a ground glass Potter-Elvehjem homogenizer in four volumes (v/v) of 50 mM Tris, pH 7.0, 1.0 mM EDTA, 100 mM NaF, 0.1 mM PMSF, 2.0 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). Homogenates were placed on ice for 10 min and centrifuged at 3000x*g* at 4 °C for 10 min. Aliquots of the supernatants were used to measure the mitochondrial enzyme activities after thawing the samples three times. Sample preparation was carried out in an Eppendorf 5415 R centrifuge (Eppendorf, Hamburg, Germany).

2.4. Mitochondrial respiratory chain complex enzyme activities

Mitochondrial enzyme activities were measured spectrophotometrically with a Varian Cary 50 spectrophotometer with temperature control (Varian Inc., Palo Alto, CA, USA). Complex I (CI) activities were measured by the rate of NADH-dependent ferricyanide reduction at 420 nm. The activities of succinate-2,6- dichloroindophenol (DCIP)-oxidoreductase (Complex II [C II]), succinate phenazyne oxidoreductase (succinate dehydrogenase [SDH]; Complex II) and cytochrome *c* oxidase (Complex IV [C IV]) were assayed as previously described elsewhere (Ronsoni et al., 2016). The activities were calculated as nanomol min^{-1} mg^{-1} protein. In order to control variations in the mitochondrial mass, the mitochondrial content of Mitofusin1 (Mfn1) was determined by western blot.

2.5. Western blot analysis of Mfn1

Because variations in the histopathologic distribution of neuronal lesions and gliosis in the brain tissue of MTLE-HS patients can influence the mitochondrial content, the mitochondrial mass variation between samples was controlled through the determination of Mfn1 content by