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Metabolic correction by pyruvate halts acquired epilepsy in multiple rodent models

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ABSTRACT

Metabolic intervention strategy of epilepsy treatment has been gaining broader attention due to accumulated evidence that hypometabolism, manifested in humans as reduced brain glucose consumption, is a principal factor in acquired epilepsy. Therefore, targeting deficient energy metabolism may be an effective approach for treating epilepsy. To confront this pathology we utilized pyruvate, which besides being an anaplerotic mitochondrial fuel possesses a unique set of neuroprotective properties as it: (i) is a potent reactive oxygen species scavenger; (ii) abates overactivation of Poly [ADP-ribose] polymerase 1 (PARP-1); (iii) facilitates glutamate efflux from the brain; (iv) augments brain glycogen stores; (v) is anti-inflammatory; (vi) prevents neuronal hyperexcitability; and (vii) normalizes the cytosolic redox state. In vivo, chronic oral pyruvate administration completely abolished established epileptic phenotypes in three accepted and fundamentally different rodent acquired epilepsy models. Our study reports metabolic correction by pyruvate as a potentially highly effective treatment of acquired epilepsies.

1. Introduction

In spite of continuing research efforts, 38% of adult epilepsy patients are resistant to anti-epileptic drugs (AEDs) (Loscher and Schmidt, 2011), while >50% of AED-responsive patients suffer from adverse side effects (Das et al., 2012); AED administration also requires gradually increased dosages. The search for an alternative and efficient epilepsy treatment is therefore very urgent.

Major risk factors for acquired epilepsy (traumatic brain injury, stroke, viral infection, status epilepticus (Pitkanen et al., 2015; Waldbaum and Patel, 2010b)) all share hypometabolic brain state as a common outcome; in turn, epileptic patients' brain reduced glucose utilization is revealed by FDG-PET analysis (Goffin et al., 2008).

Interestingly, similar early pre-symptomatic signature of neuropathology is also observed in Alzheimer's disease (AD) (Cunnane et al., 2016), where epilepsy is a common comorbidity in early-onset stages (Horvath et al., 2016). Epileptic/hyperactive phenotypes, accompanied by network-wide dysregulation of calcium homeostasis, have been shown in several mouse models of AD (Busche and Konnerth, 2016; Palop and Mucke, 2009). It has been suggested that epilepsy-related hypometabolism is associated with neuronal mitochondrial dysfunction (Kudin et al., 2009; Pan et al., 2008; Waldbaum and Patel, 2010a) presumably as a consequence of deficient glycolysis. Indeed, the phenomenon of cerebral glucose hypometabolism in interictal phases of epilepsy is well-established (Goffin et al., 2008; Pittau et al., 2014) although the reasons for reduced glucose consumption in the epileptic zone are still unclear. Anaplerosis, the replenishment of TCA-cycle substrates, has recently been suggested as an appealing strategy to address energy failure for epilepsy treatment (Borges and Sonnewald, 2012; Kovac et al., 2013). It was also suggested that pyruvate may be the most promising agent as it combines properties of a classical anaplerotic substrate with multiple neuroprotective effects (Kovac et al., 2013; Y. Zilberter et al. 2015).

Indeed, pyruvate possesses a unique set of neuroprotective properties (for review, see (Y. Zilberter et al. 2015)). Pyruvate is i) the main mitochondrial fuel and ii) potent ROS scavenger/antioxidant, specifically efficient against hydrogen peroxide (Das, 2006; Desagher et al., 1997). Moreover, it iii) prevents overactivation of Poly [ADP-ribose]

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polymerase 1 (PARP-1) (Martire et al., 2015; Wang et al., 2015) that leads to a depletion of cytoplasmic NAD^+ (Kovac et al., 2013; Mongan et al., 2003; Ying et al., 2002) and inhibition of hexokinase (Andrabi et al., 2014) with subsequent inhibition of glycolysis and ATP production; iv) facilitates glutamate efflux from the brain, reducing neurotoxicity (Gottlieb et al., 2003; Zlotnik et al., 2008; Zlotnik et al., 2012); v) augments brain glycogen stores in the brain (Koivisto et al., 2016; Shetty et al., 2012); vi) acts anti-inflammatory (Das, 2006; Kao and Fink, 2010); and vii) prevents neuronal hyperexcitability (Isopi et al., 2014; Simeone et al., 2014; Zilberter et al., 2013).

In this study, to validate the general anti-epileptic effects of metabolic correction by chronic pyruvate treatment we employed three different epilepsy models: Tetanus toxin (TeT) hippocampal injection-induced chronic refractory focal epilepsy (Benke and Swann, 2004; Jefferys et al., 1995; Nilsen et al., 2005), Pentylenetetrazole (PTZ)-induced kindling reproducing generalized chronic epilepsy (Park et al., 2006; Pavlova et al., 2006) and AD-related chronic epilepsy in APPswe/PS1dE9 transgenic mice (Minkeviciene et al., 2009; Zilberter et al., 2013).

2. Materials and methods

All animal protocols and experimental procedures were approved by the INSERM Ethics Committee for Animal Experimentation (#30-03102012) and Russian State Standard (РИСО 10993-2-2009) and carried out in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

2.1. Animals

Experiments were carried out on two species of animals – mice and rats. 116 adult male mice (94 OF1, 16 APPswe/PS1dE9 and 6 WT) and 60 adult male rats (57 Sprague Dawley) were used. The weight of animals was approximately 30–40 g for mice and 220 g for rats at the beginning of the experiments. Animals were housed in individual cages with enrichment: bedding ENVIRO-dri (Seralab), igloos (Plexx), under 12:12-h light/dark cycles, food and water were available ad libitum. All experiments were performed between 10:00 and 15:00. Weight of animals was monitored throughout the trial.

2.2. Pyruvate treatment

Mice and rats were divided by four groups: 1. control; 2. control treatment with pyruvate; 3. epileptic; and 4. epileptic treatment with pyruvate. Mice from groups 1 and 3 were fed ad libitum the standard chow (Custom diet A04, SAFE, France) and groups 2 and 4 – the pyruvate-containing chow (Custom Diet. A04 + 6 g of pyruvate/kg of chow, SAFE, France).

The 6 g/kg dose we delivered with chow led to ingestion of approximately 450 mg/kg/day after adjusting for feed intake and spillage (see Supplementary Information, "*Rationale for the dosage of pyruvate*"). Rats of the groups 2 and 4 were supplied once a day with solution of sodium pyruvate by oral gavage (110 mM, 10 mL/kg body weight). Control groups were supplied daily with 0.9% NaCl solution by oral gavage. The total duration of pyruvate treatment was 3.5 and 6–7 months for mice and rats, respectively.

3. Mice

3.1. TeT-induced mouse epilepsy

Mice were operated on twice. For anesthesia an intraperitoneal injection (0.1 mL per 10 g of body weight) of mixture (600 μl Ketamine + 100 μl Xylazine + 2 mL 0.9% (w/v) NaCl) was used. During the first operation half of the mice were injected in hippocampus using Nanofil Syringe (tip ID 35 μm, World Precision Instruments) with TeT to induce epileptogenesis. 2 μL of TeT (1 ng in 0.9% NaCl) was injected over a 10 min period (0.2 μL per min), into the ventral hippocampus (β = −3, L = 2.4, H = 4.2 mm). The other half of the mice was the control group with saline vehicle injection (2 μL of 0.9% NaCl). No behavioral or somatic hallmarks of direct tetanus intoxication were observed. One month following the TeT injection, as epilepsy already developed entirely by that time (Hawkins and Mellanby, 1987; Jefferys and Walker, 2006; Sharma et al., 2007), treatment with pyruvate started. Two months later mice were implanted with electrodes (β = −1.8 mm, L = 1 mm, h = 1.45 mm - recording electrode; β = −1.58 mm, L = 1.5 mm, h = 1.53 mm for bipolar stimulating electrode); and after the recovery period of at least one week the local field potential (LFPs) recordings started. Therefore, the period between TeT injection and initiation of recordings was about 3.5 months. Each mouse was recorded for 2–3 h/session, 3–4 sessions with a 3–5 days' gap between trials. All recordings were performed in awake, freely moving animals, which were controlled to stay awake during recordings.

3.2. AD-related chronic epilepsy

Transgenic APPswe/PS1dE9 mice ($n = 16$) were used in the study. The mice came from a colony at the University of Eastern Finland in Kuopio, based on breeders from Johns Hopkins University (Baltimore, MD, USA). The mice carried mouse/human APPswe double point mutations and human presenilin-1 gene with deleted exon 9, cointegrated in the same transgene under the mouse PrP promoter. This line is characterized by hyperexcitability and reveal epileptiform activity after 3 months of age (Minkeviciene et al., 2009). Wild type animals of the same weight and age were used for control group. The pyruvate treatment protocol started at the age of 3 months and 3 months later first EEG recordings were performed. For EEG mice were implanted with recording electrodes into dentate gyrus (DG) (β = −2 mm, L = 1 mm, h = 2.1 mm) bipolar stimulating electrodes were implanted to perforated path (β = −4 mm, L = 2 mm, h = 1.7 mm) and after the recovery period of at least one week the LFPs recordings started.

3.3. Mouse setup

LFPs were amplified with an ISO DAM-8A amplifier (WPI, USA), filtered (highpass filter 0.1 Hz, digitized at 5 kHz) and acquired using HEKA Instrutech 8 + 8 digitizer with PatchMaster software (HEKA Instruments, Germany). Shaffer collateral/commissural pathway was stimulated using the DS2A isolated stimulator (Digitimer Ltd., UK) with an implanted bipolar nichrome electrode. Stimulus current was adjusted using single pulses (50 to 500 μA, 200 μs, 0.15 Hz) to induce an observable LFPs. Synaptic stimulation consisting of a 5-s stimulus train (200 μs pulses at 20 Hz) was used to induce afterdischarges.

4. Rats

4.1. PTZ kindling

Kindling was induced with i.p. PTZ injection at a subconvulsive dose (35 mg/kg in 0.9% NaCl) every 48 h. Control rats received isotonic saline (1 mL/kg) under the same protocol. After each injection, the convulsive behavior was observed for 30 min and classified in the following stages (modified (Racine et al., 1972)): 0 – absence of reaction; 1 – facial movements, ear/whiskers twitching; 2 – clonus of the face and head; 3 – clonic convulsions of forelegs; 4 – clonic convulsions front and hind legs, the reaction Straube and "wild run"; 5 – consisted of clonic convulsions with loss of posture; 6 – repeated clonic convulsions of forelegs with loss of posture; 7 – jumping; 8 – running and jumping; 9 – running and jumping together with the following tonic convulsions; 10 – multiple tonic convulsions. If during the three consecutive PTZ injections the seizures reached at least stage 6, the animal was considered