ARTICLE IN PRESS

BBADIS-63932; No. of pages: 10; 4C: 8

Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Sulfite disrupts brain mitochondrial energy homeostasis and induces mitochondrial permeability transition pore opening *via* thiol group modification

Mateus Grings [a], Alana P. Moura [a], Alexandre U. Amaral [a], Belisa Parmeggiani [a], Juciano Gasparotto [a], José C.F. Moreira [a], Daniel P. Gelain [a], Angela T.S. Wyse [a], Moacir Wajner [a,b], Guilhian Leipnitz [a,*]

[a] *Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600-Anexo, CEP 90035-003 Porto Alegre, RS, Brazil*
[b] *Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, CEP 90035-903 Porto Alegre, RS, Brazil*

ARTICLE INFO

Article history:
Received 26 November 2013
Received in revised form 23 April 2014
Accepted 25 April 2014
Available online xxxx

Keywords:
Sulfite oxidase deficiency
Sulfite
Bioenergetic dysfunction
Mitochondrial permeability transition
Calcium
Brain mitochondria

ABSTRACT

Sulfite oxidase (SO) deficiency is biochemically characterized by the accumulation of sulfite, thiosulfate and S-sulfocysteine in tissues and biological fluids of the affected patients. The main clinical symptoms include severe neurological dysfunction and brain abnormalities, whose pathophysiology is still unknown. The present study investigated the *in vitro* effects of sulfite and thiosulfate on mitochondrial homeostasis in rat brain mitochondria. It was verified that sulfite *per se*, but not thiosulfate, decreased state 3, CCCP-stimulated state and respiratory control ratio in mitochondria respiring with glutamate plus malate. In line with this, we found that sulfite inhibited the activities of glutamate and malate (MDH) dehydrogenases. In addition, sulfite decreased the activity of a commercial solution of MDH, that was prevented by antioxidants and dithiothreitol. Sulfite also induced mitochondrial swelling and reduced mitochondrial membrane potential, Ca^{2+} retention capacity, NAD(P)H pool and cytochrome *c* immunocontent when Ca^{2+} was present in the medium. These alterations were prevented by ruthenium red, cyclosporine A (CsA) and ADP, supporting the involvement of mitochondrial permeability transition (MPT) in these effects. We further observed that N-ethylmaleimide prevented the sulfite-elicited swelling and that sulfite decreased free thiol group content in brain mitochondria. These findings indicate that sulfite acts directly on MPT pore containing thiol groups. Finally, we verified that sulfite reduced cell viability in cerebral cortex slices and that this effect was prevented by CsA. Therefore, it may be presumed that disturbance of mitochondrial energy homeostasis and MPT induced by sulfite could be involved in the neuronal damage characteristic of SO deficiency.

© 2014 Published by Elsevier B.V.

1. Introduction

Sulfite, thiosulfate and S-sulfocysteine accumulate in tissues and biological fluids of patients affected by sulfite oxidase (SO) deficiency, an autosomal recessive disorder which can arise either from the isolated deficiency of the enzyme SO or from defects in the biosynthetic pathway of its essential cofactor, a molybdenum containing pterin molecule [1,2]. SO is a mitochondrial enzyme that catalyzes the final step in the oxidative degradation of the sulfur containing amino acids cysteine and methionine, playing also an important role in detoxifying exogenously supplied sulfite, since this metabolite may be generated from compounds that are used in food and pharmaceutical industries as preservatives and antimicrobial agents [3–5].

Both forms of SO deficiency are clinically characterized by progressive neurological dysfunction, severe neonatal seizures, lens subluxation, axial hypotonia, limb hypertonicity and failure to thrive, resulting often in early childhood death [1,2,6]. Neuropathological studies reveal severe encephalopathy with neuronal loss and demyelination in the cerebral white matter accompanied by gliosis and diffuse spongiosis. Marked atrophy of the cerebral cortex, basal ganglia, thalami, as well as myelin loss in the cerebellum are also reported [1,6–8]. Furthermore, MRI scans show hypoplasia of the corpus callosum, basal ganglia and brainstem, and cystic changes and calcifications in the basal ganglia [1,9].

Abbreviations: AASA, α-aminoadipic semialdehyde; ANT, adenine nucleotide translocator; Alam, alamethicin; BSA, bovine serum albumin; CAT, catalase; CCCP, carbonyl cyanide m-chlorophenyl hydrazine; CoQ, coenzyme Q_{10}; CP, chlorpromazine; CsA, cyclosporin A; DTT, dithiothreitol; FAU, fluorescence arbitratry units; FCCP, carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone; GDH, glutamate dehydrogenase; GM, glutamate plus malate; α-KG, α-ketoglutarate; α-KGDH, α-ketoglutarate dehydrogenase; MDH, malate dehydrogenase; MEL, melatonin; MPT, mitochondrial permeability transition; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAC, N-acetylcysteine; NEM, N-ethylmaleimide; P6C, piperideine-6-carboxylate; PM, pyruvate plus malate; QUIN, quinacrine; RCR, respiratory control ratio; RR, ruthenium red; SO, sulfite oxidase; SUC, succinate; TFZ, trifluoperazine; TNB, 5-thio-2-nitrobenzoic acid; $\Delta\Psi m$, mitochondrial membrane potential

* Corresponding author. Tel.: +55 51 3308 5551; fax: +55 51 3308 5540.
E-mail address: guilhian@ufrgs.br (G. Leipnitz).

http://dx.doi.org/10.1016/j.bbadis.2014.04.022
0925-4439/© 2014 Published by Elsevier B.V.

Please cite this article as: M. Grings, et al., Sulfite disrupts brain mitochondrial energy homeostasis and induces mitochondrial permeability transition pore opening via thiol group modification, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbadis.2014.04.022

ARTICLE IN PRESS

Although brain abnormalities are predominant features in patients affected by SO deficiency, the biochemical basis of the pathogenesis characteristic of this disorder is still unclear. Nevertheless, there are evidences that accumulation of sulfite and its derivatives induces oxidative stress [10,11] and disturbs mitochondrial function [12,13] in rat brain, which may contribute for the clinical findings observed in affected patients.

Calcium homeostasis dysregulation has been suggested to play an important role in the pathophysiology of neurodegenerative disorders that are associated with excitotoxicity, bioenergetic dysfunction and oxidative stress [14–16]. Under such pathological conditions, excessive Ca^{2+} uptake by the mitochondrion directly results in organelle dysfunction characterized by exacerbated reactive oxygen species formation, dissipation of the membrane potential, altered redox potential and opening of the mitochondrial permeability transition (MPT) pore, which may lead to cell death [17,18].

Since the effects of sulfite and thiosulfate on mitochondrial function are not totally elucidated, we examined the *in vitro* effects of these compounds on ADP-stimulated state (state 3), resting state (state 4) and carbonyl cyanide m-chlorophenyl hydrazine (CCCP)-stimulated state (uncoupled state) of mitochondrial respiration, the respiratory control ratio (RCR), as well as the activities of glutamate, malate and α-ketoglutarate dehydrogenases in brain mitochondrial preparations from young rats. Considering that a mitochondrial dysfunction can compromise Ca^{2+} buffering system and that this may be involved in the pathogenesis of SO deficiency, we also investigated the influence of sulfite in the presence of micromolar concentrations of Ca^{2+} on mitochondrial membrane potential, swelling, Ca^{2+} retention capacity, matrix NAD(P)H content, membrane protein thiol group content and cytochrome *c* release. We further tested the effect of sulfite on cell viability in cerebral cortex slices.

2. Material and methods

2.1. Reagents

All chemicals, including sodium sulfite, sodium thiosulfate, glutamic acid and malic acid, were purchased from Sigma (St. Louis, MO, USA), except for calcium green-5N that was obtained from Molecular Probes, Invitrogen (Carlsbad, CA), and mouse anti-cytochrome *c* monoclonal antibody and anti-mouse IgG peroxidase-linked antibody from Abcam (Cambridge, UK). Sulfite and thiosulfate were dissolved in the buffer used for each technique and the pH was adjusted to 7.4 immediately before the experiments. The final concentrations of these metabolites in the incubation medium ranged from 1 to 500 μM.

2.2. Animals

Thirty-day-old male Wistar rats, obtained from the Central Animal House of the Department of Biochemistry, ICBS, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, were used. The animals were maintained on a 12:12 h light/dark cycle (lights on 07:00–19:00 h) in air conditioned constant temperature (22 ± 1 °C) colony room, with free access to water and 20% (w/w) protein commercial chow (SUPRA, Porto Alegre, RS, Brazil). The experimental protocol was approved by the Ethics Committee for Animal Research of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, and followed the NIH Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

2.3. Preparation of mitochondrial fractions

Forebrain and liver mitochondria were isolated from 30-day-old rats as previously described [19] with slight modifications [20]. Animals were killed by decapitation, had their brain and liver rapidly removed and put into ice-cold isolation buffer containing 225 mM mannitol, 75 mM sucrose, 1 mM EGTA, 0.1% bovine serum albumin (BSA; fatty acid free) and 10 mM HEPES, pH 7.2. Regarding to the brain, the cerebellum, pons, medulla and olfactory bulbs were removed and the remaining material was used as the forebrain. Both tissues were cut into small pieces using surgical scissors, extensively washed to remove blood and homogenized 1:10 in a Dounce homogenizer using both a loose-fitting and a tight-fitting pestle. The homogenate was centrifuged for 3 min at 2000 *g*. After centrifugation, the supernatant was again centrifuged for 8 min at 12,000 g. The pellet was suspended in isolation buffer containing 10 μL of 10% digitonin and centrifuged for 8 min at 12,000 *g*. The final pellet containing the mitochondria was gently washed and suspended in isolation buffer devoid of EGTA, at an approximate protein concentration of 20 $mg \cdot mL^{-1}$. For the measurement of malate dehydrogenase (MDH), glutamate dehydrogenase (GDH) and α-ketoglutarate dehydrogenase (α-KGDH) activities, mitochondrial preparations were submitted to a pre-incubation at 37 °C for 30 min in the absence or presence of sulfite. All experiments were performed in mitochondria with high RCR values, guarantying the full integrity of the preparations.

We carried out parallel experiments with various blanks (controls) in the presence or absence of sulfite and thiosulfate, and also with or without mitochondrial preparations in the incubation medium in order to detect any interference (artifacts) in the techniques utilized to measure the mitochondrial parameters.

2.4. Determination of mitochondrial respiratory parameters by oxygen consumption

Oxygen consumption rate was measured according to Amaral et al. [21] using a Clark-type electrode in a thermostatically controlled (37 °C) and magnetically stirred incubation chamber using glutamate plus malate (2.5 mM each), succinate (5 mM) plus rotenone (2 $\mu g.mL^{-1}$), α-ketoglutarate (5 mM) or pyruvate plus malate (2.5 mM each) as substrates. Sulfite or thiosulfate was added to the reaction medium consisted of 0.3 M sucrose, 5 mM MOPS, 5 mM potassium phosphate, 1 mM EGTA and 0.1% BSA, pH 7.4, and mitochondrial preparations (0.75 mg $protein.mL^{-1}$ using glutamate plus malate, α-ketoglutarate or pyruvate plus malate and 0.5 mg $protein.mL^{-1}$ using succinate). State 3 respiration was measured after the addition of 1 mM ADP to the incubation medium. In order to measure resting (state 4) respiration, 1 $\mu g \cdot mL^{-1}$ oligomycin A was added to the incubation medium. The respiratory control ratio (RCR: state 3/state 4) was then calculated. The uncoupled state was induced by the addition of the classical uncoupler CCCP (1 μM). States 3, 4 and CCCP-induced state were calculated as nmol O_2 consumed $\cdot min^{-1} \cdot$ mg $protein^{-1}$ and the results were expressed as percentage of control.

2.5. Determination of glutamate dehydrogenase (GDH) activity

GDH activity was assayed according to Colon et al. [22]. The reaction mixture contained mitochondrial preparations (60 μg $protein.mL^{-1}$), 50 mM triethanolamine buffer, pH 7.8, 2.6 mM EDTA, 105 mM ammonium acetate, 0.2 mM NADH, 10 mM α-ketoglutarate and 1.0 mM ADP. The reduction of NADH absorbance was monitored spectrophotometrically at 340 nm. GDH activity was calculated as μmol NADH $\cdot min^{-1} \cdot$ mg $protein^{-1}$ and expressed as percentage of control.

2.6. Determination of malate dehydrogenase (MDH) activity

MDH activity was measured according to Kitto [23]. The incubation medium consisted of mitochondrial preparations (7 μg $protein \cdot mL^{-1}$), 10 μM rotenone, 0.1% Triton X-100, 0.14 mM NADH, 0.3 mM oxaloacetate and 50 mM potassium phosphate, pH 7.4. MDH activity was determined following the reduction of NADH fluorescence at wavelengths of excitation and emission of 366 and 450 nm, respectively. MDH activity

Please cite this article as: M. Grings, et al., Sulfite disrupts brain mitochondrial energy homeostasis and induces mitochondrial permeability transition pore opening via thiol group modification, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbadis.2014.04.022

Download English Version:

https://daneshyari.com/en/article/8260125

Download Persian Version:

https://daneshyari.com/article/8260125

Daneshyari.com