Sulfite impairs redox and mitochondrial bioenergetics homeostasis in neonatal rat brain and primary astrocytes

Júlia Pramio¹, Mateus Grings¹, Amanda Gasparin da Rosa¹, Rafael Teixeira Ribeiro¹, Larissa Daniele Bobermin¹, André Quincozes-Santos¹, Moacir Wajner¹,² and Guilhian Leipnitz¹,³*

¹ PPG Ciências Biológicas: Bioquímica, UFRGS, Brazil
² Serviço de Genética Médica, HCPA, Brazil
³ PPG Ciências Biológicas: Fisiologia, UFRGS, Brazil
*e-mail: guilhian@ufrgs.br

Introduction

Sulfite oxidase (SO)

Molybdenum cofactor (MoCo)

Sulfite

Sulfate

SO: enzyme located in the mitochondrial intermembrane space of various tissues.

✓ Deficiency of SO occurs either due to:

- Mutations affecting the enzyme encoding SUOX gene
- Mutations in genes encoding enzymes responsible for MoCo biosynthesis (MOCS1, MOCS2, MOCS3 and GPHN)

Isolated sulfite oxidase deficiency (ISOD)

Molybdenum cofactor deficiency (MoCD)

✓ Inherited metabolic diseases that affect mainly the central nervous system;
✓ Rapidly progressive encephalopathy resulting in early death;
✓ Predominant tissue accumulation of sulfite.

Affected individuals present with seizures refractory to therapy, psychomotor deficit, feeding difficulties, facial dysmorphic features, ectopia lentis, microcephaly, axial hypotonia and peripheral hypertonia, which usually manifest in the neonatal period or early infancy;

(Kappler e Enemark, 2014; Claerhout et al., 2017; Durmaz e Özbakir, 2018; Alonzo Martínez et al., 2020)

✓ The pathophysiology of the neurological dysfunction observed in the neonatal period is still poorly known so that we aimed to evaluate the toxicity of sulfite in this period.
Methods

One-day-old Wistar rats received an intracerebroventricular injection of sulfite (0.5μmol/g) or vehicle and were euthanized 30min later for the evaluation of parameters in the cerebral cortex.

Primary cortical astrocyte cultures were incubated with sulfite (100-1,000μM) or vehicle for 6 and 24h

(Olivera-Bravo et al., 2014; Bobermin et al., 2016; Grings et al., 2017; Bobermin et al., 2020)

Results

Sulfite alters antioxidant defenses in cerebral cortex of newborn rats

Fig. 1 Effect of sulfite (0.5 μmol/g) administration on reduced glutathione (GSH) concentrations (a), as well as superoxide dismutase (SOD) (b), glutathione peroxidase (GPx) (c), glutathione S-transferase (GST) (d), glutathione reductase (GR) (e) and glucose-6-phosphate dehydrogenase (G6PDH) (f) activities in rat cerebral cortex 30 min after injection. Values are means ± standard deviation for five independent experiments (animals) per group. *P < 0.05, **P < 0.01, compared to rats receiving PBS (control group) (Student’s t-test for independent samples).

Fig. 2 Effect of sulfite (0.5 μmol/g) administration on heme oxygenase-1 (HO-1) immunococontent in rat cerebral cortex 30 min after injection. Values are means ± standard deviation for four to five independent experiments (animals) per group. **P < 0.01, compared to rats receiving PBS (control group) (Student’s t-test for independent samples).
Sulfite disturbs energy metabolism in cerebral cortex of newborn rats

Fig. 3 Effect of sulfite (0.5 μmol/g) administration on citrate synthase (CS) (a), succinate dehydrogenase (SDH) (b), malate dehydrogenase (MDH) (c) and creatine kinase (CK) (d) activities in rat cerebral cortex 30 min after injection. Values are means ± standard deviation for six to seven independent experiments (animals) per group. *P < 0.05, compared to rats receiving PBS (control group) (Student’s t-test for independent samples).

Fig. 4 Effect of sulfite (0.5 μmol/g) administration on complexes II (a), II-III (b) and IV (c) activities in rat cerebral cortex 30 min after injection. Values are means ± standard deviation for six to seven independent experiments (animals) per group. **P < 0.01, compared to rats receiving PBS (control group) (Student’s t-test for independent samples).
Sulfite increases ERK1/2 and p38 content in cerebral cortex of newborn rats

**Fig. 5** Effect of sulfite (0.5 μmol/g) administration on extracellular signal-regulated kinase 1/2 (ERK 1/2) (a), p38 kinase (b) and C-Jun N-terminal kinase (JNK) (c) immunocontent and phosphorylation in rat cerebral cortex 30 min after injection. Values are means ± standard deviation for four to five independent experiments (animals) per group. **P < 0.01, compared to rats receiving PBS (control group) (Student's t-test for independent samples).**

Sulfite does not alter mitochondrial dynamics in cerebral cortex of newborn rats

**Fig. 6** Effect of sulfite (0.5 μmol/g) administration on mitofusin 1 (MFN1; fusion protein) (a) and dynamin-related protein 1 (DRP1; fission protein) (b) immunocontent in rat cerebral cortex 30 min after injection. Values are means ± standard deviation for four to five independent experiments (animals) per group. No significant differences between groups were detected (Student's t-test for independent samples).
Sulfite increases ROS levels and disrupts bioenergetics in cortical primary astrocytes

Fig. 7 Effect of sulfite (100-1,000 µM) on 2',7'-dichlorofluorescin (DCFH) oxidation (a, b), extracellular lactate levels (c) and methylthiazolyldiphenyl-tetrazolium bromide (MTT) reduction (d) in cortical primary astrocyte cultures after 6 or 24 h sulfite exposure. Values are means ± standard deviation for three to four independent experiments per group. *P < 0.05, ***P < 0.001, compared to control cells (Duncan multiple range test).

Conclusion

Our results show that sulfite increases ROS production, disturbs antioxidant defenses and induces bioenergetic dysfunction in rat brain. Importantly, sulfite caused similar alterations in astrocytes, which are glial cells with a fundamental role in the maintenance of the neuronal antioxidant system. It may be presumed that these pathomechanisms are involved in pathophysiology of brain damage found in ISOD and MoCD.

References


Acknowledgements

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Pró-Reitoria de Pesquisa/Universidade Federal do Rio Grande do Sul (Propesq/UFRGS) and Instituto Nacional de Ciência e Tecnologia em Excitotoxicidade e Neuroproteção (INCT-EN).