**Supplementary information**

**Butyrate rescues oxidative-stress induced transport deficits of tryptophan – potential implication in affective or gut-brain axis disorders**

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**Short title:** Butyrate rescues tryptophan transport

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**Effect of oxidative stress and butyrate on tryptophan uptake**

***Effect of only butyrate treatment on tryptophan uptake in fibroblasts***

In order to evaluate the effects of butyrate on tryptophan transport in the absence of oxidative stress, fibroblasts were treated with different concentrations of butyrate alone for one and six hours.

Treating fibroblasts with only butyrate at micromolar concentrations (100 μM, 500 μM, 1000 μM) for one or six hours did not alter the uptake of tryptophan in human fibroblast cells compared to untreated control (**Fig. S1**). Furthermore, when fibroblast cells were treated with only butyrate at millimolar concentrations (20 mM, 40 mM) for one hour, no changes in the uptake of tryptophan were observed when compared to untreated control.

**Effect of oxidative stress and micromolar concentrations of butyrate on gene expression patterns**

***LAT1 (SLC7A5) mRNA expression***

For micromolar concentrations of butyrate treatment, the mRNA expression of *SLC7A5* of cells treated with 10 µM of H2O2 or only with butyrate (100 μM, 500 μM, 1000 μM) for one or six hours did not alter the gene expression compared to control (**Fig. S2**). Also, for millimolar concentrations of butyrate treatment, the mRNA expression of *SLC7A5* of cells treated with butyrate (20 mM, 40 mM) for one hour did not alter the gene expression compared to control. Treating oxidative stress-induced cells with millimolar concentrations of butyrate for one or six hours did not alter the mRNA expression of SLC7A5.

***LAT2 (SLC7A8) mRNA expression***

The mRNA expression of *SLC7A8* of cells treated with 10 µM of H2O2 or with only the micromolar concentrations of butyrate (100 μM, 500 μM, 1000 μM) for one and six hours did not alter the gene expression when compared to the untreated control (**Fig. S3**). Butyrate treatment for six hours of oxidative stressed cells did not result in any differences of gene expression. Treating oxidative stressed cells with millimolar concentrations of butyrate (20 mM, 40 mM) for one or six hours did not alter the gene expression of *SLC7A8* when compared to the untreated control.

***4F2hc (SLC3A2) mRNA expression***

Incubating the cells with 10 µM of H2O2 for one hour and different concentrations of butyrate (100 μM, 500 μM, 1000 μM, 20 mM, 40 mM) for one or six hours did not result in any difference in the expression of *SLC3A2* mRNA (**Fig. S4**).

**Effect of oxidative stress and butyrate on cell cytotoxicity**

In order to investigate if the effects of oxidative stress and butyrate treatments on the tryptophan transport were not influenced by the cell viablility, cell cytotoxic assays were carried out. Cells were treated with 10 μM of H2O2 for one, three or six hours and then treated with different concentrations of butyrate (100 µM, 500 µM, 1000 µM, 20 mM and 40 mM) for one and six hours respectively. Cell cytotoxicity was tested in duplicates by using lactate dehydrogenase (LDH) cell cytotoxicity assay kit according to the manufacturer protocol (Pierce™ LDH cytotoxicity assay kit, ThermoFisher Scientific, USA). Ordinary one-way analysis of variance (ANOVA) by using Dunnett’s multiple comparisons test for cell cytotoxicity assays were used to verify the existence of significant differences between groups treated with butyrate, H2O2 and the control group.

Treating the fibroblast cells with different concentrations of butyrate (100 μM, 500 μM, 1000 μM, 20 mM, 40 mM) for one and six hours and also with 10 µM of H2O2 for one, three and six hours did not induce any cell cytotoxicity when compared to spontaneous control (cells treated with water) and maximum control (cells treated with lysis buffer). Different treatment conditions with both H2O2 andbutyrate did not induce any cell cytotoxicity in fibroblast cells.

**Fig. S1:** Effect of different concentrations of butyrate on tryptophan uptake in healthy human fibroblast cells. **A**, **B** Treating the fibroblast cells with only butyrate (100 μM, 500 μM, 1000 μM) for one or six hours did not alter the uptake of tryptophan in human fibroblast cells compared to untreated control. **C** Treating human fibroblast cells with only butyrate (20 mM, 40 mM) for one hour did not alter the uptake of tryptophan in human fibroblast cells compared to control (n=6)

**Fig. S2:** Effect of oxidative stress and different concentrations of butyrate treatment for one or six hours on the *SLC7A5* mRNA expression normalized to *GAPDH* in human fibroblast cells. **A** Treating cells with 10 µM of H2O2 for one hour and with micromolar concentrations of butyrate (100 μM, 500 μM, 1000 μM) for six hours did not alter the gene expression of *SLC7A5* normalized to *GAPDH* compared to control. **B** Treating cells with millimolar concentrations of butyrate (20 mM, 40 mM) for one hour did not alter the gene expression of *SLC7A5* normalized to *GAPDH* compared to control.

**Fig. S3:** Effect of oxidative stress and different concentrations of butyrate treatment on *SLC7A8* mRNA expression normalized to *GAPDH* in human fibroblast cells.Treating cells with one hour 10 µM H2O2 and six hours micromolar concentrations of butyrate did not significantly alter the gene expression of *SLC7A8* normalized to *GAPDH* compared to control (n=2)

**Fig. S4:** Effect of oxidative stress for one hour and different concentrations of butyrate treatment for one or six hours on *SLC3A2* mRNA expression normalized to *GAPDH* in human fibroblast cells. **A, B** Treating the cells with 10 µM of H2O2 for one hour and micromolar concentrations of butyrate (100 μM, 500 μM, 1000 μM) for one or six hours did not alter the gene expression of *SLC3A2* normalized to *GAPDH* compared to control. **C, D** Treating the cells with 10 µM of H2O2 for one hour and millimolar concentrations of butyrate (20 mM, 40 mM) for one or six hours did not alter the gene expression of *SLC3A2* normalized to *GAPDH* compared to control (n=2)