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Itaconic acid decreases mitochondrial respiration and ROS generation in brain tissue

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Introduction

Itaconic acid (IA) is a recently discovered mammalian metabolite which is produced by macrophages upon pro-inflammatory activation: in quiescent bone marrow – derived macrophages (BMDMs). IA is hardly detectable but upon lipopolysaccharide (LPS) stimulation it reaches millimolar concentrations [1]. Recently reported physiological roles of IA include inhibition of bacterial enzyme isocitrate lyase (bactericidal activity) and the inhibition of a Krebs cycle enzyme succinate dehydrogenase (SDH) in the host cells (which has been shown for BMDMs) [2,3]. By inhibiting SDH, IA regulates succinate (a pro-inflammatory metabolite) levels thus remodelling the host cells metabolism during inflammation [3]. Microglia are macrophages residing in the brain, however, it is not clear whether these cells can also produce IA. In general, there has been very little research on IA effects on brain tissue. Therefore, in this study, we investigated whether IA exerts an effect on brain mitochondrial respiration and ROS generation and whether IA is neurotoxic.

Methods

For neuronal viability experiments we used primary neuronal-glial cell cultures prepared from 7-8-day old Wistar rat cerebellum (cerebellum granule cell; CGC). Cultures were treated with 3-10 mM IA for 24-72 h. The viability of neurons in the culture was measured by propidium iodide (necrotic cells) and Hoechst 33342 (homogenous nuclei – viable cells, chromatin condensed – apoptotic cells) staining.

For mitochondrial respiration and ROS generation assessment we used isolated brain mitochondria and brain tissue homogenates. Brains were taken from 2-3 months old Wistar rats and homogenized using glass-teflon homogenizer in MiR05 buffer (110 mM sucrose, 60 mM K-lactobionate, 0.5 mM EGTA, 3 mM $MgCl_2$, 20 mM taurine, 10 mM KH_2PO4, 20 mM HEPES, pH 7.1 at 30 °C, and 0.1% BSA, fatty acid free [4]). Mitochondria were isolated using differential centrifugation. Mitochondrial respiration was recorded by high-resolution respirometry (Oroboros Instruments, Innsbruck,) using 0.25mg mitochondrial protein/mL or 1mg tissue/mL and various substrates and inhibitors in the presence of 1-5 mM IA. The oxygen consumption rates (OCRs) were measured in pmol(O_2)/s*ml/0.5mg mitochondrial protein. Mitochondrial ROS generation was measured fluorometrically using Amplex Red dye. Conditions for respirometry and ROS measurements were identical (same temperature, concentrations of mitochondrial protein, substrates and inhibitors). ROS generation was recorded in 5 minute intervals over 30 minutes and mean ROS generation rate was calculated (μmol H_2O_2/min).

Statistical analyses were performed using IBM SPSS statistics 22 software. One way ANOVA with Tukey post hoc statistical test was used to compare means. The difference was considered significant when $p<0.05$.

Results

We found that during 24 h incubation up to 5 mM concentrations of IA had no effect on neuronal viability. However, 10 mM IA decreased neuronal viability to 10% of control after 48 h and to 5 % of control after 72 h. The percentage of apoptotic cells was 75% and 85% after 48 h and 72 h incubation, respectively, while necrosis was at the level of 5-10% after 24-72 h incubation. Respirometry with brain tissue homogenates showed that 1-5 mM IA decreases maximal ADP-stimulated mitochondrial respiration rates by 35-45%. Using isolated brain mitochondria we found that IA decreases mitochondrial respiration with NADH-dependent substrates (pyruvate, malate) and $FADH_2$-dependent substrates (succinate): in the control group the mean OCRs in ADP-stimulated respiration were 216.60±11.73 with complex I (CI) substrates, 70.77±1.93 with complex II (CII) substrates and the respiratory control ratios (RCRs) were 6.65±0.43 for CI and 2.18±0.11 for CII; in the 1 mM IA group the ADP-stimulated respiration was 121.46±5.67 with CI substrates, 45.86±2.52 with CII substrates and the RCRs were 4.54±0.21 for CI and 1.74±0.40 for CII; in the 5 mM IA group the ADP stimulated respiration was 82.98±15.37 with CI substrates, 26.92±1.79 with CII substrates and the RCRs were 3.15±0.38 for CI and 1.05±0.12 for CII. The ROS production rates were: 1.0918±0.171 (CI) and 1.7752±0.1561 (CII) in control group; 1.0292±0.0517 (CI) and 1.469±0.119 (CII) in 1mM IA group; 0.6405±0.0908 (CI) and 1.0804±0.1066 (CII) in 5 mM IA group.

Discussion

In our study we found that IA is not neurotoxic at concentrations lower than 10 mM, and 10 mM IA is neurotoxic only after long (>24 h) incubation. 1 mM IA supressed OCR with pyruvate and malate by about 40% and 5mM IA by about 60%. OCR with succinate was reduced by about 40% with 1 mM IA and by about 60% with 5 mM IA. Mitochondrial ROS generation was not affected by 1 mM IA, however 5 mM IA significantly decreased ROS generated by mitochondria respiring with pyruvate and malate as well as with succinate by about 40%. Based on these results we concluded that IA inhibits brain mitochondrial respiration and ROS generation with Complex I and Complex II substrates at non-toxic concentrations.

References

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