Mechanism of oxidative stress-induced intracellular acidosis in rat cerebellar astrocytes and C6 glioma cells.

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摘要

Abstract

1. Following ischaemic reperfusion, large amounts of superoxide anion (.O2-), hydroxyl radical (.OH) and H2O2 are produced, resulting in brain oedema and changes in cerebral vascular permeability. We have found that H2O2 (100 microM) induces a significant intracellular acidosis in both cultured rat cerebellar astrocytes (0.37 +/- 0.04 pH units) and C6 glioma cells (0.33 +/- 0.07 pH units). 2. Two membrane-crossing ferrous iron chelators, phenanthroline and deferoxamine, almost completely inhibited H2O2-induced intracellular acidosis, while the non-membrane-crossing iron chelator apo-transferrin had no effect. Furthermore, the acidosis was completely inhibited by two potent membrane-crossing .OH scavengers, N-(2-mercaptopropionyl)-glycine (N-MPG) and dimethyl thiourea (DMTU). Since .OH can be produced during iron-catalysed H2O2 breakdown (Fenton reaction), we have shown that a large reduction in pH1 in glial cells can result from the production of intracellular .OH via H2O2 oxidation. 3. We have ruled out the possible involvement of: (i) an increase in intracellular Ca2+ levels; and (ii) inhibition of oxidative phosphorylation. 4. Our results suggest that .OH inhibits glycolysis, leading to ATP hydrolysis and intracellular acidosis. This conclusion is based on the following observations: (i) in glucose-free medium, or in the presence of iodoacetate or 2-deoxy-D-glucose, H2O2-induced acidosis is completely suppressed; (ii) H2O2 and iodoacetate both produce an increase in levels of intracellular free Mg2+, an indicator of ATP breakdown; and (iii) direct measurement of intracellular ATP levels and lactate production show 50 and 55% reductions in ATP content and lactate production, respectively, following treatment with 100 microM H2O2. 5. Inhibition of the pH1 regulators (i.e. the Na(+)-H+ exchange and possibly the Na(+)-HCO3(-)-dependent pH1 transporters) resulting from H2O2-induced intracellular ATP reduction may also be involved in the H2O2-evoked intracellular acidosis in glial cells