THE EFFECT OF PEROXYNITRITE AND SOME ANTIOXIDANTS ON THE RATE OF OSMOTIC HEMOLYSIS OF ERYTHROCYTES

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The treatment of erythrocytes with peroxynitrite (ONOO−), a cytotoxic species formed in vivo by the reaction of nitric oxide (NO•) and the superoxide anion (O₂•−), led to an increase in the rate of hemolysis upon sudden osmotic stress. The rate of hemolysis was measured using a stopped-flow technique, and a first-order semilogarithmic plot of the decrease in turbidity that takes place during hemolysis was used to determine an apparent rate of hemolysis. The effect of peroxynitrite on this rate of hemolysis was studied. It was found that the increase in the rate of osmotic hemolysis is peroxynitrite concentration dependent (100-1000 µM). The observed increase was due to the action of peroxynitrite, and not of decomposition products contained in the peroxynitrite preparation. The peroxynitrite induced increase in the rate of hemolysis was only partially suppressed by albumin, reduced glutathione, melatonin and bovine serum, but uric acid and ascorbic acid sufficiently inhibited the increase in the rate of hemolysis. Because in the course of hemolysis the erythrocyte membrane bilayer is ruptured and its stability can be reflected in its fluidity, we checked the EPR spectra of spin labels (5DSA and 16DSA) incorporated into the lipid bilayer of the erythrocyte membranes treated with peroxynitrite. It was found that in peroxynitrite treated membranes fluidity was markedly decreased in the hydrophobic core of the bilayer.

In conclusion, this study shows that, although the details of the underlying mechanism are not clear, peroxynitrite is capable of increasing the rate of osmotic hemolysis of erythrocytes and changing the fluidity of the membranes. A comparison of our results on the protective effect of antioxidants with results published for peroxynitrite-induced hemolysis in physiological saline led to the conclusion that the rate of osmotic hemolysis actually measured a different process, probably involving rupturing of the membrane.