Short communication

Effect of Activated Oxygen Species on Mitochondria Isolated from Myocardium after Reperfusion Injury

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Activated oxygen species-induced disturbances in myocardium have been suggested indirectly occurring during the reperfusion damage (Shlafer et al. 1982; Jolly et al. 1984). Mitochondria continously forming O_2^-, H_2O_2 (Forman and Boveris 1982), and $^{\cdot}OH$ (Otani et al. 1984) should be affected directly when excessive amounts of these oxygen metabolites become generated. Effects of $^{\cdot}OH$ and H_2O_2 on oxidative phosphorylation of mitochondria isolated from nonperfused, control perfused, and ischemic and reperfused rabbit hearts were investigated. Mitochondria were isolated according to Vaghy et al. (1982). Oxygen consumption was determined polarographically (Vaghy et al. 1980). In addition, basic state swelling (Vaghy et al. 1980), the content of thiobarbituric acid reactive material (Tanizawa et al. 1981), and lipid soluble fluorescence (Fletcher et al. 1973) were measured.

If pyruvate was used as the substrate, H_2O_2 ($\geq$25 nmol/mg) and $^{\cdot}OH$ induced a reduction in respiration in state 3, in respiration during uncoupling with dinitrophenol; also, the respitatory control index (RCI), and the ADP:O ratio were diminished, the latter only if 75 nmol H_2O_2/mg or more were used (Fig. 1). The stronger decrease of RCI as compared to ADP:O (by about 70 % and 30 %, respecively, with 100 nmol H_2O_2/mg; by about 60 % and 15 %, respectively, with $^{\cdot}OH$) indicates a deterioration of the respiratory activity rather than of the phosphorylation efficiency. Moreover, a small enhancement of respiration state 4 and a slight swelling during the basic respiration were found, but no changes suggesting uncoupling of oxidative phosphorylation.

The decrease in the respiratory activity differed from that induced by inhibition of the phosphorylating system by oligomycin (0.66 μg/mg), also suggesting that the phenomena observed were not due to alterations on the phosphorylation level. No substantial changes in the content of thiobarbituric acid reactive

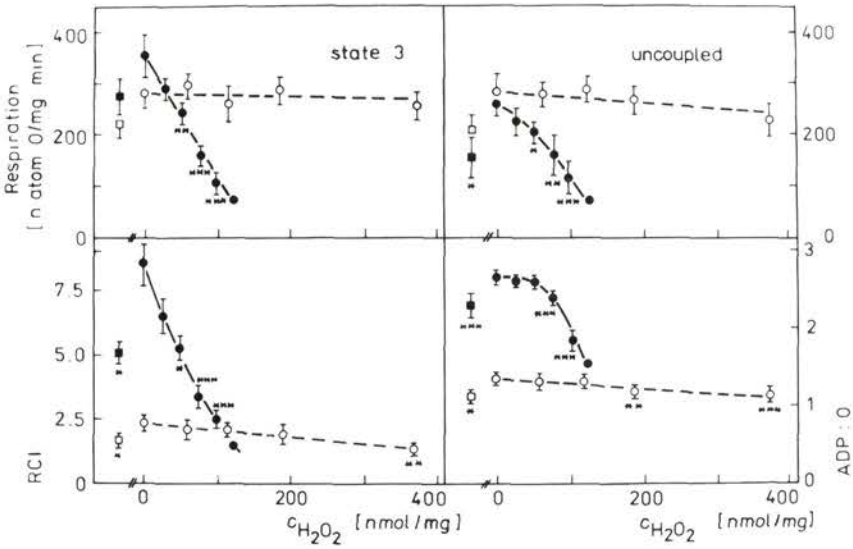

Fig. 1. Effects of H_2O_2 and $^{\cdot}OH$, formed from 6.5 nmol Fe^{2+} and 13 nmol H_2O_2/mg, on the function of isolated myocardial mitochondria using 1 mmol/l pyruvate or 5 mmol/l succinate (+5 μmol/l rotenone) as the substrate. 6.5 nmol Fe^{2+}/mg and 13 nmol H_2O_2/mg proved to be ineffective when added separately. The test medium consisted of 120 mmol/l KCl, 10 mmol/l HEPES, 2 mmol/l K—P_i, pH 7.1 at 37 °C. The mitochondria (0.75 mg protein/ml) were added immediately after H_2O_2 and Fe^{2+}. Then the mitochondria were incubated for 5 min prior to the addition of ADP (490 nmol/mg in pyruvate medium and 220 nmol/mg in succinate medium, respectively). Uncoupling with 130 nmol 2,4-dinitrophenol/mg. Mitochondria were isolated from nonperfused hearts immediately after the excision. Data are given as means ±S.E.M., $n=5$; significance levels: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ (as compared to controls without H_2O_2 or Fe^{2+} plus H_2O_2); closed circles — pyruvate medium, open circles — succinate medium.

material and in the lipid soluble fluorescence could be detected in mitochondria exposed to H_2O_2 or $^{\cdot}OH$ for 1; 5; or 10 min. This suggests that these products forming relative slowly from lipid peroxidation were not a major cause of the inhibited respiration in state 3 and during uncoupling. The functional disturbance were weaker if succinate was used as the substrate (Fig. 1). Based on this it may be suggested that the region that seems most sensitive to activated oxygen species, is the NADH-oxidizing branch of the respiratory chain.

Postischemic reperfusion under the above conditions (see legend of Fig. 2) resulted in moderate myocardial deterioration: ventricular extrasystoles were observed, the heart rate reached 79.8 % ($p<0.05$), the segmental shortening 71.6 % ($p<0.05$), and CPK leakage 546 % ($p<0.001$) compared to values measured during the control perfusion. The mitochondria showed reduced respiration state 3 and RCI as compared with control perfused hearts, whereas, respiration state 4 and the ADP:O ratio did not show changes (the latter not shown).

Nearly the same alterations were observed when the mitochondria from control perfused hearts were exposed to 50 nmol H_2O_2/mg. After the myocardial reperfusion injury, the isolated mitochondria were more sensitive against H_2O_2 compared to those isolated from hearts after the control perfusion. The mitochondria of the impaired hearts already showed functional disturbances after incubation with ≥ 1 nmol H_2O_2/mg, attaining mitochondria H_2O_2 contents occurring physiologically. The control mitochondria were more protected. Only ≥ 25 nmol H_2O_2/mg induced the disturbances. The effect of H_2O_2 was weakened by simultaneous incubation with superoxide dismutase; this enzyme catalyzes the removal of O_2^- the latter being necessary for the regeneration of Fe^{2+} for $^{\cdot}OH$ formation from H_2O_2 in the Haber-Weiss cycle. The $^{\cdot}OH$ scavenger mannitol was less effective, possibly due to its membrane impermeability and the very high reactivity of $^{\cdot}OH$ (Fig. 2). In conclusion it can be stated that mitochondrial disturbances induced by activated oxygen species were similar to those observed during myocardial reperfusion injury. These disturbances were accompanied by an impairment of the mitochondrial defence against activated oxygen species.

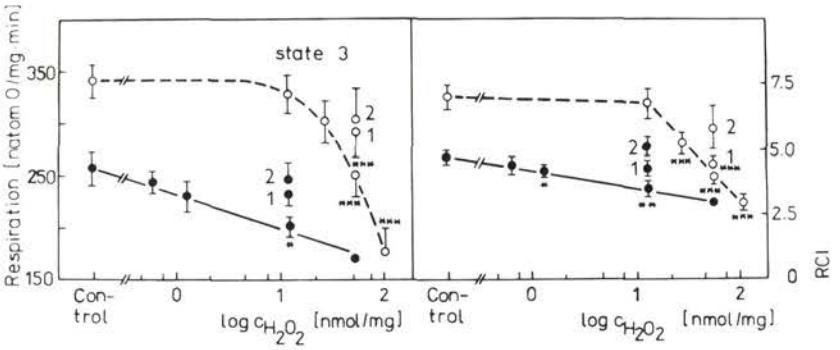

Fig. 2. Concentration-dependence of the H_2O_2 reduced respiration state 3 and RCI of mitochondria isolated from retrogradely perfused hearts in vitro according to the Langendorff technique either after ischemic perfusion for 60 min (~ 0.35 ml/min·g heart wet weight, $pO_2 \sim 20$ mmHg ≈ 2.7 kPa) followed by a 10 min reperfusion or after a 70 min control perfusion (both 12 ml/min·g heart wet weight, $pO_2 \sim 600$ mmHg ≈ 80 kPa). Krebs-Henseleit bicarbonate buffer with 11 mmol/l glucose, pH 7.4 gassed with O_2 containing 5% CO_2 (control perfusion, reperfusion) or N_2 (ischemia) at 37 °C, was used as the perfusion fluid. The perfusion pressure during control perfusion and reperfusion was between 60 and 80 mmHg (8—10 kPa). The mitochondria test medium contained 150 mmol/l KCl, 1 mmol/l pyruvate, and 2 mmol/l K—P_i, pH 7.1 at 37 °C. Mitochondria (0.75 mg/ml) were incubated for 1 min prior to the addition of 220 nmol ADP/mg; the mitochondria were added immediately after H_2O_2. M and SOD represent the interaction of 1 µmol mannitol and 6 U superoxide dismutase per mg, respectively. Both were added simultaneously to 13 nmol H_2O_2/mg (mitochondria isolated from ischemic perfused and reperfused hearts) and to 52 nmol H_2O_2/mg (mitochondria isolated from control perfused hearts). Data are means ± S.E.M., $n = 6$; significance level: $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to the corresponding control (without any effector); closed circles — mitochondria preperations from heaters after ischemic perfusion and reperfusion, open circles — mitochondria preparations from hearts after control perfusion.

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Received July 29, 1985/Accepted May 19, 1986