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REOXYGENATION INJURY IN ISOLATED HEPATOCYTES: CELL DEATH PRECEDES CONVERSION OF XANTHINE DEHYDROGENASE TO XANTHINE OXIDASE

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Reoxygenation of isolated hepatocytes from fed rats after 3 h of anaerobic incubation led to a significantly enhanced loss of cell viability. No evidence for the participation of reactive oxygen species generated by xanthine oxidase in this reoxygenation injury was found. Conversion of xanthine dehydrogenase to xanthine oxidase occurred at a time when almost all of the hepatocytes had lost their viability. Furthermore, xanthine dehydrogenase was first released from the severely injured cells and then converted to the oxidase form. The results suggest that in the intact organ participation of reactive oxygen species, generated by xanthine oxidase, in reoxygenation injury may only occur when, upon reoxygenation, hypoxic cell injury in part of the tissue has progressed to such an extent that there is a significant conversion of xanthine dehydrogenase to xanthine oxidase.

Reoxygenation of a previously hypoxic tissue - following reperfusion of an ischemic area in an organ infarct or during the transplantation of an organ - may paradoxically lead to exacerbation of tissue injury. Reactive oxygen species, such as $O_2^{-}\cdot$, H_2O_2, and $OH\cdot$, may decisively contribute to this reoxygenation injury. It has been suggested (1) that the reactive oxygen species are generated by the catalytic activity of xanthine oxidase. This proposal is based on the observation that in several tissues, such as liver, intestine and heart, a conversion of xanthine dehydrogenase to xanthine oxidase occurs under anoxic conditions and that superoxide dismutase, catalase, and allopurinol, a potent inhibitor of xanthine oxidase, diminish reoxygenation injury in some model systems (e.g. 2-5).

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It is assumed that the conversion of xanthine dehydrogenase to the oxidase form proceeds intracellularly during the hypoxic period by catalysis of proteases, activated by an increased cytosolic Ca^{2+} concentration (1). However, the involvement of reactive oxygen species in reoxygenation injury is not generally accepted. In several studies neither a conversion of xanthine dehydrogenase to xanthine oxidase nor beneficial effects of superoxide dismutase, catalase, and allopurinol on reoxygenation injury were observed (e.g. 6-8).

The present study reports on reoxygenation injury in isolated hepatocytes with special emphasis on the conversion of xanthine dehydrogenase to xanthine oxidase. The results demonstrate that only extracellular xanthine dehydrogenase, liberated from destroyed cells, is subject to this conversion. Thus, cell death precedes conversion of xanthine dehydrogenase to the oxidase form in this model system.

METHODS

Hepatocytes were isolated from fed male Wistar rats by a modified procedure of Berry and Friend (9). The integrity of the isolated hepatocytes was evaluated by the determination of lactate dehydrogenase (LDH) leakage (10). Isolated hepatocytes ($2 - 3 \times 10^6$ cells/ml) were incubated at 37 °C in Krebs-Henseleit buffer, pH 7.4, supplemented with 10 mM glucose, 2.1 mM lactate, and 0.3 mM pyruvate, in an oxystat system (10). Reoxygenation was performed at a constant oxygen partial pressure of 28 mm Hg. Xanthine dehydrogenase and xanthine oxidase activity were measured according to Kehrer et al. (7). Aliquots of cell suspension were centrifuged at 4000 x g for 20 sec and the two enzyme activities determined in the supernatant (extracellular activities) and in the cell pellet after addition of Triton X-100 (0.1 %) (intracellular activities).

RESULTS

In isolated hepatocytes from fed rats incubated under anaerobic conditions, there was a slow but continuous decrease in cell viability during the first 3 h of incubation, as indicated by leakage of lactate dehydrogenase (Fig. 1A). Subsequently, loss of cell viability increased significantly and after 5 h only a small number of viable cells were present. Reoxygenation of the anaerobic cells within the first 3 h completely prevented further cell death (data not shown).

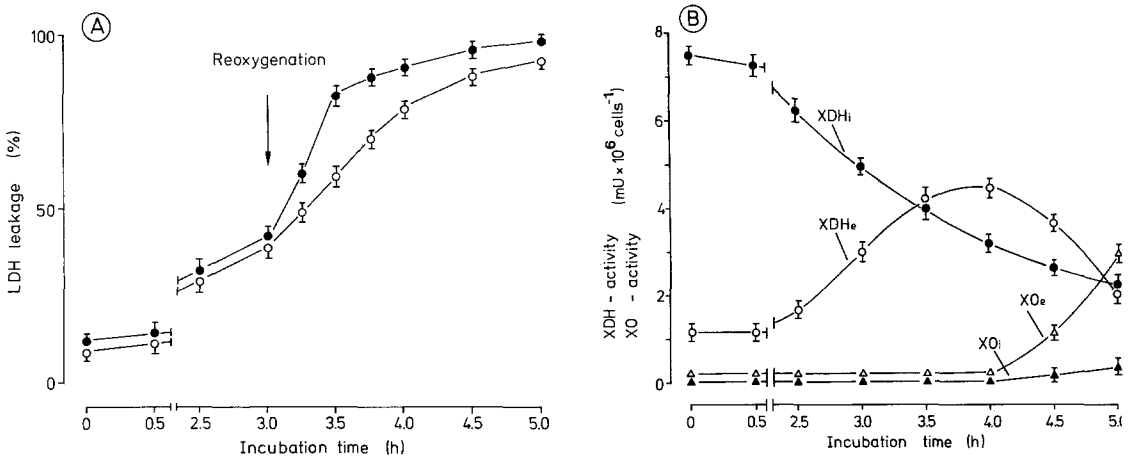

Fig. 1A

Effect of reoxygenation on lactate dehydrogenase (LDH) leakage from isolated hepatocytes incubated under anaerobic conditions. Hepatocytes were reoxygenated at a constant oxygen partial pressure of 28 mm Hg (filled circles). Control incubations (open circles) were maintained under anaerobic conditions over the whole incubation period. Each value represents a mean ± S.E.M. of at least 3 incubations.

Fig. 1B

Release of xanthine dehydrogenase and conversion of xanthine dehydrogenase to the oxidase form during incubation of isolated hepatocytes under anaerobic conditions. XDH_i, intracellular xanthine dehydrogenase; XDH_e, extracellular xanthine dehydrogenase; XO_i, intracellular xanthine oxidase; XO_e, extracellular xanthine oxidase. Each value represents a mean ± S.E.M. of at least 3 incubations.

However, reoxygenation after 3 h produced a rapid loss of cell viability, which occurred at a significantly enhanced rate compared to cells maintained under anaerobic conditions (Fig. 1A).

Release of xanthine dehydrogenase from the hepatocytes was almost negligible during the first 2.5 h of anaerobic incubation but then continuously increased and at 5 h about 70 % of the enzyme had left the cells (Fig. 1B). Conversion of xanthine dehydrogenase to xanthine oxidase could not be detected during the first 4 h of anaerobic incubation (Fig. 1B). However, after 4 h the activity of the oxidase form rapidly increased, concomitant with a decrease in xanthine dehydrogenase activity, and after 5 h about 40 % of the enzyme had been converted to the

oxidase form. This conversion was exclusively extracellular. No significant intracellular xanthine oxidase activity was detected over the whole incubation period. Similar results were obtained in experiments where the anaerobic hepatocytes were reoxygenated after 3 h. Xanthine dehydrogenase was first released from the hepatocytes and then converted to xanthine oxidase (data not shown).

DISCUSSION

Generation of reactive oxygen species by xanthine oxidase cannot be responsible for the reoxygenation injury in isolated hepatocytes depicted in Fig. 1A, since conversion of xanthine dehydrogenase to xanthine oxidase only occurred when the cells had already lost their viability (compare Figs. 1A and 1B). In contrast to the mechanism proposed by McCord (1), xanthine dehydrogenase was first released by the already severely injured cells, similar to lactate dehydrogenase, and only subsequently converted to the oxidase form. This conversion is presumably mediated by the catalytic activity of proteases also liberated from the damaged cells. Hence, a mechanism not involving the formation of reactive oxygen species by xanthine oxidase appears to be responsible for the reoxygenation injury observed here. One clue to the mechanism of this reoxygenation injury is suggested by the finding that in the isolated perfused rat heart reoxygenation injury can be prevented by KCN (7,11,12), indicating that resumption of mitochondrial function may be involved.

In the experiments with isolated hepatocytes presented here, reoxygenation injury apparently did not involve reactive oxygen generated by xanthine oxidase. In the intact liver, however, it is conceivable that reactive oxygen species contribute to cell injury. Thus, upon reperfusion xanthine oxidase may reach areas of the liver where the cells are slightly damaged or still intact. In these areas, reactive oxygen formed by the catalytic activity of xanthine oxidase may produce an additional reoxygenation injury either directly or via activation of granulocytes (13,14).

The present results could also explain the contradictory observations in various tissues concerning the inhibitory

effects of superoxide dismutase, catalase, and allopurinol on reoxygenation injury (2-8). A beneficial effect of these substances can only be expected when, in part of the organ, hypoxic cell injury has already progressed to such an extent that there is a significant conversion of xanthine dehydrogenase to xanthine oxidase. Reperfusion before that time may cause reoxygenation injury but without participation of reactive oxygen.

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