RESTORATION OF SOME ENERGY LINKED PROCESSES LOST DURING THE AGEING OF RAT LIVER MITOCHONDRIA

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Summary: Some energy linked processes partially lost during the ageing of rat liver mitochondria, such as respiratory control index, ADP:O ratio and the Ca^{++} and K^{+} uptake were restored to approximately the original level prior to ageing by addition of dithioerythritol to aged mitochondria.
It is suggested that the maintenance of mitochondrial energy linked processes may depend upon the integrity of specific pairs of vicinal thiol groups of the membrane.

It has been recently demonstrated that tellurite, added to rat liver mitochondria at concentrations ranging from 50 to 100 μM, induced a decrease of P:O ratio and of Ca^{++} and K^{+} uptake. These effects are particularly evident when the source of energy was NAD linked substrates oxidation (1).

The action of tellurite may logically result from its known capacity to react with pairs of thiol groups forming dimercaptide bonds, both directly and via dithiotellurite (2):

$$4\ RSH + H_2TeO_3 \rightleftharpoons RSSR + RS\text{-}Te\text{-}RS + 3H_2O$$

$$\downarrow$$

$$RSSR + Te$$

Considering that tellurite accelerates and amplifies some of the events occurring during the ageing of mitochondria and that dithioerythritol (DTE) reverses some of tellurite effects (3), the action of DTE on some energy linked processes during the ageing of mitochondria has been studied.

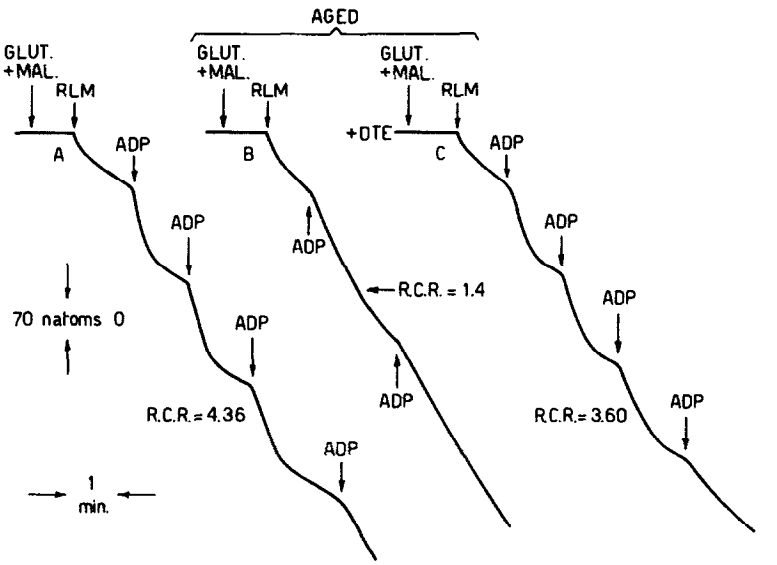

Figure 1: Effect of dithioerythrytol on the respiratory control of aged rat liver mitochondria.

The assay medium (final volume 1.8 ml, temperature 25°C) contained 10.8 mM K_2HPO_4, 2.82 mM KH_2PO_4, 9.9 mM NaF, 21.62 mM NaCl, 48.3 mM KCl, 5 mM $MgCl_2$, 5 mM glutamate, 5 mM malate. 6 mg of mitochondrial protein (RLM) were added. Where indicated 320 nmoles of ADP were added.
Trace A: Fresh mitochondria.
Trace B: Mitochondria aged at 0-4°C for 6 hours.
Trace C: Mitochondria aged at 0-4°C for 6 hours; 0.67 mM dithioerythrytol (DTE) present.

Materials and Methods

Rat liver mitochondria were isolated from Wistar strain albino rats in 0.25 M sucrose (4). Mitochondria were suspended in 0.25 M sucrose at a concentration of 90-120 mg protein/ml and were stored in ice (0-4°C). Oxygen uptake was measured at 25°C with a Clark oxygen electrode.

Ca^{++} movement was followed by measuring the synchronous variations of H^+ concentration with a combination electrode and a Beckman Expandomatic pH meter connected to a strip-chart recorder.

Results

As shown in Fig. 1 the respiratory control ratio, almost completely lost during ageing of mitochondria at

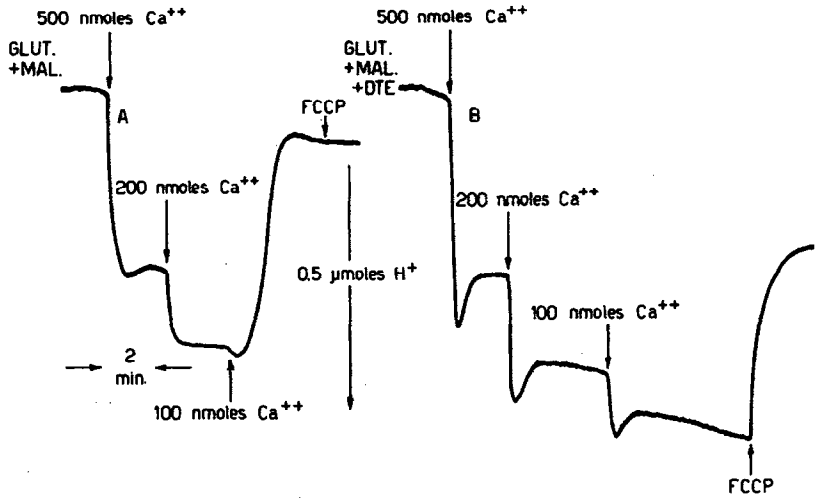

Figure 2: The effect of dithioerythrytol on Ca^{++} uptake by aged rat liver mitochondria.

The assay medium (final volume 4 ml, temperature 25°C) contained 80 mM KCl, 5 mM Tris-HCl (pH 7.4), 5 mM glutamate, 5 mM malate.
The mitochondria had been previously aged at 0-4°C for 6 hours; 10 mg of mitochondrial protein were used for each assay.
Trace A: in the absence of dithioerythrytol (DTE).
Trace B: in the presence of 0.5 mM DTE.

0-4°C (trace B), was restored to approximately the initial value by addition of 0.67 mM DTE to aged mitochondria (trace C). The ADP:O ratio was similarly increased by DTE.

The addition of DTE to aged mitochondria also restored the Ca^{++} uptake linked to the oxidation of glutamate plus malate, which was partially lost during ageing (see Fig. 2).

The typical experiment reported in Fig. 2 shows that in aged mitochondria Ca^{++} uptake was limited to 70 nanomoles of Ca^{++}/mg protein. Further addition of Ca^{++} no longer induced acidification of the suspending medium. In addition, the suboptimal amount of Ca^{++} accumulated was not retained within mitochondria during the resting respiration, but spontaneously released into the external medium (Fig. 2, trace A). In the presence of DTE more Ca^{++} was taken up and was retained untill the energy coupling mechanism was interrupted by an uncoupler (FCCP) (Fig. 2, trace B).

Similar effects have been obtained by recording the proton movements connected with K^+ uptake in the presence of valinomycin.

Mercaptoethanol, a monosulfhydryl reagent, did not produce any of the effects observed with DTE.

The participation of dithiol groups in oxidative phosphorylation has already been postulated by Fluharty and Sanadi on the basis of the observation that 2,3-dimercaptopropanol, but not monothiols, reversed the uncoupling effect of γ-(p-arsenophenyl)-n-butyrate (5). Likewise Newton has produced evidence for the involvement of dithiols in photophosphorylation (6).

The restoration by DTE of some energy linked processes, partially or completely lost during ageing of mitochondria, suggests that the partially uncoupled state resulting from ageing is, within certain limits, a reversible process.

Taking into account the reducing action of DTE on the dimercaptide bonds, it is reasonable to assume that ageing brings about an oxidation of pairs of vicinal thiol groups to dimercaptide bonds. As a consequence of such an oxidation, mitochondrial membranes become leaky and the coupling efficiency progressively lost. With a proper reduction of dimercaptide bonds to the original thiol groups, the integrity of the membrane is restored and the energy linked processes reintegrated.

From the results reported here, the primary importance of thiol groups for the integrity of mitochondrial membrane and for the conservation of the energy linked processes becomes evident.

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