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Abstract

Electron microscopy shows that intact mitochondria can be isolated from neck-muscle stored at 144 h post-mortem at 4°. Isolated mitochondria, all in the condensed configuration, have clearly defined outer and inner membranes, outer compartments and intracristal spaces; a larger proportion of swollen ones was isolated from the 144 h than from the 120 h post-mortem tissue.

Mitochondria from 96 h tissue still retained the following % of the initial values for the ADP/O ratio, respiratory control index (RCI) and state 3 respiratory rate observed for 0-5 h tissue: malate + pyruvate, 100, 72 and 53; succinate, 80, 30 and 74; ascorbate + tetracemethyl- p-phenylenediamine (TMPD), 92, 88 and 72.

Both the succinate and ascorbate-TMPD oxidase systems appear to have a "critical" storage time of about 70 h, whereas the malate + pyruvate system has one of about 96 h. A sharp decline of the ADP/O ratio, RCI and the state 3 respiratory rate occurred after this time, but these three parameters were better preserved in the ascorbate-TMPD oxidase system.

The oxidation of the citric acid cycle intermediates in the neck-muscle mitochondria thus shows a higher sensitivity to post-mortem aging with respect to cytochrome oxidase activity. This is probably due to post-mortem muscle acidification.

Introduction

Mitochondria are normally isolated from tissues immediately after slaughter of the animals, although there are no data available to substantiate the necessity for such rapid isolation. Reports of preservation of some of the functions of stored and aged mitochondria from rat-liver have been made by a few groups of investigators. Greiff and Meyers found that about 64 and 69% of the initial value of ADP/O ratio was retained in freeze-dried and frozen mitochondria. Walton et al. stored mitochondria at -196° for 60 days with 40% loss in respiratory control. Carafoli and Gazzotti found that most of the energy-linked functions were lost after 2 to 4 days with mitochondria aged at 2°, but the ATP-induced mitochondrial contraction was still preserved after 10 to 14 days. These authors also observed that ADP no longer stimulated succinate oxidation after 2 days of ageing, with the RCI decreasing by about 50% after 1 day.
Ozawa et al. working with mitochondria from ischemic livers, observed a 40% decline in phosphorylation rates during 1 h of ischemia at 22°. This was suggested to be due to mitochondrial damage partly caused by free fatty acids released during lipolysis of microsomal lipids, resulting in a complete loss of rat liver RCI observed during 4 h of ischemia at 24°. These authors also reported that approximately 50% of the RCI was preserved by bovine serum albumin (BSA) with mitochondria isolated from rat liver ischemic up to 13 h at 24° with no decline in the ADP/O ratio. However, at 37° even in the presence of BSA complete loss of respiratory control and ADP/O ratio was observed from 1½ h of ischemia.

This paper reports some of the biochemical changes in the mitochondria isolated from ox neck-muscle (M. sternomandibularis) following storage, using electron microscopic and polarographic techniques.

Materials and Methods

Chemicals

Antimycin A (Type III), oligomycin and the sodium salts of ADP, ATP, succinate and pyruvate were obtained from Sigma; sodium salts of L-ascorbate and EDTA, and TMPD from British Drug Houses; all other reagents were of analytical grade. p-Tri-fluoromethoxy-carbonylcyanide-phenylhydrazone (FCCP) was a gift from Dr. P. Heytler.

Methods

Isolation of mitochondria. Mitochondria from ox neck-muscle, both fresh and stored at 4° for various time intervals, were isolated as previously described for other skeletal muscle. The mitochondrial-containing 7000 x g pellet, free from the top pale “fluffy” layer, was then washed three times before use.

Storage of tissue. The neck-muscle, free of fatty tissue, was kept in a polythene bag at 4°. For post-mortem studies at different time intervals, about 25 g tissue was used. No bacterial growth was visible up to 144 h storage under these conditions, but any possible surface contamination was avoided by removing the external 2-3 mm of tissue.

Electron microscopy. Thin sections of the various mitochondrial preparations were examined. The mitochondria were fixed in 2% glutaraldehyde and treated with 2% osmium tetroxide for 1 h prior to dehydration with ethanol followed by propylene oxide, before being embedded in Epon 812. The sections were cut with a glass knife and stained with uranyl acetate and lead citrate before examination with an AEI (Model EM6-B) electron microscope.

Biochemical analyses. Oxygen uptake was measured polarographically with a Clark oxygen electrode at 25°. The ADP/O ratio and RCI were calculated from the electrode traces as described by Chance and Williams. Protein was determined by Folin-phenol reagent with BSA as standard.

Results

Figure 1 clearly illustrates the structure of mitochondria which were obtained from the neck-muscle before (A) and after storage at 4° for various time intervals (B-D). Except