CORRELATION OF MITOCHONDRIAL CYTOCHROME CONCENTRATION AND ACTIVITY TO OXYGEN AVAILABILITY IN THE NEWBORN

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Summary: Effect of in vivo oxygenation on mitochondrial respiratory chain capacity was studied in newborn puppies. Heart mitochondria were isolated from 0 to 7 days. As the arterial PO_2 rises sharply during the first 3-4 days, State 3 respiratory capacity and Ca^{++} transport activity decrease linearly. Cytochrome c and a + a_3 concentrations increase rapidly. Thus the enzymatic turnover of the respiratory chain decreases to one-fifth of the fetal term value in 4 days. These data are in agreement with earlier results indicating a strong controlling effect of in vivo oxygenation on mitochondrial respiratory capacity.

INTRODUCTION

During the first days of extrauterine life rapid development of many enzyme proteins occurs. At birth the concentration of mitochondrial cytochromes is quite low (1). During the first week these enzymes reach adult levels in the rat liver and heart (1). In rat brain cytochrome oxidase is still increasing after two weeks (2). It is not clear, however, whether the activity of mitochondrial enzyme systems changes during this time period.

Our earlier studies of direct effects of tissue oxygenation on mitochondrial respiratory activity would suggest alterations of kinetic activities of the mitochondrial enzymes (3, 4). Were the newborn mitochondrial enzymes affected by oxygen similarly to adult animals and humans (3) a large change should occur during the first days, when the very low fetal arterial PO_2 rises rapidly to adult levels. In an earlier report we have indicated that this is the case (5).

METHODS

Puppies were used for these studies. Fetal animals at term before breathing air served as controls. Newborns were studied at different ages up to two weeks.

Fetal animals at term were delivered by Caesarean section. The mothers were anesthetized with Nembutal. Before removal of the ascending aortic blood samples, and the hearts for mitochondrial preparations, the fetuses were not allowed to breathe.

Newborn animals were anesthetized with Nembutal. The chest was opened and ascending aortic blood was collected for determination of blood PO_2. Immediately after the withdrawal of blood samples, the heart was removed, placed in 100 ml of ice-cold medium, and cut into small pieces with scissors. Mitochondria were isolated in 0.225 M mannitol and 0.075 M sucrose supplemented with 100 μM EDTA at 0° C according to conventional methods as described earlier (3). The homogenization of fetal and newborn hearts, up to the age of ten days, was performed without Nagarse. In older heart preparations 5 mg Nagarse per 2 grams of tissue was used.

Isolated mitochondria were assayed for respiratory activity with a Clark oxygen electrode using 10 mM glutamate and malate or 10 mM succinate in the presence of 10μM Rotenone as substrates, and ADP as the phosphate acceptor. The concentrations and steady state changes of mitochondrial cytochromes were determined in a Chance dual-wavelength spectrophotometer. Cytochrome oxidase was measured at 445-460 nm and cytochrome c at 550-540 nm. Kinetics of the Ca^{++} transport activity were also measured in the dual wavelength spectrophotometer using Murexide as the Ca^{++} indicator at 540-507 nm according to the method of Mela and Chance (6).

Protein concentrations of the mitochondrial samples were determined by the Biuret method.

RESULTS

Arterial oxygen tension. The arterial PO_2 of the fetus is very low. As seen in Figure 1, the ascending aortic PO_2 of the puppy at term before breathing air varies between 8 and 15 mm Hg. During the first four days a rapid rise of PO_2 occurs. This phase is followed by a slower increase of the arterial PO_2 to normal adult level.

Quality of mitochondrial preparations from newborn hearts. Mitochondria prepared from fetal and newborn hearts using gentle short-term homogenization are well coupled, exhibiting respiratory control ratios (RCR) of 10 or above with glutamate and malate as substrates. Two samples with looser coupling (RCR = 8) were discarded. The ADP/O ratios gave values close to theoretical (3 for glutamate + malate, 2 for succinate).

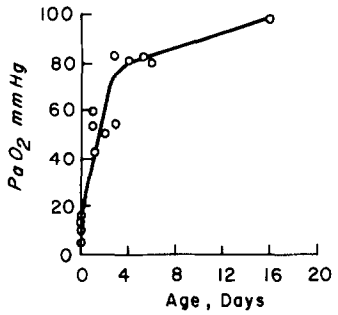

Figure 1: Changes of arterial PO_2 as a function of age in puppies. Each point indicates an individual animal.

State 3 respiratory activity. In their active state, State 3, while respiring in the presence of ADP, mitochondria reach almost maximal respiratory activities in vitro. This respiratory state, thus, is a good indicator of mitochondrial respiratory capacity.

When State 3 heart mitochondrial respiration was examined in newborn puppies, decreasing rates were found during the first 4-5 days, as seen in Figure 2. An inverse linear correlation was obtained when State 3 respiratory activities were plotted against the arterial PO_2 of the newborns.

Changes of mitochondrial cytochrome concentration. As is seen in Figure 3, also the concentrations of mitochondrial cytochromes change dramatically during the first days of life. Cytochrome c increases during the first three newborn days by almost 200%, from a fetal term value of 0.21 nmoles/mg protein to almost adult level. Adult level of 0.68 nmoles/mg protein is reached by the end of the first week. Cytochrome oxidase increase biphasically. The first, faster rise occurs between days 0 and 2 and amounts to a 100% increase. A second slower phase starts after day 2. By the end of the first week of life cytochrome oxidase reaches a concentration of 50% of adult value.

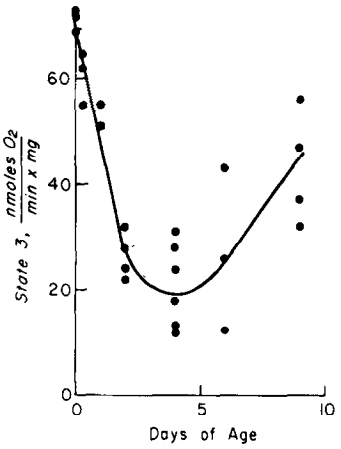

Figure 2: State 3 respiratory activities of isolated heart mitochondria as a function of age. Glutamate and malate were used as substrates. Each point indicates an individual animal.

Utilizing the data presented in Figures 2 and 3, the alterations of respiratory activity were calculated per one molecule of cytochrome oxidase. The calculated data, shown in Figure 4, indicate that an extremely large decrease of cytochrome oxidase turnover occurs in State 3 with increasing age up to 7 days. Similarly the mitochondrial capacity to accumulate calcium decreases dramatically. It should be noted that Ca^{++} transport capacity of newborn heart mitochondria in the absence of added phosphate ceases completely at day 7.

The decreasing ion transport and respiratory capacities described above are transient. During the next few weeks all mitochondrial parameters reach adult levels.

DISCUSSION

The data presented here support our earlier contention that mitochondria are sensitively affected by changes of tissue oxygen concentration in vivo (3-5). Under acute hypoxia a 60% decrease of liver tissue PO_2 in the rat

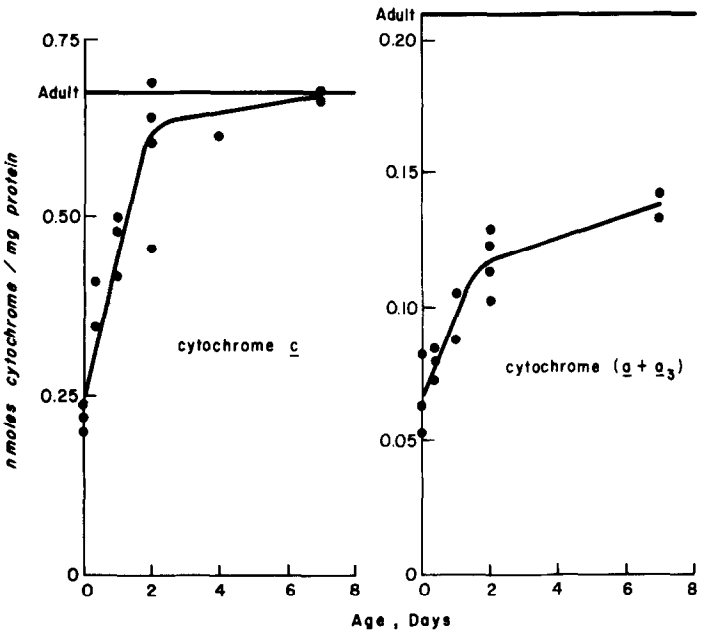

Figure 3: Concentrations of heart mitochondrial cytochrome c and a + a_3 as a function of age. For calculations 19 mM^{-1} for c and 160 mM^{-1} for a + a_3 were used as extinction coefficients. Each point indicates an individual animal.

induced twice normal respiratory capacities of liver mitochondria in 20-40 minutes (4). Chronic hypoxia also induced increased respiratory capacity of dog heart mitochondria parallel with a decrease of cytochrome a + a_3, c and b concentrations (3). Reoxygenation of hypoxic animals induced an opposite mitochondrial activity change. All these data are in agreement with each other in demonstrating that decreased *in vivo* tissue oxygenation induces increased mitochondrial respiratory capacity per respiratory chain, and that increased oxygenation in chronically hypoxic animals, as well as human, induces decreased mitochondrial respiratory capacity.

The site of interaction of oxygen with mitochondrial respiratory chain *in vivo* is presently not clear. However, our data suggest that possibly

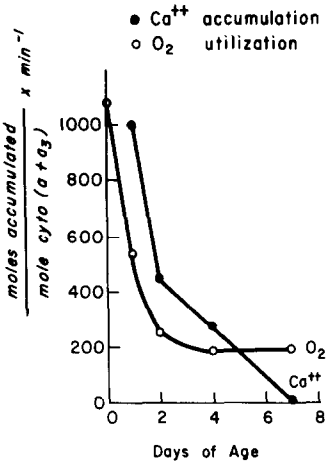

Figure 4: O_2 utilization and Ca^{++} accumulation rates per mole of cytochrome oxidase as a function of age. Each point is a mean value of 3-4 animals.

not only one, but several of the mitochondrial functions are involved. The various carrier-mediated membrane transport mechanisms are the most probable sites of interaction. These include Ca^{++} transport, as indicated in this report, and under conditions of State 3 respiration, possibly ADP and/or substrate transport.

Our findings are contradictory to those of Chance (7) and Lübbers (8), who found no effect of changing oxygen concentrations on mitochondrial respiratory activity *in vitro* unless critically low tissue PO_2 were reached. Since oxygen is not capable of affecting mitochondrial respiratory or ion transport capacities directly *in vitro*, a cytoplasmically mediated *in vivo* control mechanism seems necessary.

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