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Postnatal Development of Energy Metabolism in the Rat Brain

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Summary

The activities of cytochrome c oxidase and F_oF_1-ATPase as well as the content of cytochromes cc_1, aa_3, and b were investigated in free brain mitochondria in the course of postnatal development and aging. The results show an increase of V_{max} of both enzymes during postnatal development (between day 5 and 30). During the following phase ending at the age of 6 months, a decrease of F_oF_1-ATPase and cytochrome c oxidase activity occurs. From 6 to 12 months of age the activity of these enzymes did not change. The K_M for both enzymes remained unchanged during the whole period observed. The content of cytochromes increased from the low values found in young rats, reached the highest values at around one month, and decreased till the age of 3 months. Later, their content in brain mitochondria did not markedly change. Our results suggest that the metabolic maturation of brain mitochondria differs in several aspects from the same process in other tissues, mainly in the time course. This is probably due to the unique role of neural tissue in the organism.

Key words

Brain mitochondria • Energy metabolism • Cytochrome c oxidase • Cytochromes • F_oF_1-ATPase

Introduction

Cell energy metabolism matures during the postnatal development of mammals. The rate of this process differs in various tissues. In the brain, maturation lasts for several weeks of postnatal life, e.g. contrary to the liver tissue where adult characteristics of the mitochondrial enzyme equipment are attained very fast.

The increase of cytochrome c oxidase and various mitochondrial enzymes and the increase of cytochromes aa_3, b, cc_1 and the coenzyme Q content are reported in brain homogenates (Klee and Sokoloff 1967, Gregson and Williams 1969, Chepelinski and Rodriguez de Lores Arnaiz 1970, MacDonnel and Greengard 1974, Dobešová and Mourek 1980, 1986, Brown *et al.* 1991). The age-dependent increase of cytochrome c oxidase activity and content of cytochromes indicated that brain mitochondria attain the adult characteristics at about 30 days of postnatal life in rats.

The brain is a very complicated organ containing heterogeneous cell population. Metabolic differences were also detected between neuronal (free) and synaptosomal mitochondria (Lai and Clark 1976, Lai *et al.* 1977). It is therefore very difficult to correlate data obtained in

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homogenates with those of isolated mitochondria and to fully characterize quantitative and qualitative changes occurring in brain mitochondria during postnatal development and aging. Nevertheless, it is possible to decide whether developmental changes detected in isolated mitochondria could be explained by an increase in the surface area of the mitochondrial inner membrane (decreased ratio between matrix and membrane proteins) or whether they are due to increased amount of particular enzyme proteins in the mitochondrial inner membrane.

We have therefore isolated the non-synaptosomal fraction of brain mitochondria and measured the cytochrome c oxidase activity and content of cytochromes in isolated membrane particles. We also measured the activity of F_oF_1-ATPase and correlated the changes in the activity of cytochrome c oxidase, which indicates the mitochondrial oxidative capacity, and the activity of F_oF_1-ATPase, which indicates the capacity for aerobic ATP production.

Methods

Male Wistar rats aged 5 days, 1, 3, 6 and 12 months were used for the experiments. Rat brain free (non-synaptic) mitochondria (from the forebrain) were prepared according to the method described by Ragusa *et al.* (1989). In brief, the crude mitochondrial pellet was washed twice and layered on the top of a discontinuous sucrose gradient (0.8, 1.0, 1.2, 1.4 M sucrose solutions) to obtain the purified mitochondrial pellet after centrifugation at 75 000 x *g* for 2 h. Inside-out submitochondrial particles were prepared by sonication of brain mitochondria in the presence of EDTA (ESMP) according to Lee and Ernster (1968).

The ATP hydrolase activity was determined spectrophotometrically at 37 °C using a regenerating system containing excess of phosphoenolpyruvate and pyruvate kinase as described by Kopecký *et al.* (1983). The activity of cytochrome c oxidase and the content of cytochromes were determined as described previously (Kalous *et al.* 1989). The protein content was determined according to Lowry *et al.* (1951) using bovine serum albumin as standard. Pyruvate kinase and lactate dehydrogenase used in the F_oF_1-ATPase test were purchased from Boehringer-Mannheim (Germany). All other chemicals were from Sigma (U.S.A.).

Results

Changes in the activity of cytochrome c oxidase during development and aging of the rat brain are shown in Figure 1. The lowest activity of this enzyme was found in the brains of 5-day-old animals and the activity increased during further development. The increase of activity during the first month of postnatal life is about 29 % (from 2.88 to 3.72 μmol/min/mg proteins). This value remains practically unchanged until three months of age. After this period the cytochrome c oxidase activity of brain mitochondria slowly decreases. In 6-month-old rats, the activity is 90 % and in 12-month-old rats it is 82 % of the maximal values of 1-month-old animals. We did not find any differences in K_M values of mitochondrial cytochrome c oxidase during developmental periods studied.

Changes in the activity of cytochrome c oxidase during development and aging in rat brain mitochondria correspond well to the changes in the content of mitochondrial cytochromes. As is shown in Table 1, the content of cytochromes aa_3, cc_1 and b increased during the first month of life and then declined till 12 months of age. The relatively highest increase was found for cytochrome cc_1 where values for 5-day-old rats represent 50 % of maximum values obtained in 1-month-old rats in comparison with 67 and 69 % for cytochrome aa_3 and b, respectively.

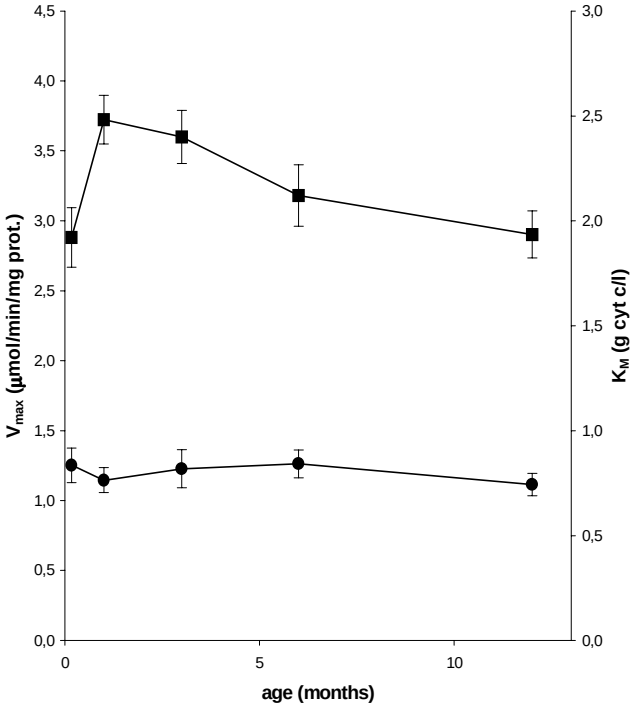

Fig. 1. *Changes of kinetic parameters of cytochrome c oxidase during development. Values are given as means ± S.D, n=5. (squares are V_{max}, circles show values of K_M)*

Table 1: Changes in the content of cytochromes in the rat brain during development.

Age	Cytochromes (nmole/mg prot.)			Ratio	
	cc_1	b	aa_3	aa_3/ cc_1	aa_3 /b
5 days	0.31±0.03*	0.22±0.03	0.42±0.04*	0.74	0.52
1 month	0.63±0.05$^+$	0.33±0.03*	0.61±0.03*	1.03	0.54
3 months	0.54±0.07	0.23±0.02	0.52±0.02	1.04	0.44
6 months	0.53±0.03	0.24±0.03	0.50±0.02	1.06	0.48
12 months	0.36±0.05*	0.19±0.02$^+$	0.43±0.04*	0.84	0.44

Data are means ± S.D. (n=5). Significantly different from 3-month-old animals: $^+p<0.01$, $*p<0.001$.

Values of the relative ratio between cytochrome aa_3 and b or cc_1 in rat brain mitochondria during aging are shown in Table 1. The presented data support the idea that changes of all cytochromes run in parallel. Only the relative content of cytochrome cc_1 in 5-day-old animals is relatively lower when compared with those of cytochrome b and aa_3.

Beside the cytochrome c oxidase we also followed changes in the activity of mitochondrial F_oF_1-ATPase during postnatal development and aging. The values of V_{max} and K_M for this enzyme are shown in Figure 2. Concomitantly with changes of cytochrome c oxidase, we found the lowest values in 5-day-old animals and the highest values in 1-month-old animals. However, the increase was higher compared to cytochrome c oxidase. Values of F_oF_1-ATPase activity in 5-day-old animals represented 45 % of maximal values in 1-month-old animals compared to 78 % of the cytochrome c oxidase activity. This means that F_oF_1-ATPase activity of rat brain mitochondria increased two times during a relatively short period of time. We did not find any changes in K_M during the same period. The decline of F_oF_1-ATPase activity was similar to that of cytochrome c oxidase. Values in 1-year-old animals represented 70 and 78 % of maximal values of 1-month-old rats for F_oF_1-ATPase and cytochrome c oxidase, respectively. Furthermore, in the course of aging the K_M values of F_oF_1-ATPase did not change (Fig. 2).

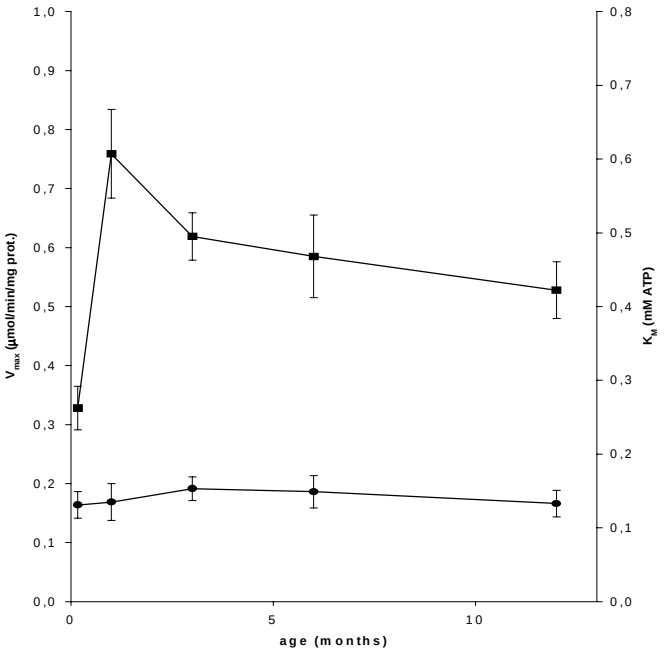

Fig. 2. *Changes of kinetic parameters of F_oF_1-ATPase during development. Values are given as means ±S.D, n=5. (squares are V_{max}, circles show values of K_M)*

Discussion

It has been shown that the oxidative metabolism of different tissues and activity of various enzymes participating in ATP production increase during postnatal development in association with their functional state. The reconstruction of energy metabolism of the cell in relation to tissue maturation and changes of tissue functional activity is necessary and many data have shown that the rate of this process is tissue-specific.

In the liver, the maturation of pre-existing mitochondria, i.e. the acquisition of ultrastructural, molecular, and functional characteristics occurs very rapidly within the first hours after birth (Valcarce *et al.* 1988, Izquierdo *et al.* 1990). The oxidative metabolism in the rat kidney sharply increases about 2 weeks after delivery, apparently in connection with the transition to solid food and increasing energetic demands necessary for intensive kidney transport processes (Brabcová *et al.*

1971, Delaval *et al.* 1990). A similar situation was found in the brain where adult values of the followed parameters were achieved at about 3 weeks of postnatal life.

It is quite evident that the increase of oxidative capacity of a tissue includes both an increase of the quantity of mitochondrial protein per cell as well as an increase of the amount of a particular enzyme in the mitochondrial membrane. At least two mechanisms may be considered in this process of mitochondrial maturation: a) increase of the capacity of cell energy metabolism (transformation of fetal anaerobic glycolytic type of ATP production into aerobic ATP production by mitochondrial oxidative phosphorylation) in connection with the increased energy demands of mature tissue, b) modification by mitochondrial membrane enzyme equipment as adaptation to nutritional factors (Drahota *et al.* 1965) and increased energy demands of the cell. Similar mechanisms also participate during regeneration processes in the liver after partial hepatectomy (Červinková *et al.* 1998).

Our data have shown that a process of mitochondria maturation occurs during development, since we used isolated membranes in our experiments, which eliminated possibility that changes in proportion between matrix and membrane proteins are involved. Our data have also indicated that the oxidative capacity of mitochondrial membranes of 5-day-old rats is more maturated (closer to adult values) than the phosphorylative capacity. Contrary to the developmental changes, alterations that occur during aging are more proportional when changes of oxidative and phosphorylative capacity are considered. This indicates that while developmental maturation is related to a specific program of membrane biogenesis in various cell populations, changes of mitochondrial enzyme activities in aging are probably a result of more general changes induced, e.g. by a decreased cell defense system efficacy against mitochondrial generation of reactive oxygen substances (Kalous and Drahota 1996).

Our results have also shown that maturation of mitochondria in the brain is a much slower process than e.g. in the liver. This corresponds with the hypothesis that the liver plays a crucial role in metabolic adaptation of newborn mammals to extrauterine life (Girard *et al.* 1992). Therefore, the shift of brain energy metabolism from anaerobic glycolysis to oxidative phosphorylation can be much slower, this being evidently also connected with higher resistance of neonatal brain to anoxia (Drahota *et al.* 1991).

Several authors found an increased content of cytochromes during the early postnatal development in the brain. However, the question remains to be solved, whether this increase is due to the increased amount of mitochondria in brain cells or due to modified properties of mitochondria (Chepelinsky and Rodriguez de Lores Arnaiz 1970, Brown *et al.* 1991). We have shown that the increase of oxidative and phosphorylative capacity of mitochondrial membrane participates in the maturation of brain cell energy metabolism.

The peak of maximal mitochondrial membrane oxidative and phosphorylative capacity in 1-month-old rats correlates well with functional brain maturation and with the fact that postnatal myelinization is completed at this age (Carey 1982). During the first month of life, energy metabolism of the rat brain must besides functional energy demands also cover energetic demands connected with proteosynthesis and synthesis of membrane phospholipids and lipids necessary for myelinization. The decrease in F_oF_1-ATPase activity of brain mitochondria that we found in the period between 1-6 months of life could be due to the fact that brain maturation had finished and energy demands are lower, just maintaining the level required for the maintenance of its physiological functions.

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