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SHORT COMMUNICATION

Developmental Changes of Cytochrome c Oxidase and Citrate Synthase in Rat Heart Homogenate

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Summary

Activity of cytochrome c oxidase and citrate synthase in rat heart homogenates was determined in 5-, 15-, 28- and 60-day-old rats. The activity of both enzymes increased during postnatal development but their changes followed different kinetics. The membrane-bound cytochrome c oxidase reached its adult values during the early postnatal period, i.e. between days 5 and 15, whereas soluble matrix-localized citrate synthase also continued to increase between days 15 and 60. Our data indicate a relative excess of cytochrome c oxidase in neonatal cardiocytes.

Key words

Rat heart • Postnatal development • Cytochrome c oxidase • Citrate synthase

Whereas the heart of adult homeotherms is almost exclusively aerobic and can utilize a wide spectrum of substrates including lipids, carbohydrates, amino acids and long-chain fatty acids as the predominant energy source, the metabolism of the immature myocardium is primarily anaerobic (Bass *et al.* 2001). The high stores of glycogen in the immature myocardium are essential for enhancing cardiac tolerance to oxygen deprivation, but this property decreases rapidly after birth (Ošťádalová *et al.* 1998).

The ontogenetic development of cardiac metabolism is characterized by quantitative and qualitative changes of cardiac mitochondria. There is an increase in size and number of mitochondria *per* cell during the neonatal period in the rat (Olivetti *et al.*1980) and during the first two months in dogs (Legato 1979). Irregular localization of mitochondria in neonatal rat heart changes stepwise into two functionally distinct populations during the first three weeks of postnatal life: subsarcolemmal mitochondria, residing beneath the plasma membrane, and interfibrillar mitochondria, located between the myofibrils (Ošťádal and Schiebler 1971, Palmer *et al.* 1977). ADP-stimulated rates are greater in interfibrillar mitochondria, whereas the coupling of respiration to phosphorylation is similar in both populations (Palmer *et al.* 1985). Both populations also differ in the value of mitochondrial membrane potential (Škárka and Ošťádal 2002, Škárka *et al.* 2003). Unfortunately, no distinct structural marker for each population has been identified so far.

Simultaneously, the activities of cardiac mitochondrial enzymes are changing significantly during ontogenetic life. Schagger *et al.* (1995), Kalous *et al.* (2001) and Škárka *et al.* (2003) have described an increase of cytochrome c oxidase activity (COX) in cardiac mitochondria during 4 weeks of postnatal life. Lang (1965) and Bonne *et al.* (1993) also described age-dependent increase of COX in cardiac homogenate. Nevertheless, more information on the development of various mitochondrial enzymes participating in providing energy is desirable.

In a previous paper (Bass *et al.* 2001), we followed the developmental changes of two mitochondrial enzymes of aerobic metabolism, citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase, in rat heart homogenate. Both enzyme activities increased from birth up to adulthood, with the steepest change between days 15 and 60 of postnatal life. The purpose of this study was to complete our previous measurements by parallel determination of CS and COX activities in rat heart homogenates. We correlated ontogenetic changes of two mitochondrial enzymes: membrane-bound COX and soluble CS localized in mitochondrial matrix.

All the investigations conform with the "Guide for the Care and Use of Laboratory Animals", published by the United States National Institutes of Health (NIH publication No 85-23 revised 1985).

Male Wistar rats (Velaz, Prague, Czech Republic) 5-, 15-, 28-, and 60-day-old were used (day 5 – proliferation activity of myocytes disappears, day 15 – structural arrangement of mitochondria, day 28 – end of the weaning period, day 60 – sexual maturation). All animals had free access to water and a standard laboratory diet (DOS 2B, Velaz, Prague, Czech Republic). The animals were weighed and killed by decapitation. Their hearts were removed, washed in 0.9 % NaCl, and separated by the method of Fulton *et al.* (1952) into the right and left ventricles and septum and weighed. Left ventricles were used for the experiments. From the pooled samples (10 ventricles from 5-day-old rats, 5 ventricles from 15-day-old rats and 2 ventricles from 60-day-old rats) homogenates were prepared. Ventricles were cut by scissors on ice into small pieces and gently homogenized in a Teflon-glass homogenizer in a cold sucrose medium containing 250 mM sucrose, 10 mM Tris-HCl, 1 mM EDTA of pH 7.4. Protein and enzyme activities were determined in aliquots of 5 % homogenate. Activity of two mitochondrial enzymes was determined: a) membrane-bound COX, a terminal enzyme of the mitochondrial respiratory chain, indicative of the capacity of the mitochondrial energy provision system, and b) CS, a soluble enzyme localized in the mitochondrial matrix, participating in Krebs cycle function. We measured both enzymes in homogenate, because isolated mitochondria represent only a fraction of total mitochondrial population of cardiocytes (Nedergaard and Cannon 1979). COX activity was determined spectrophotometrically according to Wharton and Tzagaloff (1967) with small modifications (Stieglerová *et al.* 2000). Enzyme activity was determined at 25 °C in 60 mM K-phosphate and 0.01 % lauryl maltoside by measuring the rate of cytochrome c oxidation at 550 nm. The CS activity was measured according to Srere (1969) and proteins by the method of Lowry *et al.* (1951), using bovine serum albumin as standard. For statistical evaluation of differences between different groups, two-way analysis of variance with the Student-Newmann-Keuls multiple range test was used.

Table 1 summarizes the basic weight parameters of individual age groups used for enzyme activity measurements. All parameters increased significantly between days 5 and 60, indicating postnatal growth of the rat heart.

Table 1. Developmental changes of body weight (BW), heart weight (HW), and left ventricular weight (LV).

Age (days)	n	BW (g)	HW (mg)	LV (mg/wet wt.)	LV (mg protein)
5	15	13.5 ± 0.9**	76.0 ± 4.1**	30.7 ± 4.4**	4.3 ± 0.08**
15	11	30.5 ± 3.2**	144.5 ± 8.4**	88.2 ± 3.8**	13.7 ± 2.22**
28	6	135.0 ± 3.5**	466.7 ± 12.8*	245.8 ± 9.4**	44.8 ± 1.31**
60	3	247.7 ± 1.2	690.0 ± 65.5	383.3 ± 7.2	62.7 ± 1.24

Data are means ± S.E.M., n number of animals, * $p<0.01$ vs. day 60, ** $p<0.001$ vs. day 60

Table 2. Developmental changes of COX and CS activities in left ventricle.

Age (days)	n	COX (activity/ mg protein)	COX (activity/LV)	CS (activity/ mg protein)	CS (activity/LV)	COX/CS
5	15	3.62 ± 0.25 **	15.4 ± 0.7 **	0.26 ± 0.03 **	1.1 ± 0.11 **	14.3 ± 1.25
15	11	6.01 ± 0.41	79.6 ± 7.3	0.48 ± 0.04	6.6 ± 0.54	12.7 ± 0.78
28	6	5.11 ± 0.22	228.1±17.3 **	0.59 ± 0.04 **	26.3 ± 3.03 **	8.7 ± 0.30 *
60	3	4.75 ± 0.31	287.2 ± 1.8 **	0.78± 0.04 **	48.9 ± 3.53 **	6.0 ± 0.40 **

Activity of COX is expressed as nmoles cytochrome c oxidase per min per mg protein. CS activity as nmoles acetylCoA utilized per min per mg protein, * $p<0.01$ vs. day 15 , ** $p<0.001$ vs. day 15; LV-left ventricle of rat heart.

Table 3. Body weight (BW), heart weight (HW), left ventricle (LV) protein mass increase and total COX and CS activity increase during various developmental periods.

Age (days)	BW (g/day)	HW (mg/day)	LV (mg protein/day)	LV (COX/LV/day)	LV (CS/LV/day)
5-15	1.7 (100 %)	6.9 (100 %)	0.94 (100 %)	6.4 (100 %)	0.55 (100 %)
15-28	8.0 (471 %)	24.8 (359 %)	2.40 (255 %)	11.4 (178 %)	1.50 (272 %)
28-60	3.5 (205 %)	7.0 (101 %)	0.56 (60 %)	1.6 (24 %)	0.70 (127 %)

All values were calculated from the data presented in Tables 1 and 2.

Developmental changes of COX and CS in homogenates of the left ventricle are shown in Table 2. A significant increase of both specific and total activity of COX was found between days 5 and 15 after birth, no further increase of specific COX activity could be found between postnatal days 15 and 60. Total COX activity increased in parallel with the increase of left ventricular protein content (Tables 1 and 2). In contrast with COX, an increase of both specific and total CS activity was observed during the whole developmental period studied (Table 2). Different developmental kinetics of COX and CS is also evident from calculations of COX/CS ratio (Table 2), which showed continuous decrease during development due to the CS changes.

Our data indicate significant developmental differences in the activity of two mitochondrial enzymes: membrane-bound COX and soluble CS. COX activity (per mg of homogenate protein) reaches its maximum value on postnatal day 15; on the other hand, the activity of CS increased until day 60 of postnatal life. This suggests that the capacity of membrane-bound respiratory chain enzymes is completed sooner than that of auxiliary soluble matrix enzymes. The highest relative oxidative capacity detected in heart homogenates of 15-day-old rats could be explained by the fact that in all the parameters tested, the relative increase per day (calculated from the data presented in Tables 1 and 2), were the highest during the period between days 15 and 28 (Table 3). From the data shown in Table 2 it is also evident that in 5-day-old rats, when the cardiocyte metabolism is still primarily anaerobic (Bass *et al.* 2001), specific COX activity attained 76 % of adult values, whereas that of CS was only 33 % (Table 2). This suggests that during the neonatal period, characterized by lower energy demands and irregular mitochondrial-myofibrillar interactions (Ošťádal and Schiebler 1971), cardiocytes already have a rather high COX activity, taking into account that most of the energy produced is of glycolytic origin.

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Reprint requests

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