ONTOGENY OF TCA CYCLE ENZYMES IN HUMAN FETAL HEART

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ABSTRACT

In order to establish a relationship between development and myocardial energy metabolism, the enzymes viz succinate dehydrogenase (SDH), isocitrate dehydrogenase (IDH) and α-ketoglutarate dehydrogenase (α-KGDH) involved in TCA cycle have been measured in the human fetal heart over 13 to 36 weeks of gestation and compared with adult heart. These three enzymes show similar developmental profiles in the mitochondrial fraction, exhibiting maximum activity at the later period of gestation. High activity was observed in the adult heart. IDH shows gradual increase in activities, with gestational ages in all the fractions studied viz cytoplasm and mitochondrial fractions. Contrary to this, α-KGDH and SDH activities decrease in cytoplasm with increase in fetal age.

INTRODUCTION

THE importance of glucose metabolism in developing heart has been emphasized by several investigators[1,2]. Reports are now available indicating that the cardiac metabolism in new born and fetus, particularly in the immature animal, is greatly dependent on glycolysis for energy production[3,4]. Recently, we have reported that human fetal heart has a very high glycolytic activity particularly during the early and mid-gestation period[5]. However, little is known about the relationship between oxidative metabolism in heart muscle and development. The mitochondrial respiratory enzyme activities are low in the early fetal guinea pig heart and increase two-fold postnatally, indicating complete development of mitochondria in this organ with the attainment of maturity during adult life[6]. The mitochondrial content is low in fetus than in the adult rat heart[7,8]. The activities of oxidative enzymes of Krebs' cycle are also very low in the fetuses of rat, sheep and guinea pig[9,10] which increase many-fold during postnatal life.

However, information regarding the changes in mitochondrial enzyme activity during fetal heart development is sparse and most studies are concentrated on laboratory animals. The present study was therefore undertaken to ascertain the changes in the activities of some oxidative enzymes viz isocitrate dehydrogenase (IDH), α-ketoglutarate dehydrogenase (α-KGDH), succinate dehydrogenase (SDH) in cytoplasmic and mitochondrial fractions of human fetal heart with gestational ages to clarify the potential capability to utilize TCA cycle and to explain the development of this organ during fetal maturity.

MATERIALS AND METHODS

Chemicals: All chemicals and reagents used were of analytical grade, purchased either from E. Merck or BDH, UK. Fine chemicals were purchased from Sigma Chemical Co., USA.

Collection of samples: The fetuses were obtained from therapeutic abortions (hysterotomized) up to 24 weeks from different nursing homes and MTP clinics in Calcutta. Fetuses above 24 weeks were collected as still birth. The ages were assayed from the menstrual cycle history of mothers. The method provides data to $\pm$ 1 week in a majority of cases[11]. According to gestational ages, the fetuses were distributed as follows: group I, 13–16 weeks; group II, 17–20 weeks; group III, 21–24 weeks; group IV, 25–28 weeks; group V, 29–32 weeks; and group VI, 33–36 weeks. Adult (postmortem) heart tissues were collected from N.R.S. Hospital in Calcutta.

The bodies of the fetuses and adult heart tissues were kept in a freezer (−4°C) immediately after collection and the heart tissues were removed from the fetuses as quickly as possible and immediately utilized for enzyme preparation.

Cytoplasmic and mitochondrial fractions were separated as in Sprengers *et al*[12]. Briefly, the frozen tissue was homogenized in a teflon homogenizer (0–4°C) with ice-cold 0.25 M sucrose (20% w/v). The homogenate was centrifuged for 10 min at 1,000 *g* to remove cell debris and nucleus and the

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supernatant was centrifuged for 15 min at 12,000 g. The 12,000 g pellet was referred to as the mitochondrial fraction. The pellet was washed with the same solution and mitochondria was resuspended on 0.25 M sucrose. The 12,000 g supernatant was used as the cytoplasmic fraction.

IDH activity was measured by the method of Ochoa[13]. α-KGDH was determined by the method of Kaufman[14] and SDH was assayed according to the method of Slater and Bonner[15].

Protein determination: Protein was measured by the method of Lowry *et al*[16]. All the assays were performed in duplicate or triplicate on freshly homogenized tissues. Homogenate were kept at 0–4°C throughout. The linearity of the reaction with time and protein concentration was checked for each enzyme assay.

RESULTS AND DISCUSSION

The activities of some key enzymes of TCA cycle in two fractions i.e. cytoplasm and mitochondria of the developing human fetal heart are shown in tables 1 and 2.

The activities of $NADP^+$-dependent IDH exhibit an almost similar pattern of development in each of the two fractions studied. At the early stage of fetal life, it has low activity which increases simultaneously with progression of gestation; very high activity was observed in adult life (table 1). It was reported earlier that in the case of rat, the heart mitochondrial $NADP^+$-dependent IDH is greatly induced in the adult life[17], which is in conformity with the results reported here. The activities of α-KGHD and SDH in mitochondrial fraction show similar type of development as that of IDH, but in contrast

Table 1 *Developmental changes of isocitrate dehydrogenase activity in human fetal and adult heart*

Gestational ages (weeks)	Specific activity of IDH (nmole/min/mg protein)	
	Cytoplasm	Mitochondria
13–16 (5)	0.124 ± 0.005	0.289 ± 0.010
17–20 (5)	0.158 ± 0.008*	0.336 ± 0.025
21–24 (5)	0.391 ± 0.027*	0.598 ± 0.051*
25–28 (4)	0.790 ± 0.031*	1.395 ± 0.099*
29–32 (4)	1.005 ± 0.075*	1.666 ± 0.102
33–36 (4)	1.972 ± 0.101*	3.043 ± 0.150*
Adult (3)	1.103 ± 0.090	5.125 ± 0.391

Figures in the parentheses indicate the number of cases studied; For calculation of *P* value the result of each value compared with the result of the preceding group.
*$P < 0.001$ (highly significant).

both the enzymes α-KGDH and SDH show gradual decline in the cytoplasm (table 2). Although, no major differences in the overall enzymatic profiles are apparent, nevertheless significant changes in the activities of individual enzymes during development occur. The general trend of increasing activities of these enzymes in mitochondrial fraction may indicate that the fetal heart mitochondria tend to mature with increase in fetal age. Though the exact function of heart mitochondrial $NADP^+$ linked IDH is not known, it may be that, the $NADP^+$-dependent IDH is probably linked with the function of heart in O_2 deficiency. In O_2 deficiency when NADH/NAD ratio becomes high, there is a stop in production of NADH and in that case $NADP^+$-linked IDH starts functioning to meet the demands of the tissue. It is also of interest to note that very high activities of IDH and α-KGDH have been observed during the

Table 2 *Developmental changes of α-KGDH and SDH activity in human fetal and adult heart*

Gestational ages (weeks)	Specific activity of α-KGDH (nmole/min/mg protein)		Specific activity of SDH (nmole/min/mg protein)	
	Cytoplasm	Mitochondria	Cytoplasm	Mitochondria
13–16 (5)	0.044 ± 0.0020	0.846 ± 0.05	0.0104 ± 0.0009	4.46 ± 0.21
17–20 (5)	0.027 ± 0.0021*	1.449 ± 0.08*	0.0065 ± 0.0005*	8.58 ± 0.75*
21–24 (5)	0.011 ± 0.0008*	3.442 ± 0.25*	0.0043 ± 0.0002*	11.81 ± 1.09*
25–28 (4)	0.009 ± 0.0005	4.876 ± 0.31*	0.0008 ± 0.00007*	13.07 ± 1.10
29–32 (4)	—	4.905 ± 0.29	—	14.75 ± 1.03
33–36 (4)	—	5.356 ± 0.37	—	19.01 ± 0.90*
Adult (3)	—	5.410 ± 0.45	—	20.21 ± 1.90*

Figures in the parentheses indicate the number of cases studied; For calculation of *P* value the result of eac' value compared with the result of the preceding group.
*$P < 0.001$ (highly significant).

late period of gestation (33–36 weeks) whereas SDH shows parallel behaviour with α-KGHD but it has comparatively high activity throughout the gestation. Similar observation i.e. very high activity of SDH, compared to that of IDH and α-KGDH in other tissues have also been reported[18,19]. Further, SDH is located histochemically in the muscle fibre of the respective chambers of the heart; therefore, the activities of this enzymes increase in the content of cardiac muscle fibre, involved in contraction as the heart tissue develops with the advancement of gestation[20]. In addition, with the advent of postnatal life and its establishment of a new type of circulation there is an additional functional load placed upon the heart during adult life. In order to maintain the proper functioning of the heart, there is a corresponding increase in the activities of those enzymes involved in the turnover of TCA cycle.

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ANNOUNCEMENT

SYMPOSIUM ON NEUROENDOCRINE REGULATION IN FERTILITY CONTROL—9–11 NOVEMBER 1987

The Symposium will be held at the Institute of Self-Organising System and Biophysics, North-Eastern Hill University, Shillong 793 001, India, the following topics will be covered: structure and function of GnRH and its analogues; pituitary function regulation; prospects for fertility regulation; comparative endocrinology of GnRH; extrahypothalamic GnRH; and immunochemistry and immunobiology of GnRH. The symposium is open for free communications and poster presentation. The proceedings of the symposium will be published. Those interested, may contact Dr Vinod Singh, the Organising Secretary of the symposium.