DEVELOPMENTAL PATTERN OF THE GLYCOLYTIC ENZYMES IN THE HUMAN FETAL TESTES

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ABSTRACT

The activities of some important glycolytic enzymes viz hexokinase (HK), phosphoglucoisomerase (PGI), phosphofructokinase (PFK), aldolase and lactate dehydrogenase (LDH) have been determined in the human fetal testis at different periods of gestation. The enzyme HK shows high level of activity at 21–24 weeks of gestation. Aldolase and PFK show similar developmental profile i.e. considerable high activity at 14–16 weeks of gestation. But, another peak of activity for PFK is observed at 25–28 weeks of gestation. PGI and LDH possess maximum activity at 25–28 weeks and 17–20 weeks of gestation respectively. Therefore, ontogeny of these glycolytic enzymes with energy demand and general functional development in human fetal testis has been considered.

INTRODUCTION

GLUCOSE is metabolized by EM pathway, TCA cycle and pentose phosphate pathway. The energy derived from the catabolism of glucose is utilized for different cellular processes in the normal as well as in the developing organism. A large number of reports are now available indicating the presence and importance of EM pathway of glucose metabolism in the adult testis during maturation and differentiation of germ cells. The presence of EM pathway has been demonstrated in testis of a number of animals[1]. In the adult testis, EM pathway predominates[2]. The enzyme phosphofructokinase was found to be very active during germ cell maturation and spermatogenesis[3]. The hexokinase activity is higher in the testis of chicken than in rabbit testis[1]. Lactate dehydrogenase was shown by Niemi and Ikonen[4] and Ito[5] to be located mainly in the interstitial cells of both the fetal and the adult rat testis. In the bull testis, this enzyme has been found mainly in the spermatogonia and spermatocytes[6] with less activity in the interstitial cell. High activity of LDH is present in the neonatal period of rat which rapidly declines during spermatogonial and spermatocytes differentiation[7]. Between 20 and 30 days of age there is an increase in the LDH activity in the interstitial and tubular tissue of rat testis[8].

Though a wealth of information has been accumulated on glycolytic enzymes of different animal species, little attention has been paid to the study on human fetal testis. The present study was therefore undertaken to assess the developmental pattern of some key enzymes involved in glycolytic pathway in human fetal testis. Five important glycolytic enzymes namely hexokinase, phosphoglucoisomerase, phosphofructokinase, aldolase and LDH have been selected for this study and their activities have been followed in the isolated human fetal testis during different gestation period.

MATERIALS AND METHODS

Chemicals: All chemicals and reagents used were of analytical grade, purchased either from E. Merck, or B.D.H., U.K. Fine chemicals were purchased from Sigma Chemical Co., USA.

Collection of samples: The fetuses were obtained from therapeutic abortions up to 20 weeks from different nursing homes and MTP clinics in and around Calcutta. Fetuses above 20 weeks were collected as still birth. The ages were assayed from the menstrual cycle history of mothers. The method provides data to ± 1 week in the majority of cases[9]. According to gestational ages, the fetuses were distributed as follows: Group I 14–16 weeks, Group II 17–20 weeks, Group III 21–24 weeks, Group IV 25–28 weeks and Group V 29–32 weeks.

The fetuses after collection were stored in freezer (−20°C). Both the testes were dissected out as quickly as possible and used for the assay of the enzymes.

The testes were homogenized in ice-cold distilled water to make 5% homogenate (w/v) and the homogenate was centrifuged at 1,000 g for 15 min at 4°C to remove the cell debris and nucleus. The supernatant was used as the source of enzymes.

Enzymatic assays: Hexokinase activity was assayed by the method of Joshi and Jagannathan[10]. Phosphoglucoisomerase was determined by the method of Roe *et al*[11]. Phosphofructokinase was measured according to the coupled enzyme assay method of Ling *et al*[12]. Aldolase was determined by the colorimetric method of Beck[13]. LDH was assayed by the method of Kornberg[14].

Protein determination: Protein was measured by the method of Lowry *et al*[15]. All the assays were performed in duplicate or triplicate on freshly homogenized tissues. Homogenates were kept at 0–4°C throughout.

RESULTS

Results presented in table 1 indicate that the specific activity of hexokinase in human fetal testis is low at 14–16 weeks, then gradually rises, and attains the peak value at 21–24 weeks of gestation after which the activity of hexokinase declines but at a level higher than the initial. The specific activity of phosphoglucoisomerase is also low at 14-16 weeks of gestation, then it rises and the maximal level is reached at 25–28 weeks of fetal life. Thereafter specific activity of this enzyme declines. The specific activity of phosphofructokinase and aldolase exhibit similar time sequence during the development. Both the enzymes have a high level of activity at early stages of development (i.e. 14–16 weeks of gestation). But another peak of activity of PFK has been observed at 25–28 weeks of gestation.

The activity pattern of lactate dyhydrogenase in human fetal testis increases from 14–16 weeks of gestation and the maximum activity is observed between 17 and 20 weeks of gestation. After that the specific activity of this enzyme falls.

DISCUSSION

The results described here indicate the changes in specific activity of a few glycolytic enzymes during development of human fetal testis. It is interesting to mention that amongst several types of cells, only interstitial cells in human testes differentiate during fetal life[16]. Several authors have pointed out that LDH of testes is of Leydig cell origin[17, 18]. In addition to LDH, glycolytic enzymes are mainly localized in the interstitial cell of the testes[3]. Histological studies further reveal that (result not shown) human fetal testes mainly contain interstitial cell along with primordial germ cells. It is well established that these germ cells do not differentiate before puberty. Thus it is evident that any change in enzyme profiles in the course of development during fetal life, reflects the change in the functional potential of interstitial cell. Therefore the EM pathway of glucose metabolism plays a distinct role in the development of human fetal testes, providing energy for the different metabolic and synthetic function of interstitial cells.

However, while considering the develoment of enzymes, the high level of hexokinase activity at

Table 1 *Age related changes of glycolytic enzymes activity in human fetal testis*

	Specific activity				
Group gestational ages (in weeks)	Hexokinase (nmol of glucose-6--phosphate produced/ min/mg protein)	Phosphoglucoisomerase (nmol of fructose-6--phosphate produced/ min/mg protein)	Phosphofructokinase (nmol of fructose-1, 6--diphosphate produced/ min/mg protein) 10×10^{-1}	Aldolase (nmol of dihydroxyacetone phosphate produced min mg protein)	Lactate dehydrogenase (nmol of NADH consumed min mg protein
14–16 (6)	1.4 ± 0.01	8.2 ± 0.05	4.1 ± 0.22	4.51 ± 0.30	20.08 ± 0.18
17–20 (5)	2.2 ± 0.02[1]	10.3 ± 0.08	2.3 ± 0.15[1]	3.01 ± 0.25[1]	33.71 ± 0.32[1]
21–24 (5)	2.3 ± 0.02[1]	9.8 ± 0.07[1]	1.6 ± 0.10[1]	0.88 ± 0.07[1]	18.45 ± 0.16[1]
25–28 (5)	1.6 ± 0.01[1]	14.2 ± 0.13[1]	3.4 ± 0.20[1]	0.37 ± 0.03[1]	11.04 ± 0.11[1]
29–32 (4)	1.8 ± 0.012[1]	5.2 ± 0.04[1]	1.0 ± 0.08[1]	0.08 ± 0.01[1]	10.55 ± 0.12[1]

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 preceeding group [1] $P < 0.001$ (Highly significant)

21–24 weeks of gestation, indicates the greater production of glucose-6-phosphate. Considerable low activity of phosphofructokinase was also observed at this stage. Aldolase shows the maximum activity at 14–16 weeks and then decreases sharply. Hexokinase activity is inversely related to glucose-6-phosphate levels and this level is generally governed by phosphofructokinase[19]. It is well known that phosphofructokinase is an important site of hormonal and metabolic regulation in glycolysis. Newsholme and Randle[20] further suggested that the decrease in fructose-6-phosphate concentration associated with an increase in fructose-1, 6-diphosphate concentration is associated with faster glycolytic flow. Thus the findings that both aldolase and phosphofructokinase have high activity at 14–16 weeks of gestation suggest the maximum glycolytic flow during this period of fetal life. The second peak of activity in phosphofructokinase and phosphoglucoisomerase indicates that the biphasic nature of the ontogeny of these enzymes is very difficult to explain. This type of development is supported by Hahn and Skala[21] in human fetal brain, suggesting that energy metabolism proceeds in phases, the energy requirement going up to a high level and declining thereafter and increasing again to reach the previous level. The specific activity of LDH is found to be maximum at 17–20 weeks of gestation. Dubowitz[22] suggested that the increase in the LDH activity may correspond to the appearance of contractile activity. Brinster[23] also showed that the principal reactions of LDH (i.e. lactate and pyruvate) are of prime importance as energy sources during the initial stages of cell multiplication following fertilization. Unlike adult tissue, constant relationship between the activities of the enzymes does not exist since the degradation rates of enzymes may not be the same in the growing tissue. According to Schimke *et al*[24], the control of enzyme levels by regulation of the rate of degradation is more important in developing mammalian system. On the basis of the above findings, the distinctive differences in the developmental pattern of the enzymes studied here can be clarified.

In conclusion, it can be said that the ontogeny of these glycolytic enzymes which are shown to have the maximum activity at early stages of development, reflects the importance of EM pathway to utilize glucose for providing energy at the stage of cellular differentiation and maintenance required for the proper functioning of human fetal testis.

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