Proc. Indian natn. Sci. Acad. **B54** No. 1 pp. 39–41 (1988)

Ontogeny of Some Krebs Cycle Enzymes in Human Fetal Testes

CHHABI DATTA, JAYATI DASGUPTA, TRISHNA SENGUPTA and D SENGUPTA*
Department of Biochemistry, University College of Science, University of Calcutta
35, Ballygunge Circular Road, Calcutta 700019

(Accepted on 12 January 1988)

The ontogeny of isocitrate dehydrogenase (IDH), α-ketoglutarate dehydrogenase (α-KGDH) and succinate dehydrogenase (SDH) activities, has been studied in adult human testis and human fetal testis collected at 14–32 weeks of gestation. These three enzymes in the particulate fraction show high activity with development of testis. $NADP^+$ dependent IDH activities in the cytoplasm and particulate fractions increase gradually with development of testis. These data suggest increasing participation of Krebs cycle as the human testes develops.

Key Words: Human fetal testis, Krebs cycle enzymes, Ontogeny

Introduction

Like the testes of a number of animal species (Baldwin & Ewing 1967), the human fetal testes have high glycolytic activity (Datta et al. 1987). In adult testicular tissue, TCA cycle enzymes exhibit high activities. Oxidative enzymes are present in germinal and non-germinal cells of fetal testis of different animals (Niemi & Ikonen 1962, Hitzeman 1962). However, the changes in oxidative enzyme activities during fetal testicular development are scarcely studied. In the present study, changes in NAD^+ dependent isocitrate dehydrogenase (IDH), $NADP^+$ dependent isocitrate dehydrogenase, α-ketoglutarate dehydrogenase (αKGDH) and succinate dehydrogenase (SDH) activities in developing human fetal testis have been reported.

Materials and Methods

Collection of samples: The fetuses were from therapeutic abortion cases of upto 20 weeks of gestation from clinics in Calcutta and those above 20 weeks of gestation were stillborn ones. The fetuses were grouped viz. 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.

Human adult testes were obtained about 3–8 hr after death from NRS Hospital, Calcutta, from 25–35 years old healthy persons, who died in accidents. Both the testes were collected immediately and utilized.

Preparation and Enzymatic Assays: Cytoplasmic and particulate fractions were separated by centrifuging (12,000 × g, 15 mins) after removal of cell debris and nuclei. The pellet (particulate fraction) was washed with and resuspended in 0.25 M sucrose; the supernatant was used as the cytoplasmic fraction, and kept at 0–4°C. The protein (Lowry et al. 1951), $NADP^+$ dependent IDH (Ochoa 1955), NAD^+ dependent IDH (Plaut & Sung 1955), SDH (Slater & Bonner 1952) and α-KGDH (Reed & Mukherjee 1969) were determined. For calculation of 'P' values, each value was compared with the previous one (table 1).

Results and Discussion

The $NADP^+$ dependent IDH exhibits almost similar pattern of development in both the fractions studied. It has low activity at early stage of fetal life and increases gradually with development of testis. The activities of α-KGDH and SDH show parallel behaviour with NAD^+ dependent IDH. In the particulate fraction, the activity increases gradually, whereas in the cytoplasmic fraction it decreases with the development of testes. Although no major differences in the overall enzymatic

*For correspondence

Table 1 *Developmental changes (nmol min^{-1} mg^{-1} protein) in the activities of Isocitrate dehydrogenase (IDH) α-ketoglutarate dehydrogenase (α-KGDH) and Succinate dehydrogenase (SDH) in fetal and adult human testes*

Gestational ages (weeks)	IDH				α-KGDH		SDH	
	$NADP^+$ Linked		NAD^+ Linked		Cyto-plasmic	Parti-culate	Cyto-plasmic	Parti-culate
	Cyto-plasmic	Parti-culate	Cyto-plasmic	Parti-culate				
14–16 (6)	0.22 ± 0.02	1.21 ± 0.11	0.03 ± 0.002	1.22 ± 0.15	0.04 ± 0.003	1.50 ± 0.09	0.05 ± 0.003	0.91 ± 0.08
17–20 (5)	0.39 ± 0.03*	1.76 ± 0.15*	0.02 ± 0.002*	1.57 ± 0.11	0.03 ± 0.002*	2.12 ± 0.18*	0.03 ± 0.002*	1.20 ± 0.09
21–24 (5)	0.42 ± 0.04	2.30 ± 0.20*	0.01 ± 0.001*	2.21 ± 0.18*	trace	3.04 ± 0.28*	0.01 ± 0.001*	2.43 ± 0.19*
25–28 (4)	0.58 ± 0.04*	3.13 ± 0.24*	trace	3.01 ± 0.26*	trace	3.67 ± 0.37	trace	4.66 ± 0.42*
29–32 (4)	0.79 ± 0.06*	5.20 ± 0.48*	ND	4.92 ± 0.40*	ND	4.90 ± 0.22*	ND	6.75 ± 0.50*
Adult (3)	1.13 ± 0.11	5.46 ± 0.49	ND	5.18 ± 0.45	ND	5.71 ± 0.52	ND	12.32 ± 0.89

Figures in the parenthesis indicate the number of cases
*P<0.001 (highly significant); ND–not detectable

profiles are apparent, nevertheless significant changes in the activities of individual enzyme during development occur (table 1).

The human fetal testes contain interstitial cells along with primordial germ cells, which are confined to seminiferous tubules. The mitochondria which are present in germinal and nongerminal cells of testes increase in number as the tissue tends to mature (Gondos 1977).

The gradual increase in the activities of NAD^+ linked IDH, SDH and α-KGDH (table 1) in the particulate fraction may indicate the dependence of maturing testicular tissue on the oxidative metabolism. Similar observations have been reported by others in experimental animals (Koudstaal et al. 1967, Richter 1961). The high activity of cytoplasmic $NADP^+$ dependent IDH, may be associated with the formation of NADPH required for the conversion of progesterone to testosterone (Lynn & Brown 1958). NADPH generating IDH are very much active during the late gestation than at any time after birth (Hitzeman 1965). Higher $NADH^+$/NAD ratio inhibits the production of NADH and in such case $NADP^+$ linked IDH becomes operative (Andres et al. 1980). The higher activity of $NADP^+$ as compared to NAD^+ dependent IDH may be associated with the need of 2-oxo-glutarate and NADPH for glutamate production (Chico et al. 1977). As in other tissues (Koudstaal et al. 1967, Dasgupta et al. 1987) at later stages of gestation activity of testicular SDH is higher than that of other enzymes.

The buoyant density of particulate fractions changes during ontogeny (Corash & Gross 1974). During the early stage of testicular development, the mitochondria are not properly formed and do not sediment at 12,000 × g for 15 minutes, so considerable activity remains in the supernates. As the development progresses, the buoyant density increases with a decrease in the activities of these enzymes in the cytoplasmic fraction and concomitant increase in the particulate fraction.

Glucose in the fetal testis is mainly metabolised through the glycolytic pathway and with its development TCA cycle also becomes increasingly important. These and other findings (Datta et al. 1987) indicate that glycolytic pathway is important in the early and mid stages of testicular development, whereas the TCA cycle becomes more active later in gestation and in adult.

Acknowledgements

The authors thank Dr S Dey for collection of human fetuses and the Council of Scientific and Industrial Research, New Delhi, for funds.

References

Andres A, Satrustegui J and Machado A 1980 Development of NADPH producing pathways in rat heat; *Biochem. J.* **186** 1799–1803

Baldwin D M and Ewing L L 1967 An enzymatic comparison of glucose metabolism in the rabbit and chicken testes and kidney cortex; *Comp. Biochem. Physiol.* **23** 569–582

Chico E, Satrustegui J and Machado A 1977 Constancy of NADP linked isocitrate dehydrogenase/trans hydrogenase ratio of mitochondria of different mammalian tissues; *Biochimie.* **59** 933–934

Corash L and Gross E 1974 Subcellular constituents of human placenta II isolation and density distribution of lysosomes of first trimester tissue; *Pediatr. Res.* **8** 774–779

Dasgupta J, Datta C, Sengupta T, De S and Sengupta D 1987 Ontogeny of TCA cycle enzymes in human fetal heart; *Current Science* **56** 466–468

Datta C, Sengupta T, Dasgupta J, De K, De S and Sengupta D 1987 Developmental pattern of the glycolytic enzymes in the human fetal testes; *Current Science* **56** 261–263

Gondos B 1977 Testicular development; in *The Testis Vol IV* pp 1–37 eds A D Johnson and W R Gomes (New York: Academic Press)

Hitzeman S J 1962 Development of enzyme activity in the Leydig cells of the mouse testes; *Anat. Rec.* **143** 351–359

Hitzeman S J 1965 Production and utilization of reduced nicotinamide adenine dinucleotide phosphate in the mouse testes; *J. Exptl. Zool.* **160** 107–116

Koudstaal J, Frensdorf E L, Kremer J, Mudde J M and Hardonk M J 1967 The histochemical pattern of the human adult testes; *Acta Endocrinologica* **55** 415–426

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with Folin phenol reagent; *J. Biol. Chem.* **193** 265–275

Lynn W S Jr and Brown R H 1958 The conversion of progesterone to androgens by testes; *J. Biol. Chem.* **232** 1015–1030

Niemi M and Ikonen M 1962 Cytochemistry of oxidative enzyme systems in the Leydig cells of the rat testes and their functional significance; *Endocrinology* **70** 167–174

Ochoa S 1955 *Methods in Enzymology* Vol I pp 699–703 eds S P Colowick and O N Kaplan (New York: Academic Press)

Plaut G W E and Sung S C 1955 *Methods in Enzymology* Vol I pp 710–714 eds S P Colowick & O N Kaplan (New York: Academic Press)

Reed L J and Mukherjee B B 1969 *Methods in Enzymology* Vol 13 pp 58–59 eds M Lowensteen (New York: Academic Press)

Richter D 1961 Enzyme activity during early development; *Brit. Med. Bull.* **17** 118–121

Slater E C and Bonner W D 1952 The effect of fluoride on the succinic oxidase system; *Biochem. J.* **52** 185–196