Arch. Gerontol. Geriatr., 6 (1987) 27–32
Elsevier

AGG 00158

Regulation of aspartate aminotransferase isoenzymes by hydrocortisone in the liver of aging rats

Ramesh Sharma [a] and S.K. Patnaik [b]

[a] *Department of Life Sciences, Manipur University, Canchipur, Imphal 795003, India and*
[b] *Biochemistry Division, Department of Pharmaceutics, Institute of Technology, Banaras Hindu University, Varanasi 221005, India*

(Received 26 February 1986; revised version received 13 September 1986; accepted 15 September 1986)

Summary

The activities and induction patterns of the isoenzymes of aspartate aminotransferase of the liver of male rats of various ages were studied. The activity of cytoplasmic aspartate aminotransferase increases gradually till adulthood and remains constant thereafter with increasing age of the rat. However, the activity of mitochondrial isoenzyme remains constant throughout the life span of the rat. Adrenalectomy decreases and hydrocortisone increases the activity of cytoplasmic isoenzyme in rats of all ages. In contrast, the above treatments do not shown any affect on the activity of mitochondrial isoenzyme in the liver of rats of all ages.

Aspartate aminotransferase; Aging; Hydrocortisone

Introduction

The activities of several enzymes decrease and of several others increase as a function of age of an organism. A possible reason for such changes may be due to the decrease and increase, respectively in the template activity of the corresponding genes (Kanungo, 1980). Two homologous and genetically independent isoenzymes of aspartate aminotransferase (AsAT; L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) have been found in animal tissues, one in the cytosol (c-AsAT) and the other in the mitochondrial (m-AsAT) fraction (Fleisher et al., 1960; Boyd, 1961). This enzyme affects the reversible transfer of an amino group from L-aspartate or L-glutarate to α-ketoglutarate and oxaloacetate. Liver AsAT is responsible for the

Correspondence address: R. Sharma, Department of Life Sciences, Manipur University, Canchipur, Imphal 795003, India.

synthesis of glucose from non-carbohydrate precursors (Lardy et al., 1965). There are reports on the hormonal regulation of AsAT (Nakata et al., 1964; Herzfeld and Greengard, 1971). However, information on age-related differential regulation of AsAT isoenzymes is scanty. We have reported (Sharma and Patnaik, 1982a) the properties of liver c-AsAT of the aging rats. In addition to that we report here the differential regulation by hydrocortisone of both isoenzymes of aspartate aminotransferase in the liver of rats through different phases of the life span.

Materials and Methods

Animals

Male albino Wistar rats of three different age groups (6, 30 and 90 weeks) were used. They were kept at $24 \pm 2°C$ under a controlled illumination programme that provided a 12-h light period followed by a 12-h dark period. The rats were fed with a freshly prepared diet containing wheat flour and vitaminised milk powder in the ratio of 4:1 usually given at 6 p.m. daily. The food and water were supplied ad libitum. All the chemicals used were of analytical grade. The biochemicals were purchased from Sigma Chemical Co., USA.

Effect of hormone

Pilot experiments were undertaken to find the time- and dose-dependence of this enzyme on hydrocortisone in rats of various ages. Maximum response of the enzyme was obtained 3 days after hormone administration at a dose of 5.0 mg/100 g body weight. The rats of each age group were divided into three sets, each containing 4–5 rats. The set I rats served as the normal. The rats of sets II and III were bilaterally adrenalectomised (A/d). These rats were given 0.9% NaCl ad libitum instead of water for 10 days. On the 11th day, the set II rats were administered 1.0 ml of 0.9% NaCl intraperitoneally (i.p.) and these rats served as control for the induction studies. The rats belonging to set III were given an i.p. dose of hydrocortisone (5.0 mg/100 g body weight, suspended in 1.0 ml of 0.9% NaCl) at the fixed time of day for 3 days. All the rats were killed 3 h after the final hormone injection.

Tissue preparation

The rats were killed by cervical dislocation. Their livers were removed, washed in normal saline and blotted dry on a filter paper. A 10% (w/v) homogenate of the liver was prepared in ice-cold 0.25 M sucrose solution using a Potter Elvehjem homogeniser fitted with a teflon pestle. The homogenate was filtered through a double layered cheese cloth and centrifuged at $700 \times g$ at 0°C for 15 min. The resulting supernatant was further centrifuged at $14000 \times g$ for 30 min at 0°C to sediment mitochondria. The supernatant thus obtained was used for the assay of c-AsAT. The mitochondrial pellet was washed twice and suspended in a solubilising

medium which contained potassium phosphate buffer (10.0 mM, pH 7.5), sucrose (0.25 M) and Triton X-100 (0.5%) for 3 h, and was used for the assay of m-AsAT.

Assay of AsAT isoenzymes

Both the isoenzymes of AsAT were assayed (Karmen, 1955; Herzfeld and Greengard, 1971) using a DBGT-Beckman model spectrophotometer at 25°C. Protein content (mg/g wet weight) of the cytoplasmic and mitochondrial fractions were estimated (Lowry et al., 1951). The activities of both the isoenzymes are expressed as U/mg protein. All the data were statistically analysed (Garrett, 1966). The level of significance (p) between two sets of data was calculated according to Student's t test. p values, which were 5% or lower for two sets of data were taken as significant.

Results and Discussion

Alterations in the levels of enzymes and their inducibility by certain hormones are an age-related phenomenon (Kanungo, 1980). Our data (Table I) show that the specific activity (U/mg protein) of c-AsAT of the liver increases by 48% until adulthood (30 weeks) and remains constant thereafter with advancing age of the rat. Table III shows that the protein content (mg/g wet weight) of the cytoplasmic and mitochondrial fractions of the liver remains almost unchanged in rats of all ages. An earlier report (Herzfeld and Greengard, 1971) shows that the fetal tissues of the rat in general have a low level of AsAT as compared to the adult tissues. It has been reported that the activity of c-AsAT increases in the liver during development and aging of rats (Nakata et al., 1964; Sharma and Patnaik, 1982a). The higher activity

TABLE I

Activity of aspartate aminotransferase (AsAT) isoenzymes of the liver of normal male rats of different ages

Age (wk)	Enzyme activity (U/mg protein) $\times 10^2$			
	c-AsAT		m-AsAT	
	mean SD	p	mean SD	p
6	51.78 ± 0.57		23.20 ± 2.46	
		< 0.001		NS
30	76.98 ± 1.30 (+48%)		26.55 ± 1.29 (NE)	
		NS		NS
90	78.47 ± 0.97 (NE)		26.35 ± 1.39 (NE)	

The data were collected from 4 rats of each age group. Four separate assays were performed on each rat. Standard deviation (SD) and the levels of significance ($p < 0.05$) are given. +, increase; −, decrease; NE, no effect; NS, not significant.

TABLE II

Effect of adrenalectomy (A/d) and hydrocortisone (HC) on the activity of aspartate aminotransferase isoenzymes of the liver of male rats of various ages

Iso-enzyme	Treatment	Enzyme activity (U/mg protein) $\times 10^2$					
		6 wk		30 wk		90 wk	
		mean SD	p	mean SD	p	mean SD	p
c-AsAT	normal	51.78 ± 0.57	< 0.001	76.98 ± 1.30	0.001	78.47 ± 0.97	< 0.001
	A/d	39.55 ± 1.32 (−24%)	< 0.001	58.05 ± 1.49 (−25%)	0.001	51.81 ± 1.12 (−34%)	< 0.001
	A/d + HC	70.86 ± 1.24 (+79%)		96.85 ± 2.32 (+66%)		76.85 ± 2.33 (+48%)	
m-AsAT	normal	23.20 ± 2.46	NS	26.55 ± 1.29	NS	26.35 ± 1.39	NS
	A/d	19.56 ± 2.87 (NE)	NS	25.96 ± 1.43 (NE)	NS	27.38 ± 1.25 (NE)	NS
	A/d + HC	23.45 ± 1.96 (NE)		29.36 ± 2.87 (NE)		26.57 ± 1.16 (NE)	

Abbreviations: see Table I.

of c-AsAT in the liver of adult and old rats may be correlated with a higher degree of transamination during these phases in the life span of the rat. An almost similar pattern in the activity of c-AsAT was observed in the brain of aging rats (Sharma and Patnaik, 1985). Since hepatic c-AsAT participates in the process of gluconeogenesis (Lardy et al., 1965), a higher level of this isoenzyme in the liver of old rats may contribute to the higher concentration of blood glucose in old age (Porter and Langley, 1926; Johannessen, 1940). The activity of m-AsAT, on the other hand, remains constant in the liver of rats of all ages. Hence, it is likely that the process of transamination in the mitochondria of rat liver remains constant throughout its life span. In contrast to this, the activity of m-AsAT decreases gradually in the brain as a function of age of the rat (Sharma and Patnaik, 1985). It has been reported (Nakata et al., 1964) that the ratio of cytoplasmic to mitochondrial isoenzymes of AsAT increases during development and growth of the rats.

Removal of the hormone-secreting organs from the animal causes a change in the levels of many enzymes in different tissues (Kanungo, 1980; Sharma and Patnaik, 1982a,b, 1984). Our results (Table II) indicate that adrenalectomy decreases and hydrocortisone treatment to adrenalectomised rats increases significantly the activity of c-AsAT in the liver of rats of all ages. However, the degree of induction is highest in the liver of young rats and decreases with increasing age. This decrease in the inducibility of the isoenzyme by hydrocortisone as a function of age of the rat may be due to a gradual decrease in the level of corticosteroid receptors (Singer et al., 1973; Roth and Adelman, 1975) and/or certain regulatory changes occur in the genome which may decrease its responsiveness towards the hormone. The decrease

TABLE III

Protein content (mg/g wet weight) of the liver of normal male rats of different ages

Age (wk)	Cytosolic fraction		Mitochondrial fraction	
	mean SD	p	mean SD	p
6	72.45 ± 3.15		42.35 ± 2.10	
		NS		NS
30	75.36 ± 2.45		40.64 ± 3.25	
		NS		NS
90	73.65 ± 1.76		44.28 ± 1.85	

The data were collected from 4 to 5 rats of each age group. Standard deviation (SD) and the level of significance (p) are given. NS, not significant.

in the degree of induction of this isoenzyme and the other gluconeogenic enzymes (i.e. cytoplasmic malate dehydrogenase and phosphoenolpyruvate carboxykinase), by hydrocortisone in the liver of aging rats, has been reported (Sharma and Patnaik, 1982a,b, 1984). In contrast to this, the above treatments do not show any significant influence on the activity of mitochondrial-AsAT of the liver of rats of all ages (Table II). These findings clearly indicate that both the isoenzymes of AsAT differ from one another, even in the same tissue, so far as their responses towards hydrocortisone and also age of the rat are concerned. These findings are in agreement with the earlier report (Braunstein, 1973) that both isoenzymes of AsAT are genetically independent.

On the basis of these findings it may be concluded that the endogenous levels of both isoenzymes of AsAT are dependent on different types of physiological controls. The responsiveness of the isoenzymes of AsAT to hydrocortisone depends on the stage of development and age of the rat and it undergoes specific changes at different phases of life span. Such differential regulation of the enzymes may be brought about by factors like hormones according to a specific programme (Kanungo, 1980) and may lead to the aging of an organism.

Acknowledgements

The authors are grateful to Professor M.S. Kanungo of the Department of Zoology, Banaras Hindu University for helpful advice and facilities. This research was supported by grants from University Grants Commission, New Delhi under special assistance programme.

References

Boyd, J.W. (1961): The intracellular distribution, latency and electrophoretic pattern of L-glutamate oxaloacetate transaminase from rat liver. Biochem. J., 81, 434–441.

Braunstein, A.E. (1973): In: The Enzymes, vol. 9, pp. 397–481. Editor: P.D. Boyer. Academic Press, New York.

Fleisher, G.A., Potter, C.S. and Wakim, K.G. (1960): Separation of 2-glutamic oxaloacetate transaminase by paper electrophoresis. Proc. Soc. Exp. Biol. Med., 103, 229–231.

Garrett, H.E. (1966): Statistics in psychology and education, David McKay Company Inc., New York.

Herzfeld, A. and Greengard, O. (1971): Aspartate aminotransferase in rat tissues; changes with growth and hormones. Biochim. Biophys. Acta., 237, 88–98.

Johannessen, E.C. (1940): Hypoglycemia without glycosuria in older people. Nord. Med. Stockholm, 8, 2231.

Kanungo, M.S. (1980): Biochemistry of Aging. Academic Press, London.

Karmen, A. (1955): A note on the spectrophotometric assay of glutamate oxaloacetate transaminase in human blood serum. J. Clin. Invest., 34, 131–133.

Lardy, H.A., Paetkan, V. and Walter, P. (1965): Paths of carbon in gluconeogenesis and lipogenesis: the roll of mitochondria in supplying precursors of phosphoenolpyruvate. Proc. Natl. Acad. Sci. USA, 53, 1410–1415.

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

Nakata, Y., Suematsu, T. and Sakamoto, Y. (1964): Transaminase activities in some rapidly growing tissue. J. Biochem., 55, 199–201.

Porter, E. and Langley, G.J. (1926): Studies in blood sugar. Lancet, 2, 947.

Roth, G.S. and Adelman, R.C. (1975): Age-related changes in hormonal binding by target cells and tissues, possible role in altered adoptive responsiveness. Exp. Gerontol., 10, 1–11.

Sharma, R. and Patnaik, S.K. (1982a): Properties of liver cytoplasmic aspartate aminotransferase of rats of various ages. Biochem. Int., 5, 561–566.

Sharma, R. and Patnaik, S.K. (1982b): Differential regulation of malate dehydrogenase isoenzymes by hydrocortisone in the liver and brain of rats of various ages. Develop. Growth Differ., 24, 501–505.

Sharma, R. and Patnaik, S.K. (1984): Regulation of citrate synthase and phosphoenolpyruvate carboxykinase by hydrocortisone in the liver of aging rats. Arch. Gerontol. Geriatr., 3, 167–174.

Sharma, R. and Patnaik, S.K. (1985): Age-dependent regulation of aspartate aminotransferase isoenzymes by hydrocortisone in the brain of male rats. Mol. Physiol., 7, 195–200.

Singer, S., Ito, H. and Litwack, G. (1973): ^{3}H-cortisol binding by young and old human liver cortisol protein in vitro. Int. J. Biochem., 4, 569–573.