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AGE-DEPENDENT REGULATION OF ASPARTATE AMINOTRANSFERASE ISOENZYMES BY HYDROCORTISONE IN THE BRAIN OF MALE RATS

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Summary

The activities and induction patterns of the isoenzymes of aspartate aminotransferase (AsAT) of the brain of male rats of various ages were studied. The activity of cytoplasmic AsAT of the brain increases until adulthood and remains constant thereafter. However, the activity of mitochondrial AsAT decreases gradually as a function of age of the rats. Adrenalectomy decreases and hydrocortisone treatment increases the activity of cytoplasmic-AsAT of the brain of young and adult but not of old rats. The hormone-mediated induction of this isoenzyme is actinomycin D-sensitive. However, these treatments do not show any significant effect on the activity of mitochondrial AsAT of the brain of rats of all ages.

Introduction

Alterations in the levels of enzymes and their inducibility by certain hormones are age-related phenomena (Wilson, 1973; Adelman, 1975; 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 catalyzes the reversible transfer of an amino group from L-aspartate or L-glutamate to α-ketoglutarate and oxaloacetate. It is of much importance for brain tissue because approx.

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75% of the free amino acids of the brain are accounted for by aspartic and glutamic acid and their derivatives. There are reports on the hormonal regulation of liver AsAT (Nakata et al., 1964a; Herzfeld and Greengard, 1971; Sharma and Patnaik, 1982a). However, information concerning age-related differential regulation of this enzyme in rat brain is scanty. We report here the differential regulation of AsAT isoenzymes by hydrocortisone in the brain of rats through different phases of the life span.

Materials and Methods

Animals. Male albino rats of Wistar strain (*Rattus norvegicus albinus*) of three different ages (6, 30 and 90 weeks) were used. They were maintained at $24 \pm 2°C$ with 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 vitamin-supplemented milk-powder in the ratio of 4 : 1 in water with added table salt. The diet was supplemented with gram (*Cicer arietinum*) on alternate days. Tap water was supplied ad libitum. All the chemicals used were of analytical grade and the biochemicals were purchased from Sigma Chemical Co., U.S.A.

Effect of hormone. Pilot experiments were undertaken to find out the time- and dose-dependence of the enzyme towards hydrocortisone in rats of various ages. Maximum response of the enzyme was obtained 3 days after the hormone administration at a dose of 5.0 mg/100 g body wt. The rats of each age group were divided into four sets, each having 4–5 rats. Set I rats served as the control. Sets II, III and IV rats were bilaterally adrenalectomized and were given 0.9% NaCl ad libitum instead of water for 10 days following adrenalectomy. On the 11th day, set II rats received 1.0 ml of 0.9% NaCl intraperitoneally (i.p.) instead of the hormone and these rats served as the control for the induction studies. Sets III and IV rats were given an i.p. dose of hydrocortisone (5.0 mg/100 g body wt., suspended in 1.0 ml of 0.9% NaCl) at a fixed time of the day (i.e., 1700 h) for 3 days. The set IV rats were also given actinomycin D (10.0 μg/100 g body wt., suspended in 1.0 ml of 0.9% NaCl), 1 h prior to hydrocortisone administration for 3 days. All the rats were killed after 3 h of the final hormone injection and their brains were taken out, washed in normal saline and blotted dry on a filter paper.

Assay of AsAT isoenzymes. A 10% (w/v) homogenate of the cerebral hemispheres was prepared in ice-cold 0.32 M sucrose solution at $2 \pm 1°C$, using a Potter Elvehjem homogenizer 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 to sediment nuclei. 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 was suspended in a solubilizing 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.

Both the isoenzymes of AsAT were assayed spectrophotometrically (Karmen, 1955; Herzfeld and Greengard, 1971) using a DBGT-Beckman model spectropho-

tometer at 25°C. Protein content of the soluble and mitochondrial fractions were estimated (Lowry et al., 1951) and the activity of both the isoenzymes was expressed as units (μmol NADH oxidized per min) per mg protein at 25°C. Each set of data was collected from 4–5 rats of specific age groups. All the data were statistically analysed (Garrett, 1966). The level of significance (P) between two sets of data was calculated according to the 't'-test. P values, which were 5% or lower for two sets of data, were taken as significant.

Results and Discussion

Several physiological and biochemical changes are known to occur during aging of an organism (Kanungo, 1980). Since enzymes are responsible for specific functions, the initiation, duration and termination of various phases of the life-span of an organism such as differentiation, development and reproductive maturity may depend on the appearance or disappearance or alterations in the levels of specific enzymes or their isoenzymes.

Our data show that the activity of c-AsAT of the brain increases by 32% until adulthood (30 weeks) and remains constant thereafter with the advancing age of the rat (Table 1). Earlier reports (Herzfeld and Greengard, 1971; Sharma and Patnaik, 1982a) showed 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 cytoplasmic AsAT increases in the brain during development and growth of rats (Pasquini et al., 1967). The higher activity of c-AsAT in the brain of adult and old rats may be correlated with a higher degree of transamination during these phases of the life-span of the rat. The activity of mitochondrial AsAT, on the other hand, decreases as a function of age. Hence, it is likely that the process of transamination in the mitochondria diminishes in the brain of aging rats. The present findings are in

TABLE 1

Effects of adrenalectomy (A/d), hydrocortisone (HC) and actinomycin D (A) on the activity (Units/mg protein)$\times 10^2$ of aspartate aminotransferase isoenzymes of the brain of male rats of various ages

Isoenzymes	Treatments	6 Weeks	30 Weeks	90 Weeks
c-AsAT	Normal	90.08 ± 2.08	118.00 ± 6.83	122.80 ± 17.22
	A/d	71.35 ± 2.93 [a]	95.55 ± 3.92 [a]	123.30 ± 4.04
	A/d + HC	120.50 ± 4.02 [a]	146.50 ± 0.63 [a]	115.60 ± 6.78
	A/d + A + HC	61.04 ± 2.93 [a]	105.10 ± 5.80 [a]	116.10 ± 6.14
m-AsAT	Normal	35.17 ± 1.11	28.21 ± 2.20	19.51 ± 1.03
	A/d	32.88 ± 1.09	30.42 ± 1.31	20.24 ± 1.95
	A/d + HC	35.99 ± 1.10	28.29 ± 1.69	21.82 ± 1.51
	A/d + A + HC	34.52 ± 1.25	26.43 ± 0.81	21.51 ± 1.16

The data were collected from 4–5 rats of each age group. Results are expressed as means ± S.D.

[a] Significant statistically (t'-test); treated rats are compared with adrenalectomized rats and adrenalectomized rats are compared with the normal rats.

agreement with the earlier report (Nakata et al., 1964b) that the ratio of cytoplasmic to mitochondrial isoenzymes of AsAT of the liver increases during development and growth of the rats.

It has been reported that removal of the hormone-secreting organs from an animal causes a change in the levels of many enzymes in different tissues (Kanungo, 1980; Yadav and Singh, 1983; Sharma and Patnaik, 1982a, b; 1983). Our investigations show that adrenalectomy decreases significantly the activity of c-AsAT of the brain of young and adult rats but not in the old rats (Table 1). Administration of hydrocortisone to adrenalectomized rats increases the activity of this isoenzyme significantly in the brain of young and adult rats (Table 1). The degrees of decrease and increase in the activity of this isoenzyme following adrenalectomy and hydrocortisone treatments are higher in the young rat.

This hormone-mediated induction of c-AsAT is actinomycin D-sensitive which shows that hydrocortisone induces this isoenzyme by stimulating transcription of the mRNA(s) responsible for the synthesis of this isoenzyme. The magnitude of induction of c-AsAT following hydrocortisone treatment decreases in the brain as a function of age of the rat. This may be due to the gradual loss in the level of hydrocortisone receptors (Singer et al., 1973; Roth and Adelman, 1974, 1975) and/or certain regulatory changes occurring in the genome which may decrease its responsiveness towards the hormone receptor complex. The decrease in the degree of induction of this isoenzyme and the other enzymes by hydrocortisone in the liver and brain of old rats has been reported earlier (Sharma and Patnaik, 1982a, b; 1983). However, these above-mentioned treatments do not show any significant effect on the activity of mitochondrial AsAT of the brain of rats of all the ages (Table 1). These findings indicate that both c- and m-AsAT differ from one another even in the same tissue as far as their responses towards hydrocortisone and also the age of the rat are concerned. These findings are thus in agreement with the earlier findings of Braunstein (1973) who reported that both isoenzymes of AsAT are genetically independent.

On the basis of these studies, it may be concluded that the endogenous levels of both isoenzymes of AsAT are dependent on the 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 undergoes specific changes at different phases of the life-span. Such alterations in the levels of this enzyme may be due to the regulatory changes in the template activities of the corresponding gene(s) which are brought about by various factors such as hormones according to a specific programme (Kanungo, 1980).

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