DIFFERENT PATTERNS OF INDUCTION OF THE TWO ISOZYMES OF ALANINE AMINOTRANSFERASE OF LIVER OF RAT AS A FUNCTION OF AGE[1]

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SUMMARY - Hydrocortisone-mediated induction of mitochondrial (m-) and cytoplasmic (c-) isozymes of alanine aminotransferase (AAT) of the liver of immature, adult and old adrenalectomised rats was studied. mAAT is not induced in the young and the adult, but is induced in the old. cAAT, on the contrary, is induced throughout the life span, but the degree of its induction in the old is considerably less than in the adult. The data are discussed in relation to differential gene activity as a function of age.

INTRODUCTION - The levels of several enzymes decrease and of several others increase as a function of age of animals (1, 2). Such alterations may be due to a decrease or an increase, respectively, of the template activity of corresponding genes. Induction of enzymes is a useful tool to study the functional changes in the activities of genes. The lag period for the induction of tyrosine aminotransferase of the liver of old rats was shown to be longer than that of young rats (3). Similar impairment of induction in old age has been reported for glucokinase (4) and acetylcholinesterase (5). We have reported that, whereas the induction of cytoplasmic malate dehydrogenase (cMDH) of the liver gradually decreases with age, that of mitochondrial MDH is totally impaired in old age of the rat (6). There is no report so far on any enzyme that is not inducible in the young, but is inducible in the old. We report for the

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first time that mitochondrial isozyme of alanine aminotransferase (E.C. 2.6.1.2; AAT) is such an enzyme.

AAT catalyses the reversible conversion of L-alanine and α-ketoglutarate to L-glutamate and pyruvate. It has two isozymes, cytoplasmic (cAAT) and mitochondrial (mAAT). cAAT, but not mAAT, is induced by prednisolone acetate in normal adult rats (7). Administration of hydrocortisone elevates the level of total AAT (8). These experiments were, however, not done after adrenalectomy. We show here that in adrenalectomised rats, (a) hepatic mAAT, unlike all other enzymes studied so far, is not inducible by hydrocortisone in the young, but is inducible in the old, and (b) the induction of cAAT decreases after adulthood.

MATERIALS AND METHODS - Immature (6-), adult (28-) and old (83-week) female albino rats of Wistar strain, kept at 24 $\pm$ 2°C and under an illumination programme of 12 h light beginning at 7.00 A.M. followed by a 12 h dark period, were used. They were fed standard Anidiet 'A' (Chelsea Chemical Laboratory, Poona) and gram (Cicer arietinum) ad libitum.

The rats were killed at a fixed time of the day, the liver was excised and homogenized in 0.25 M sucrose solution. It was centrifuged at 700 g in an International refrigerated centrifuge (PR-6) for 15 min. The supernatant was centrifuged at 14,000 x g for 30 min. The supernatant so obtained formed the sAAT fraction. The pellet was suspended in 0.01 M potassium phosphate buffer, pH 7.3, and labeled as mAAT fraction. Both the isozymes were assayed spectrophotometrically at 340 nm (9). Protein contents of the two fractions were determined (10), and the activity of each isozyme was expressed as specific activity (units/mg protein), units/mg DNA, and units/g wet wt.

TABLE 1. EFFECTS OF ADRENALECTOMY (Aȡ) HYDROCORTISONE (Hc) AND ACTINOMYCIN D (A) SPECIFIC ACTIVITY (x 10^3) OF c- and mAAT OF THE LIVER OF 6-, 28- AND 83-WEEK OLD FEMALE RATS

		6 weeks		28 weeks		83 weeks	
		Mean ± S.D.	p	Mean ± S.D.	p	Mean ± S.D.	p
cAAT	Normal	68.0 ± 0.85	< .005	242.0 ± 8.0	< .005	90.0 ± 9.5	< .005
	Aȡ	36.0 ± 2.0 (-47)	< .005	83.1 ± 7.1 (-65.7)	< .001	48.0 ± 2.6 (-46.7)	< .001
	Aȡ+Hc	106.0 ± 12.4 (+194.43)	< .1 (NS)	358.0 ± 6.0 (+330.8)	< .001	144.0 ± 6.3 (+200)	< .005
	Aȡ+A+Hc	62.5 ± 14.9 (NE)		68.0 ± 1.0 (-81)		97.0 ± 2.4 (-32.6)	
mAAT	Normal	116.0 ± 0.0	< .4 (NS)	231.0 ± 23.9	< .2 (NS)	97.2 ± 4.9	< .001
	Aȡ	100.0 ± 17.6 (NE)	> .25 (NS)	185.0 ± 3.0 (NE)	< .5 (NS)	37.0 ± 10.2 (-61.9)	< .005
	Aȡ+Hc	125.0 ± 8.5 (NE)	> .1 (NS)	219.0 ± 37.5 (NE)	> .7 (NS)	115.0 ± 4.0 (+210.8)	> .01
	Aȡ+A+Hc	163.0 ± 11.5 (NE)		234.0 ± 10.0 (NE)		86.0 ± 4.7 (-25.3)	

Data were collected from 4-5 rats for each set of experiments. p values 0.05 between two sets of data were taken as significant. Numbers in parentheses denote per cent increase (+) or decrease (-) as compared to the data in the preceding column. NE, no effect; NS, not significant.

The rats of each age were divided into four groups. Group I rats were injected 0.9% NaCl i.p. Group II, III and IV rats were bilaterally adrenalectomized (Aø) and maintained for 10 days on 0.9% NaCl instead of water as it is known that the blood has no hydrocortisone after this period (11). They were given the usual diet during this period. On the 11th day, group II rats were given 0.9% NaCl. Group III rats were given hydrocortisone acetate (3 mg/ 100 g body wt. in 1.0 ml of 0.9% NaCl) at 24 h intervals for 3 days. Group IV rats received actinomycin D (10 µg/100 g body wt. in 1.0 ml of 0.9% NaCl) 1 h prior to hydrocortisone administration for 3 days. The rats were killed on the 4th day for the assay of the isozymes. Group II rats served as control for calculating the per cent induction by hydrocortisone.

RESULTS AND DISCUSSION - Our data (Table) show that the specific activities of c- and mAAT are highest in the adult. When the data are expressed as units/mg DNA or units/g wet wt., the differences obtained are similar. The normal level of mAAT is two-fold higher than that of cAAT in the immature rat, but no such difference exists in the adult and the old. The activity of total AAT is known to increase till 48 weeks (12). However, whether this is due to an increase of one or both the isozymes of AAT is not known. Our data show that both the isozymes increase in activity till the fully grown adult stage (28-weeks) is reached. Also the rate of increase of cAAT is greater (4-fold) than that of mAAT (2-fold) till adulthood It is possible that a more rapid increase in cAAT during growth may increase glutamate formation and thus aid protein synthesis necessary during this period.

It is of interest to note that adrenalectomy causes a considerable decrease in the activity of mAAT of the liver of old rats only.

Also, administration of hydrocortisone to old adrenalectomised rats causes a three-fold induction. This induction is partially suppressed by actinomycin D. However, neither adrenalectomy nor hydrocortisone have any effect on mAAT of immature and adult rats.

The activity of cAAT decreases after adrenalectomy and increases after the administration of hydrocortisone considerably in all the three ages. Both these effects are highest in the adult in which the endogenous level of the enzyme is also the highest. Actinomycin D inhibits the induction by the hormone.

These studies show that hydrocortisone induces the synthesis of both the isozymes by stimulating the transcription of the corresponding messenger RNAs. Also, it appears that this adrenal hormone is responsible, atleast in part, for the maintenance of the level of the two isozymes.

The two isozymes of AAT are in separate compartments of the cell. Though both are induced by the same hormone, the patterns of their induction are different; cAAT is induced throughout the life span, and mAAT is induced only after the growth phase. This may be because the two isozymes are under the control of two separate genes whose responsiveness to the hormone is different in various phases of the life span. The decrease in induction of cAAT in old age may be due to the appearance of a repressor of its gene, and the non-inducibility of mAAT till the end of growth phase may due to a repressor that is synthesised during this period and blocks its gene. Alternatively, the receptors which stimulate the transcription of the two genes after binding with hydrocortisone may be different. The synthesis of the receptor for cAAT may decrease in old age, whereas that of mAAT may increase. This is consistent with the "gene regulation" theory of aging (1) which proposes that factors produced during earlier phases of the life span

stimulate certain genes and repress others and thus account for the changing levels of enzymes during aging.

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