Develop., Growth and Differ., **24** (5), 501–505 (1982)

Development Growth & Differentiation

Differential Regulation of Malate Dehydrogenase Isoenzymes by Hydrocortisone in the Liver and Brain of Aging Rats

(MDH isoenzymes/aging/hydrocortisone)

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The activities and induction patterns of the isoenzymes of malate dehydrogenase (MDH) of the liver and brain of male rats of various ages were studied. The activities of both the isoenzymes of MDH of the liver and brain show a gradual increase with increasing age of the rats. Adrenalectomy decreases and hydrocortisone treatment increases the activity of cytoplasmic MDH of the liver and brain of rats of all the ages except that of the brain isoenzyme of old rats. This hormone-mediated induction of the isoenzyme is actinomycin D-sensitive. Furthermore, adrenalectomy decreases and hydrocortisone treatment increases the activity of mitochondrial MDH of the liver of young and adult rats but not in old rats. However, these treatments do not show any significant effect on the activity of mitochondrial MDH of the brain of rats of all the ages.

The activities of several enzymes decrease and of several others increase as a function of age of an organism (1). A possible reason for such changes may be due to the decrease and increase, respectively in the template activity of the corresponding genes (2). Since age-related changes are tissue and sex specific (3), we have studied the changes in the isoenzyme patterns of malate dehydrogenase (MDH; L-malate; NAD^+-oxidoreductase; EC 1.1.1.37) and their differential responsiveness toward hydrocortisone in the liver and brain of male rats of various ages as a model system to get some insight into this process.

MDH is localized in both the cytoplasmic and mitochondrial fractions of several animal tissues (4, 5). The cytoplasmic malate dehydrogenase (c-MDH) differs from that of the mitochondrial one (m-MDH) so far as its catalytic (4, 5, 6), and physicochemical (7) properties are concerned. Both the isoenzymes are NAD^+-dependent. DAVIDSON and CORTNER (8, 9) have shown that c- and m-MDH are under different genetic controls. MDH of the fish has two types of subunits (A and B) which are under the control of two separate genes (10).

The cytoplasmic isoenzyme is important for gluconeogenesis in the cytoplasm since it converts malate to oxaloacetate, which is then converted to phosphoenolpyruvate (11). The mitochondrial isoenzyme is actively involved in Krebs cycle. Thus, the two forms of MDH are located in two separate compartments of the cell and perform two distinct metabolic function. So it is reasonable that they may be subjected to different control mechanisms that may change with age of an organism.

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MATERIALS AND METHODS

Animals Young (6-week), adult (30-week) and old (90-week) male albino rats of Wistar strain kept under controlled conditions of temperature and light, were used. They were fed with a freshly prepared standard diet (per cent composition of protein, fat and carbohydrate are 19, 13 and 59 respectively). Tap water was supplied *ad libitum*. All the chemicals used were of analytical grade. The biochemicals were purchased from Sigma Chemical Co., U. S. A.

Pilot experiments were undertaken to find out the time and dose dependence of these isoenzymes towards hydrocortisone in rats of various ages. Maximum response of these isoenzymes 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. The set I rats served as the normal. The sets II, III and IV rats were bilaterally adrenalectomized. These rats were given with 0.9% NaCl *ad libitum* instead of water for 10 days. On the 11th day, the set II rats were administered with 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. The rats belonging to sets III and IV were given with 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 for 3 days. The set IV rats were also given with actinomycin D (10 μg/100 g body wt. suspended in 1.0 ml of 0.9% NaCl), one hr prior to the hydrocortisone administration for 3 days. All the rats were killed by cervical dislocation after 3 hr of the final hormone injection and their tissues were taken out.

Enzyme preparation A 10% (w/v) homogenates of the tissues (for liver, 0.25 M and for brain, 0.32 M sucrose) were prepared at 2 ± 1°C using Potter-Elvehjem homogenizer. The homogenates of both the liver and brain were filtered through double layered cheese cloth and centrifuged for 15 min at $700\times g$ at 0°C to sediment nuclei. The resulting supernatant was centrifuged further at $14{,}000\times g$ for 30 min at 0°C to sediment mitochondria. The supernatant thus obtained was used for the assay of c-MDH. The mitochondrial pellet was washed twice and was suspended in 5.0 mM potassium phosphate buffer, pH 7.5, containing 0.25 M sucrose for 3 hr and was used for the assay of m-MDH.

Assay of MDH isoenzymes Both the isoenzymes of MDH were assayed spectrophotometrically according to the method of KITTO (12). The activity of both the isoenzymes was expressed as units/mg protein. Protein content of the soluble and mitochondrial fractions were estimated (13). Each set of data was collected from 4–5 rats of specific age groups. All the data were statistically analysed (14). The level of significance ('p') between two sets of data was calculated according to 't' test. 'p' values, which were 5% or lower for two sets of data, were taken as significant.

RESULT AND DISCUSSION

Since enzymes are responsible for specific functions, the initiation, duration and termination of various phases of the lifespan 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 results indicate that the specific activities of c- and m-MDH show a gradual increase in the liver and brain as a function of age of the rat (Fig. 1, 2). Since, m-MDH is an enzyme of a metabolic pathway which is oxygen-dependent, it is likely that the increase in its level in old age may be an adaptation to an increase in the aerobicity of these tissues. These findings are in agreement with the earlier reports (15, 16) that the M4 isoenzyme of lactate dehydrogenase of the brain and liver decreases in old age, thereby making these tissues more aerobic. Further, KANUNGO and GANDHI (17), using female rats have shown that the activities of both the isoenzymes of MDH are significantly higher in the liver of 70 week old rat compared to that of the 9-week old rat. However, the present observations indicate that this

increase in the activities of both the isoenzymes of MDH are not so much higher in the old male rats. The concentration of glucose in the blood increases in old age (18). It is possible, therefore, that the higher activity of c-MDH in old rat may be a contributory factor for this physiological change since it is involved in gluconeogenesis (11).

It has been reported that removal of the hormone secreting organ from an animal causes a change in the levels of many enzymes in different tissues (2). Our investigations show that adrenalectomy (A/d) decreases and hydrocortisone treatment increases the activity of c-MDH of the liver and brain of rats of all the ages except that this hormone shows no significant effect on the activity of this isoenzyme of the brain of old rat. (Fig. 2). The per cent increase in the level of this isoenzyme following the hormone treatment is lower in the brain as compared to that of the liver which may be either due to the differential responsiveness of the gene(s) for this isoenzyme in both the tissues and/or concentration of the hydrocortisone receptor is higher in the liver than the brain (19). The magnitude of induction of c-MDH following hydrocortisone treatment decreases in the liver and brain as a function of age of the rat. This may be due to a gradual loss in the level of hydrocortisone receptors (20, 21, 22). Another possibility for such a gradual decrease in the inducibility of c-MDH of the liver and brain by hydrocortisone may be due to the decrease in the responsiveness or depression of the gene(s) responsible for the synthesis

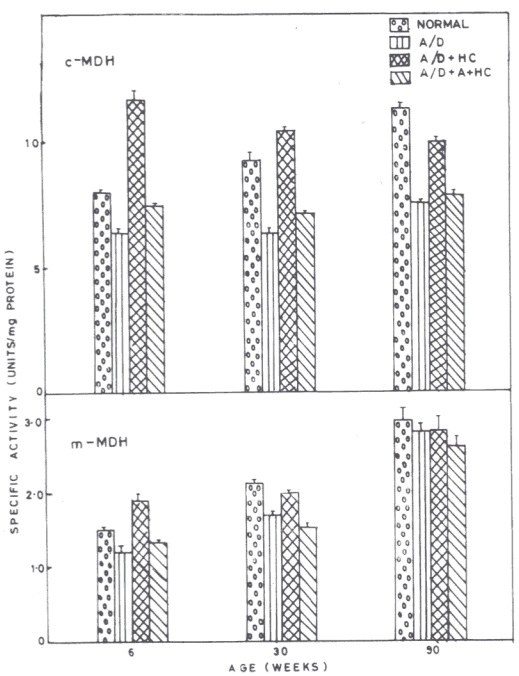

Fig. 1. Induction of MDH isoenzymes by hydrocortisone in the liver of aging rats.

Effects of adrenalectomy (A/D), hydrocortisone (HC) and actinomycin D (A) on the specific activity (units /mg protein) of malate dehydrogenase isoenzymes (c- and m-MDH) of the liver of male rats of various ages: Symbols are same for the Fig. 2.

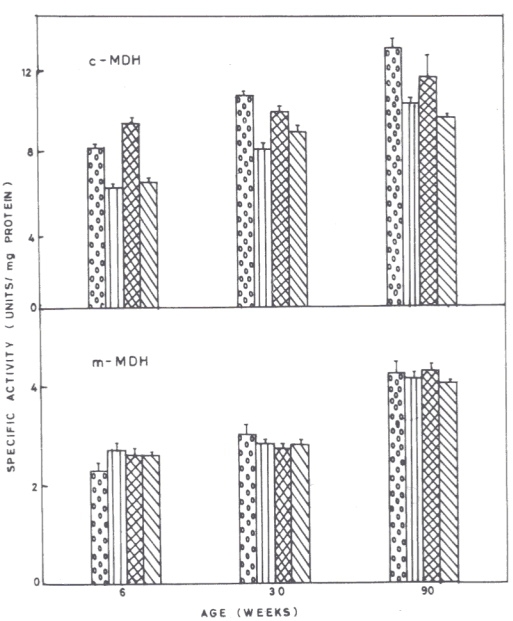

Fig. 2. Induction of MDH isoenzymes by hydrocortisone in the brain of aging rats.

Effects of adrenalectomy (A/D), hydrocortisone (HC) and actinomycin D (A) on the specific activity (units /mg protein) of malate dehydrogenase isoenzymes (c- and m-MDH) of the brain of male rats of various ages.

of this isoenzyme as a function of age of the rat. It has also been reported that some of the non-histone chromosomal proteins (NHCP) undergo changes in old age (23) and it is well known that hormone-receptor complex binds to the NHCP fraction of the chromatin. Hence, the loss of NHCP fraction may also be responsible for the impaired induction of MDH isoenzymes in old age. The decrease in the degree of induction of this isoenzyme by cortisone in the liver of old female rats has been reported earlier (17). The hormone-mediated induction of c-MDH 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. On the other hand, adrenalectomy decreases and hydrocortisone treatment increases the activity of m-MDH of the liver of young and adult rats but not in old rats (Fig. 1). However, these treatments do not show any significant effect on the activity of m-MDH of the brain of rats of all the ages (Fig. 2). The degree of induction of m-MDH by hydrocortisone is significantly lower than that of c-MDH. It has been reported earlier that c- and m-MDH are under the control of two separate genes (8, 9). The lower degree of induction of m-MDH by hydrocortisone may be due to the differential responsiveness of both the genes of c- and m-MDH towards hydrocortisone, such as, their location on the chromosome, accessibility for the inducer, nature of the mediator etc. The impairement of induction of m-MDH in the liver of old rat and in the brain of rats of all the three ages shows a total un-responsiveness of the gene(s), responsible for the synthesis of this isoenzyme at these phases of the life-span.

On the basis of these studies, it may be concluded that different organs of the rat respond differentially to the same hormone at different phases of its life-span. Therefore, the alterations in the levels and induction of enzymes that occur as a function of age may be due to the changes in the template activities of the corresponding gene(s) which are brought about by various factors such as hormones at different phases of the life span of an organism (24).

The authors are indebted to Professor M. S. Kanungo, Head 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; Nuffield Foundation, London and PL–480, U. S. A.

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(Received for publication: July 9, 1982)