INDUCTION OF PARTICULATE AND SOLUBLE ISOENZYMES OF TYROSINE AMINOTRANSFERASE BY HYDROCORTISONE IN THE LIVER OF RATS AS A FUNCTION OF AGE

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ABSTRACT - The induction of soluble cytoplasmic (c-), and particulate mitochondrial (m-) and nuclear (n-) isoenzymes of tyrosine aminotransferase (TAT) by hydrocortisone in the liver of 6-, 35- and 76-week old rats was studied. In contrast to the earlier reports, both the particulate isoenzymes (m- & n-TAT) are induced by hydrocortisone. This induction is actinomycin D sensitive. The degree and pattern of induction of the three isoenzymes of TAT vary with age. The possibility of separate regulatory mechanisms for the synthesis of the three isoenzymes is discussed.

INTRODUCTION - Tyrosine aminotransferase (TAT; E.C.2.6.1.5) has three isoenzymes which are located in different compartments of the cell: the soluble cytoplasmic (c-) form, and the particulate mitochondrial (m-) and nuclear (n-TAT) forms (1-5). Much data on the molecular, kinetic and induction of c-TAT of the liver of normal adult rat have accumulated (6-9). However, little is known about the particulate isoenzymes (m- & n-TAT) which constitute a greater proportion of the total TAT pool in the liver of the rat, and the effect of age on their induction. c-TAT appears just before birth whereas m-TAT appears in the fetus long before birth (10,11). The data available on induction of m- and n-TAT are conflicting. Hydrocortisone or cortisol has been reported to have either little or no effect on the induction of both the particulate isoenzymes of TAT, whereas c-TAT is greatly induced within five hours of hormone administration (1,4,9,12). The present report decribes the pattern of induction in vivo of c-, m- and n-TAT by hydrocortisone in the liver of rats of various ages.

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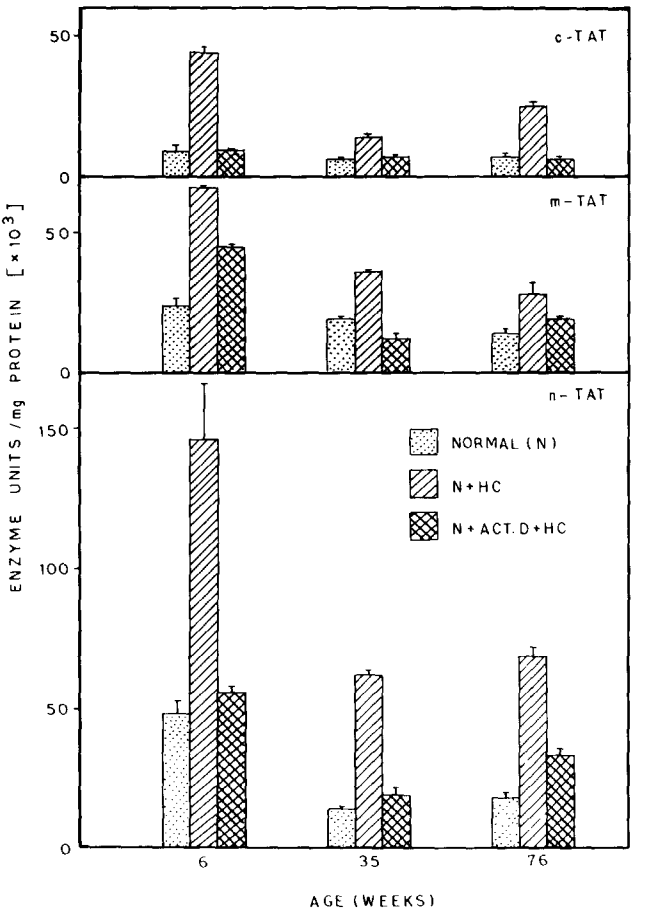

Fig. Comparative effects of hydrocortisone (HC) and actinomycin D (ACT. D) on the specific activity (units/mg protein) of TAT isoenzymes in the liver of normal male rats of various ages.

MATERIALS AND METHODS - Male albino rats of Wistar strain of three different ages, 6-, 35- and 76-week, maintained under standard conditions in the animal colony, were used. Rats of each age group were divided into three sets. Set I rats were given 1.0 ml of 0.9% NaCl and served as control. Set II and III rats were administered hydrocortisone (5 mg/100 g body wt.) in 1.0 ml of normal saline at 1500 hrs. Group III rats were also given actinomycin D (0.1 mg/100 g body wt.) 1.0 hr prior to the hormone administration. All the injections were given intraperitoneally. The rats were sacrificed by cervical dislocation at 2000 hrs, that is, 5 hr after hydrocortisone administration.

The livers were immediately removed and washed in ice-cold normal saline. 10% homogenates were prepared with 0.25 M sucrose using a Potter-Elvehjem homogenizer fitted with a teflon pestle. The cytoplasmic, mitochondrial and nuclear fractions were prepared by differential centrifugation. The enzyme activities (c-, m- and n-TAT) were assayed (7,13) and the protein content was estimated spectrophotometrically (14) in each fraction. One unit of enzyme was defined as that amount which catalyzed the formation of

1 umole of the product p-hydroxy phenylpyruvate/min at 37°C, and was expressed as specific activity (units/mg protein). The date for each set were collected from 4-5 rats and were statistically analyzed.

RESULTS AND DISCUSSION - The specific activities of the three isoenzymes are highest at 6-week and decrease significantly with increasing age (Fig.). Administration of hydrocortisone causes induction of all the isoenzymes in the three ages. All the isoenzymes are induced at each age, but they differ in the degree of their induction. c- and m-TAT are induced maximally (388% and 168%, respectively) in the 6-week old rats, whereas n-TAT is maximally induced at 35 weeks (336%). The induction of c-TAT is the least at 35 weeks. There is no difference in the induction of m-TAT of 35- and 76-week old rats. Though the induction of n-TAT at 76 week is less than that at 35 week, that of c-TAT is considerably higher at 76 week than at 35-weeks.

The induction of each isoenzyme by hydrocortisone is repressed by actinomycin D. The degree of repression is proportional to the induction of c- and m-TAT. This observation is in contradiction to an earlier finding that the induction of m-TAT by hydrocortisone or cortisol in the liver of the rat is small (2,12). However, the degree of induction, and their repression by actinomycin D appear to be age-dependent. The induction and repression of both c- and m-TAT are highest at 6-week. This is consistent with the finding of Finch et al (15) that the normal level and the rate of induction of hepatic c-TAT by hydrocortisone or cold stress are higher in young mice.

The induction of TAT by hydrocortisone has been shown to be due to its effect at the transcriptional level (9). Our studies indicate that the genes responsible for the synthesis of the three isoenzymes are responsive to hydrocortisone at all the three ages studied. However, the degree of response is different in different ages. Such age-

related alterations in the induction pattern of several enzymes and isoenzymes by different hormones have been reported from this laboratory (16-19).

Alanine aminotransferase has been shown to have cytoplasmic and mitochondrial forms which show different induction patterns after the administration of hydrocortisone at different ages of the rat (18). Our studies show that the three isoenzymes of TAT also show different patterns of induction as a function of age of the rat. In the patients suffering from tyrosinemia, hepatic c-TAT is totally absent, and m-TAT is elevated (4). This indicates that m-TAT is regulated by a mechanism different from that of c-TAT. Hence it is concluded that (a) the three isoenzymes of TAT (c-, m- & n-TAT) are induced in the liver of rats by hydrocortisone throughout the life span, and (b) the degree of induction of the isoenzymes varies with age of the rat which may be due to different regulatory mechanisms. This may be due to age-related variations in the expression of the respective genes brought about by effectors produced at different phases of the life span (20).

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REFERENCES

1. Kenney, F.T. (1962) J. Biol. Chem., 237:1610-1614.
2. Litwack, G., Sears, M.L. & Diamondstone, T.I. (1963) J. Biol. Chem., 238:302-305.
3. Miller, J.E. & Litwack, G. (1969) Arch. Biochem. Biophys., 134:149-159.
4. Fellman, J., Vanbellinghen, P.J., Jones, R.T. & Koler, R.D. (1969) Biochemistry, 8:615-619.
5. Miller, J.E. & Litwack, G. (1971) J. Biol. Chem., 246:3234-3240.
6. Kenney, F.T. (1962) J. Biol. Chem., 237:1605-1609.
7. Hayashi, S., Granner, D.K. & Tomkins, G.M. (1967) J. Biol. Chem., 242:3998-4006.
8. Rosenberg, J.S. & Litwack, G. (1970) J. Biol. Chem., 245:5677-5684.
9. Gelehrter, T.D. (1971) In Enzyme synthesis and degradation in mammalian systems (Ed. Recheigles, M.) University Park Press, U.S.A., pp. 165-199.

10. Koler, R.D., Vanbellinghen, P.J., Fellman, J.H., Theodre, J.R. & Behrman, R.E. (1969) Science, 163:1348-1350.
11. Holt, P.G. & Oliver, I.T. (1970) FEBS Lett., 8:99-100.
12. Miller, J.E. & Litwack, G. (1969) Biochem. Biophys. Res. Commun., 36:35-41.
13. Diamondstone, T.I. (1966) Anal. Biochem., 16:395-401.
14. Sutherland, E.W., Cori, C.F., Haynes, R. & Olson, N.S. (1949) J. Biol. Chem., 19:825-837.
15. Finch, C.E. (1969) Cellular activities during ageing mammals, Ph.D. thesis, The Rockfeller University, New York; MSS Information Corpn., New York, U.S.A.
16. Kanungo, M.S. & Gandhi, B.S. (1972) Proc. Nat. Acad. Sci. (U.S.A.) 69:2035-2038.
17. Moudgil, V.K. & Kanungo, M.S. (1973) Biochem. Biophys. Res. Commun., 52:725-730.
18. Patnaik, S.K. & Kanungo, M.S. (1974) Biochem. Biophys. Res. Commun., 56:845-850.
19. Chainy, G.B.N. & Kanungo, M.S. (1976) Biochem. Biophys. Res. Commun., 72:777-781.
20. Kanungo, M.S. (1975) J. Theo. Biol., 53:253-261.