TRANSAMINASE OF BRANCHED CHAIN AMINO ACIDS

II. PHYSIOLOGICAL CHANGE IN ENZYME ACTIVITY IN RAT LIVER AND KIDNEY

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It is well known that in animal metabolism valine is glycogenic, while leucine is ketogenic and isoleucine has both properties. Rowsell suggested that transamination is the first step in the metabolism of these amino acids (Rowsell, 1956). Recently, we have shown that in hog heart these three amino acids are transaminated by a specific enzyme (Ichihara et al., 1966). Taylor and Jenkins purified the enzyme extensively and reached a similar conclusion (Taylor et al., 1966). It was also shown that this enzyme is localized in both the supernatant and mitochondrial fractions of heart muscle and that the enzymes in the both fractions are isozymes (Aki et al., 1966). From these facts it was interesting to see whether the transaminase is regulated under various physiological conditions, in which gluconeogenesis or ketogenesis are induced (Nichol et al., 1964).

The present report shows that in liver hydrocortisone or a high protein diet can induce the transaminase activity, while in kidney only the diabetic condition causes induction.

Experimental

Treatment of rats ---- Male Wistar strain rats, weighing about 150 g, were maintained on laboratory chow and treated as follows. Hydrocortisone treatment: Hydrocortisone acetate(10 mg per 100 g body weight) was injected intraperitoneally into rats. Puromycin hydrochloride(15 mg per 100 g body weight) or actinomycin S(20 μg per 100 g body weight) was injected one hour before

and after and together with hydrocortisone. Therefore, the total doses of puromycin and actinomycin injected were 45 mg and 60 μg per 100 g body weight respectively. Rats were sacrificed 5 hours after hydrocortisone injection. Starvation and high protein diet: Rats were starved for 2 days and then given a 50 per cent casein diet ad libitum for a week. Alloxan diabetes: Alloxan (20 mg per rat) was injected intraperitoneally into rats after one day's starvation. After a week, when the blood glucose concentration was elevated to over 300 mg per 100 ml, the rats were sacrificed. Some of these diabetic rats were then administered with protamine-Zn-insulin(4 U per rat) daily for 4 days before sacrifice.

Enzyme assay ---- The liver and kidney of the rats were homogenized and fractionated into supernatant and mitochondrial fractions according to the method of Hogeboom (Hogeboom, 1955). Both fractions were dialyzed for several hours against the buffer described previously (Ichihara et al., 1966). Enzyme activity was assayed as reported previously (Ichihara et al., 1966), except that 40 μmoles of 2-mercaptoethanol and 30 μmoles of L-amino acid were added to the reaction mixture. Enzyme activity was expressed as mμmole keto acid formed per 10 minutes per mg protein. Protein was measured by the method of Lowry et al. (Lowry et al., 1951).

Results and Discussion

Various transaminases are known to be induced under various conditions such as cortisone treatment, starvation, a high protein diet or diabetes, which cause gluconeogenesis or ketogenesis. Five hours after hydrocortisone injection the transaminase activity for leucine was enhanced greatly in the supernatant fraction of the liver only, while the mitochondrial activity remained constant (Table I). Differences in inducibility of various isozymes localized in the supernatant and mitochondrial fractions of liver have been reported previously (Katunuma et al., 1966, Shrago et al., 1966). This in-

Table I. Induction of Transaminase Activity for Leucine in Rat Liver by Hydrocortisone Treatment

Treatment	No. of rats	Activity for Leucine	
		Supernatant	Mitochondria
		(mμmole keto acid/10 min/mg protein)	
None	7	6.0 ± 0.2*	19.1 ± 0.8
Hydrocortisone	5	25.2 ± 2.5	19.7 ± 0.6
Hydrocortisone + puromycin	5	6.8 ± 1.2	
Hydrocortisone + actinomycin	4	5.8 ± 1.8	

*Means ± standard errors

Table II. Induction of Transaminase Activity for Leucine in Rat Liver and Kidney Supernatants under various Physiological Conditions

Treatment	No. of rats	Activity for Leucine	
		Liver	Kidney
		(mμmole keto acid/10 min/mg protein)	
None	9	6.2 ± 0.8*	105.3 ± 9.8
Hydrocortisone	3	20.3 ± 1.5	123.5 ± 10.6
Starvation for 2 days	5	5.8 ± 1.1	
50 % casein diet for 2 days	7	15.5 ± 2.4	98.0 ± 8.7
Diabetes	10	5.8 ± 1.0	323.5 ± 43.2
Diabetes + insulin	8	5.1 ± 0.7	122.5 ± 18.8

*Means ± standard errors

duction of the supernatant enzyme was inhibited completely by simultaneous administration of puromycin or actinomycin S, indicating that this induction represented de novo synthesis of the enzyme protein. The induction of the

enzyme was rapid and reached a maximum 5 to 6 hours after hydrocortisone injection and then the activity decreased to the normal level within a few hours. This type of transaminase induction has been shown with tyrosine transaminase (Lin et al., 1958), but the activity for branched chain amino acids and that for tyrosine can be separated by DEAE cellulose column chromatography.

Transaminase activity for leucine was also induced by feeding rats on a high protein diet (Table II). However, starvation for 2 days did not cause any alteration in the activity. Induction by a high protein diet reached a maximum after 2 days and the level decreased again to the normal level on the 5th day. This rapid decrease to the normal level, together with the observed induction by hydrocortisone, might be related to repressor formation (Garren et al., 1964, Kenney et al., 1965). These treatments caused no induction of the kidney enzyme.

In alloxan diabetes it is interesting that the kidney enzyme is induced while the liver enzyme is not. This raised level of the kidney enzyme could

Table III. Substrate Specificity of induced Transaminases

Tissue	Treatment	Activity for		
		Valine	Leucine	Isoleucine
		(mμmole keto acid/mg protein/10 min)		
Liver*	None	3.1	5.8	2.8
	Hydrocortisone	2.7	20.4	2.1
Kidney*	None	93.5	114.2	95.1
	Diabetes	297.1	358.0	307.9

*Supernatant enzymes were used.

be reduced to the normal level by injection of insulin. None of these treatments caused significant change in the activity of the mitochondrial enzymes.

It should be mentioned that in liver, only the activity for leucine was induced under these conditions and the activities for valine and isoleucine

were unchanged, while in the kidney, diabetes caused concomitant increase in the activities for the three amino acids (Table III). Recently, we have purified the liver enzyme and found that the supernatant activity could be separated into two fractions by DEAE cellulose column chromatography. One of these fractions contained activity for all three amino acids and the other for leucine only. It is tempting to consider that the latter enzyme may be increased under these conditions. Experiments to test this possibility are now under way and will be published elsewhere with details of the properties of these two enzymes. It is also noteworthy that the transaminase of liver and kidney are induced independently or rather reciprocally under various conditions. Krebs emphasized the importance of the kidney in gluconeogenesis (Krebs, 1963). From these results it is obvious that the metabolism of these amino acids is regulated, at least, at the step of transamination.

References

Aki,K., Ogawa,K., Shirai,A., Takahashi,H.,and Ichihara,A., Proc. 18th Symp. Enz. Chem.,(in Japanese), p.319 (1966)

Garren,L.D., Howell,R.R., Tomkins,G.M.,and Crocco,R.M., Proc. Natl. Acad. Sci.(U.S.), 52, 1121 (1964)

Hogeboom,G.H., in Colowick,S.P.,and Kaplan,N.O.,Eds.,"Methods in Enzymology", Academic Press, New York, vol. I, p.16 (1955)

Ichihara,A.,and Koyama,E., J. Biochem., 59, 160 (1966)

Katunuma,N., Okada,M.,and Nishii,Y., in Weber,G.,Ed.,"Advances in Enzyme Regulation", Pergamon Press, New York, vol. IV, p.317 (1966)

Kenney,F.T.,and Albritton,W.L., Proc. Natl. Acad. Sci.(U.S.), 54,1693 (1965)

Krebs,H.A., in Weber,G.,Ed.,"Advances in Enzyme Regulation",Pergamon Press, New York, vol. I, p.385 (1963)

Lin,E.C.C.,and Knox,W.E., J. Biol. Chem., 233, 1186 (1958)

Lowry,O.H., Rosenbrough,N.J., Fair,A.L.,and Randall,R.J., J. Biol. Chem., 193, 265 (1951)

Nichol,C.A.,and Rosen,F., in Litwack,G.,and Kritchevsky,D.,Eds.,"Actions of Hormones on Molecular Processes", Wiley Inc., New York, p.234 (1964)

Rowsell,E.V., Biochem. J., 64, 235 (1956)

Shrago,E.,and Lardy,H.A., J. Biol. Chem., 241, 663 (1966)

Taylor,R.T.,and Jenkins,W.T., J. Biol. Chem., 241, 4396 (1966)