Biochimica et Biophysica Acta, 315 (1973) 61–65
© Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

BBA 66932

SPECIFIC CHANGES IN THE ACTIVITY OF TYROSINE AMINOTRANSFERASE ISOENZYMES IN RAT LIVER AFTER CORTISOL TREATMENT AND PARTIAL HEPATECTOMY

N. P. MERTVETSOV, V. N. CHESNOKOV AND R. I. SALGANIK

Institute of Cytology and Genetics, Siberian Branch of the USSR Academy of Sciences, Novosibirsk (USSR)

(Received December 18th, 1972)

(Revised manuscript received March 27th, 1973)

SUMMARY

Changes in isoenzyme spectrum of tyrosine aminotransferase in rat liver have been investigated under cortisol induction and during liver regeneration after partial hepatectomy. Electrophoresis in agar gels reveals two groups of isoenzymes of liver tyrosine aminotransferase. One of them, moving to the anode during electrophoresis in agar gel, exhibits a sharp rise of activity after cortisol administration. The second one, moving to the cathode, does not exhibit any change of activity. When the action of the hormone-inductor is stopped, the activity of inducible anode isoenzymes falls to its initial level, while the activity of cathode isoenzymes remains unchanged. During liver regeneration, the rise of activity only of anode isoenzymes of tyrosine aminotransferase is noted, while that of cathode ones remains unchanged. The increase of total activity of tyrosine aminotransferase after cortisol induction and partial hepatectomy is a result of an increase in the activity of anode isoenzymes only.

INTRODUCTION

It is known that treatment of animals by glucocorticoids produces an induction of glycogenic enzymes in their livers, among which tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) exhibits the most rapid and intensive rise in activity[1–3]. The induction of tyrosine aminotransferase is a result of DNA-dependent synthesis of RNA and is inhibited by actinomycin D and puromycin[3–4].

Previously we have shown that during liver regeneration the induction of tyrosine aminotransferase also takes place, but it is not mediated by glucocorticoids since adrenalectomy does not abolish it[5].

Some authors have shown molecular heterogeneity of animal tyrosine aminotransferase[6–9]. It is known that electrophoresis in agar gel reveals two groups of liver

tyrosine aminotransferase isoenzymes which differ significantly from each other in their mobility in an electric field and in their activity[6–9].

In this work we have investigated the changes in activity of different liver tyrosine aminotransferase fractions after cortisol induction and during liver regeneration.

MATERIALS AND METHODS

Cortisol sodium acetate was obtained from "Richter" (Hungary), agar was purchased from "Difco" (USA), L-tyrosine and NAD were from "Reanal" (Hungary) and glutamate dehydrogenase from "Koch-light" (England).

Wistar albino male rats (120–150 g) were used. Partial hepatectomy was performed according to Higgins and Anderson[10]. Some of the animals were subjected to bilateral adrenalectomy 5 to 7 days prior to study and adrenalectomized animals were given 0.5% NaCl solution to drink.

Cortisol was injected once a day intraperitoneally in a dose of 5 mg per 100 g of body weight. The animals were decapitated 3–20 h after cortisol treatment or 24 h after partial hepatectomy. The liver was taken just after decapitation, washed in cold 0.14 M KCl, weighed and homogenized with 6 vol. of 0.14 M KCl in a Potter's glass homogenizer. The homogenates were filtered through nylon cloth and centrifuged at 18 000 × *g* for 30 min. The supernatant was used for tyrosine aminotransferase electrophoresis.

Horizontal electrophoresis was carried out in a 1% agar gel containing 0.05 M phosphate buffer, pH 7.1, for 2.5 h, with a potential gradient of 5 V/cm. The dimensions of the gel block were 6 cm × 10 cm × 0.7 cm. Aliquots of the supernatant containing 10 mg of protein were applied at the start. The protein amount was measured by the method of Kalckar[11]. After electrophoresis the agar block was cut into equal sections, 0.5-cm wide. To estimate tyrosine aminotransferase activity, each gel section was homogenized and incubated at 37 °C for 1 h in a mixture prepared according to Lin *et al.*[12]. From previous experiments it was found that the reaction is linear for 1.5 h (ref. 7). The tyrosine aminotransferase activity was expressed in μmoles of *p*-hydroxyphenylpyruvate formed due to enzymic transamination during 1 h of incubation.

Tyrosine aminotransferase activity was also revealed by a histochemical method described earlier[7].

RESULTS AND DISCUSSION

By electrophoresis in 1% agar gel, two tyrosine aminotransferase fractions were revealed: one of them, moving to the anode, was localized in a gel section 0.5–1.0 cm from the line of origin; the second one, moving to the cathode was observed 1 cm from the origin and occupied a zone of 1.0–1.5 cm. The activity of the anode enzyme fraction in liver of intact rats was, on average, two times higher than that of the cathode one (Fig. 1, Table I). The histochemical reaction revealed two clear bands in each zone (Fig. 2). Hence it appears that there are two anode isoenzymes (A-isoenzymes), and two cathode isoenzymes (C-isoenzymes). The distribution of tyrosine aminotransferase activity in the limits of A- and C-isoenzymes,

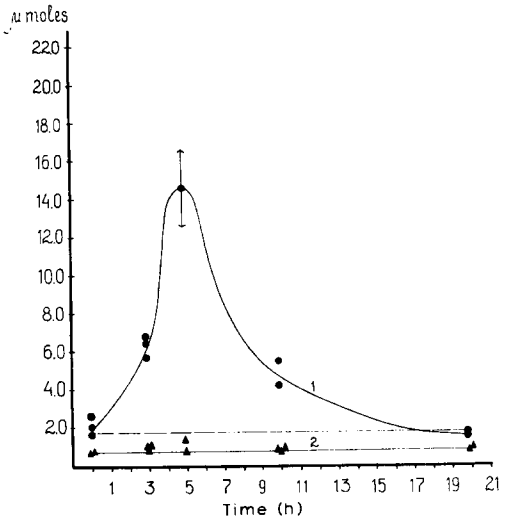

Fig. 1. The activity of tyrosine aminotransferase isoenzymes in rat liver after cortisol injection. 1, A-isoenzymes activity; 2, C-isoenzymes activity; – – –, A-isoenzymes activity in untreated control.

TABLE I

EFFECTS OF CORTISOL, ADRENALECTOMY AND LIVER REGENERATION ON THE ACTIVITY OF TYROSINE AMINOTRANSFERASE ISOENZYMES IN RAT LIVER

10 mg protein of supernatant were applied at the start in electrophoresis. Activity of tyrosine aminotransferase in supernatant of rat liver: control, 0.43 ± 0.05 μmoles/mg protein for 1 h of incubation at 37 °C; adrenalectomy, 0.28 ± 0.01 μmoles/mg; cortisol, 5 h, 0.97 ± 0.08 μmoles/mg; regeneration after partial hepatectomy, 24 h, 0.79 ± 0.06 μmoles/mg. In the experimental conditions used 70%, on average, of the activity of tyrosine aminotransferase applied for electrophoresis was detected by the enol–borate method.

Experimental conditions	*Number of animals*	*Tyrosine aminotransferase activity (μmoles p-hydroxyphenylpyruvate during 1 h of incubation at 37 °C)*	
		A-isoenzymes	*C-isoenzymes*
Control	4	2.23 (± 0.15)	0.78 (± 0.04)
Adrenalectomy	4	1.26 (± 0.12)	0.73 (± 0.03)
Cortisol, 5 h after injection	4	5.74 (± 0.17)	0.84 (± 0.07)
Regeneration after partial hepatectomy, 24 h	4	4.68 (± 0.10)	0.83 (± 0.08)

determined by scanning of the blocks after the histochemical reaction, coincides with that for enol–borate (Fig. 3).

It is known that 5 h after a single cortisol administration, the general tyrosine aminotransferase activity in rat liver reaches a maximum, but after 15–20 h, when the hormone is eliminated, the tyrosine aminotransferase activity falls back to its initial level[13]. Our experiments have shown that cortisol injection in liver stimulates a significant rise only in A-isoenzymes activity (Fig. 1, Table I). Thus, 3 h after hor-

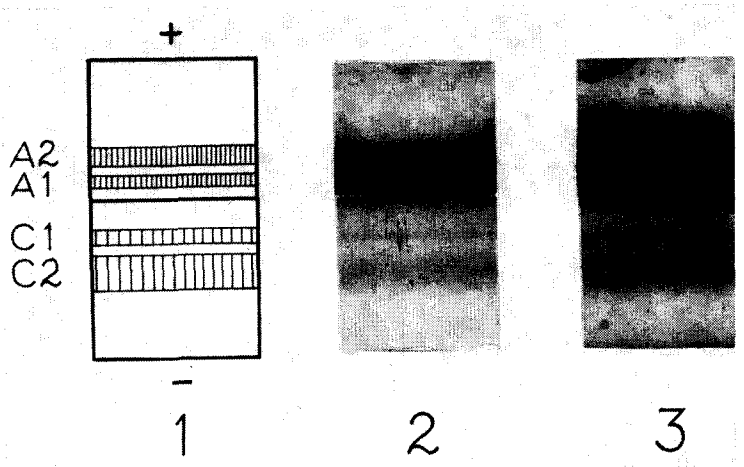

Fig. 2. Electrophoregrams of tyrosine aminotransferase isoenzymes in rat liver. 1, scheme; 2, control; 3, cortisol, 5 h. A1, A2, isoenzymes of A group. C1, C2, isoenzymes of C group.

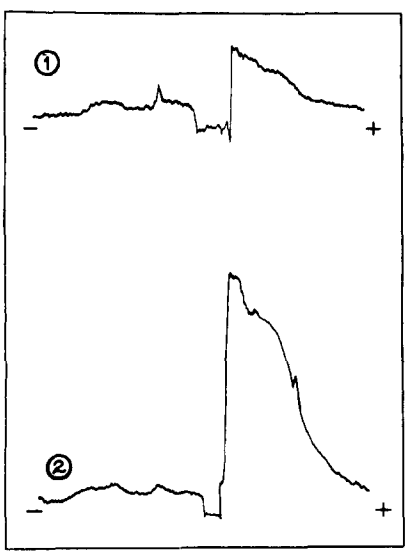

Fig. 3. Densitograms of tyrosine aminotransferase isoenzymes in rat liver. 1, control; 2, cortisol 5h.

mone administration, the activity of A-isoenzymes was three times as high as the initial activity and 5 h later almost ten times higher than the initial level. Results presented in Fig. 1 and Table I were obtained with different periods of time; this fact may explain the difference in the degree of increase of tyrosine aminotransferase activity under cortisol treatment in these two sets of experiments. But 10 h after cortisol injection, the activity of A-isoenzymes was only three times as high as the background and 20 h after injection it came back to the initial level. At this time, cortisol administration does not change the activity of the C-isoenzymes. Similar

results were obtained when tyrosine aminotransferase isoenzymes were revealed on phoregrams by histochemical reactions (Figs 2–3). 5 h after cortisol injection the intensity of staining of A-isoenzymes bands sharply increases, while that of C-isoenzymes remains unchanged.

Adrenalectomy results in some decrease of A-isoenzymes activity as compared with the normal level (Table I). The synthesis and activity of tyrosine aminotransferase A-isoenzymes seem to be selectively regulated by glucocorticoids.

It is known that the rise of tyrosine aminotransferase activity in rat liver after cortisol injection is a result of the synthesis of the enzyme mediated by DNA-dependent synthesis of RNA[1–2]. It may be supposed that the synthesis of tyrosine aminotransferase A-isoenzymes is programmed by certain cistrons in a liver cell genome, and their transcription is controlled by cortisol. Cistrons responsible for C-isoenzymes synthesis seem to maintain "the constitutive" level of tyrosine aminotransferase in liver and do not respond to cortisol injection.

It has been shown previously that tyrosine aminotransferase induction takes place in regenerating rat liver and, obviously, the glucocorticoids are not responsible for the induction since the effect is not abolished in adrenalectomized rats[5], suggesting the emergence of a special inductive "regenerating factor".

Our aim was to clarify whether during liver regeneration and, under cortisol action, the same tyrosine aminotransferase isoenzymes are induced. The experiments have shown that in regenerating rat liver 24 h after hepatectomy, when the substantial rise of tyrosine aminotransferase activity takes place, only an increase of A-isoenzymes can be seen and the activity of C-isoenzymes does not change. These data suggest that both cortisol and "regenerating factor" induce the same cistrons programming A-isoenzymes of tyrosine aminotransferase.

REFERENCES

1 Lin, E. C. C. and Knox, W. E. (1957) *Biochim. Biophys. Acta* 26, 85
2 Kenney, F. T., Wicks, W. D. and Greenman, D. L. (1965) *J. Cell Comp. Physiol.* 66, Suppl. 1, 125
3 Kenney, F. T. and Kull, F. (1963) *Proc. Natl. Acad. Sci. U.S.* 50, 493
4 Greengard, O. and Smith, M. A. (1963) *J. Biol. Chem.* 238, 1548
5 Mertvetsov, N. P., Salganik, R. I. and Argutinskaya, S. V. (1966) *Genetika (Moscow)* 1, 89
6 Levin, Ph. B. (1966) *DAN USSR* 168, 1422
7 Mertvetsov, N. P. and Saprikin, V. A. (1971) *Labor. delo* 1, 18
8 Holt, P. G. and Oliver, L. T. (1969) *FEBS Lett.* 5, 89
9 Blake, R. L. (1970) *Biochem. Genetics* 4, 215
10 Higgins, G. M. and Anderson, R. M. (1931) *Arch. Pathol.* 12, 186
11 Kalckar, H. M. (1947) *J. Biol. Chem.* 167, 461
12 Lin, E. C. C., Pitt, B. M., Civen, M. and Knox, W. E. (1958) *J. Biol. Chem.* 233, 668
13 Rosen, F. and Nichol, Ch. (1963) *J. Biol. Chem.* 238, 3725