MULTIPLE FORMS OF TYROSINE AMINOTRANSFERASE IN RAT LIVER AND THEIR HORMONAL INDUCTION IN THE NEONATE

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1. Introduction

Since the discovery by Lin and Knox [1] that tyrosine aminotransferase (L-tyrosine 2-oxoglutarate aminotransferase, EC 2.6.1.5) is inducible by hydrocortisone in adult rat liver, pyridoxine [2] insulin [3], glucagon [3,4], adrenalin and 3′,5′-cyclic AMP [5] have also been shown to increase enzyme activity in intact animals, perfused liver and liver explants in organ culture. Since the effects of some of these compounds are prevented by actinomycin D, induction of the enzyme at the genome level has been suggested. Hager and Kenney [6] and Wicks [5] have suggested multiple mechanisms for the induction of this enzyme in explanation of the diversity of inducing agents. Holt and Oliver [7,8] have reported a number of observations on tyrosine aminotransferase synthesis in fetal and postnatal rats which are inconsistent with a unitary mechanism of induction and it was further suggested [8] that multiple forms of the enzyme with distinct and specific inductive mechanisms might better explain the apparent complexity of the system. Data presented in this paper confirm the existence of multiple forms of tyrosine aminotransferase activity and indicate differential specificity of various inducers for the various forms. Miller and Litwack [9] have also indicated the existence of at least two forms of soluble tyrosine aminotransferase in rat liver using monoiodotyrosine and tyrosine as substrates.

2. Experimental

Rats of the Wistar albino strain of *Rattus norwegicus* were used. Animals were two days old and approx. 7 g weight unless otherwise specified. Litter mate animals were divided into two groups and each group (4–5 animals) was injected with inducers as given in the results. Injections were made intraperitoneally using a micrometer syringe. A total volume of 50 μl was given to each animal regardless of the number of compounds being administered. The compounds used in induction experiments were injected either alone or in various combinations at the following doses per animal: Adrenalin 15 μg, hydrocortisone hemisuccinate 1.0 mg, glucagon 0.1 mg, insulin 4.0 milliunits, pyridoxin hydrochloride 7.5 mg, N^6-2′-*O*-dibutyryl 3′,5′-cyclic AMP 0.2 mg and actinomycin D 5.0 μg. After injection, animals were maintained in a humidicrib at 37° for 4 hr and then killed. All experiments were begun at 9 a.m.

Livers from each group were pooled and homogenized in 0.25 M sucrose (1 ml/g liver). The homogenates were centrifuged at 25,000 × *g* for 30 min at 4° and the supernatants removed for electrophoresis in polyacrylamide gel. Gels measuring 9 × 3 × 1/10 in. were polymerized between glass plates from the following mixture: 200 ml of water containing 60 g acrylamide and 1.8 g methylene-bis-acrylamide, 200 ml of buffer containing 0.6 ml *N-N-N′-N′*-tetramethyl ethylenediamine and 400 ml water containing 1.1 g ammonium persulphate. The buffer solution (pH 8.6) contained Tris (0.093 M), disodium EDTA (0.26 mM) and boric acid (5.8 mM) and was also used in the electrode vessels. A divider made from rubber tubing was used at the top surface of the gel to enable liver extracts from animals treated in different ways to be compared in the same gel slab. Distilled water was added to the gel surface and 1.0 ml of each liver supernatant layered beneath it.

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Electrophoresis at 100 mA and approx. 70 V through the gel was started with the cathode wick in the water layer and anode in the lower buffer. After 10 min, during which time a band of hemoglobin enters the gel, the residual enzyme was washed off the gel surface, the divider removed and buffer added. Electrophoresis was then resumed for 2–3 hr and the gels immediately cut into 2 mm strips perpendicular to the direction of electrophoresis with a motor-driven multiblade cutter. Each strip was cut into small pieces for determination of transaminase activity.

Incubation of the gel sections was carried out with shaking at 37° in 2.0 ml of the assay media of Sereni et al. [10]; the reaction was started by addition of α-oxoglutarate. The product, *p*-hydroxyphenyl pyruvate, was detectable within 15 min and production was linear throughout the incubation period 20 to 60 min. The reaction was stopped after 60 min by icing the tubes and immediate addition of 2 drops of 6N H_2SO_4.

The reaction mixture was extracted twice with 5 ml ethylether, the combined ether extracts were washed with 2 ml of 0.05 M H_2SO_4 and then extracted with 3 ml of 0.1 N NaOH. Alkaline extraction removes the *p*-hydroxyphenyl pyruvate which then undergoes oxidation to generate a chromophore with maximum absorbance at 331 mμ. Absorbancies were determined 15 min after alkaline extraction of the ether solution and referred to a standard curve obtained with synthetic *p*-hydroxyphenyl pyruvate treated with 0.1 M NaOH. The linear range of the procedure was 1 to 60 μg of *p*-hydroxyphenyl pyruvate.

3. Results and discussion

Tyrosine aminotransferase activity was found to occur only in 10–13 gel sections anodal to the hemoglobin band which was used as an internal marker. Attempts to stain for the enzyme by the method of Hayashi et al. [11] lead to the appearance of bands cathodal to the hemoglobin marker which appeared even in the absence of tyrosine.

Two-day old rats in our colony have very low activities of liver tyrosine aminotransferase but increased activity is induced by injection of pyridoxine, insulin, 3′,5′-cyclic AMP and adrenaline [8]. Enzyme activity

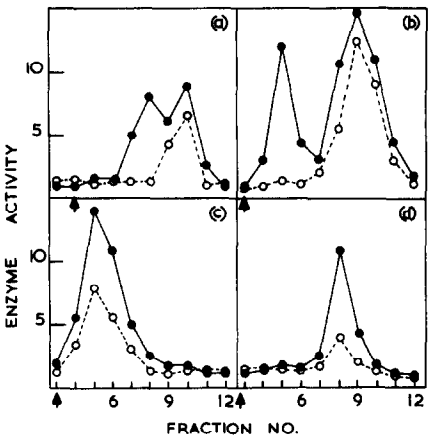

Fig. 1. Polyacrylamide gel electrophoresis of liver extracts from 2-day old rats after injection with the following compounds at the doses given in the experimental section: (a) •—• hydrocortisone + adrenalin, ○---○ adrenaline; (b) •—• pyridoxine + adrenalin, ○---○ adrenalin; (c) •—• pyridoxine + insulin, ○---○ insulin; (d) •—• adrenalin + N^6-2′-*O*-dibutyryl 3′,5′-cyclic AMP, ○---○ N^6-2′-*O*-dibutyryl 3′,5′-cyclic AMP. Enzyme activities are μg of *p*-hydroxyphenyl pyruvate per hour. Haemoglobin is indicated by the arrow.

can rarely be demonstrated in gels run with liver extracts from untreated animals.

Fig. 1 shows that treatment with the various inducers produces markedly different enzyme profiles in polyacrylamide gels. Using hemoglobin as a marker, fig. 1a shows that a fast peak of activity is induced by adrenalin while two peaks appear after the adrenalin-hydrocortisone mixture. Two peaks are also produced by a mixture of adrenalin and pyridoxin (fig. 1b) while a single slow-moving peak is produced by pyridoxin either alone or in combination with insulin (fig. 1c). A single fast-moving peak is also produced by dibutyryl 3′,5′-cyclic AMP which appears identical with the adrenalin peak (fig. 1d).

Fig. 2a shows that simultaneous treatment with pyridoxin, hydrocortisone and adrenalin gives rise to 3 components and that the omission of hydrocortisone causes deletion of the intermediate peak.

High doses of glucagon induce both the intermediate and fast forms of the enzyme and the intermediate peak is more sensitive to actinomycin D (fig. 2b). Induction of tyrosine aminotransferase by cyclic AMP and adrenalin has previously been shown to be insensitive to actinomycin D [8].

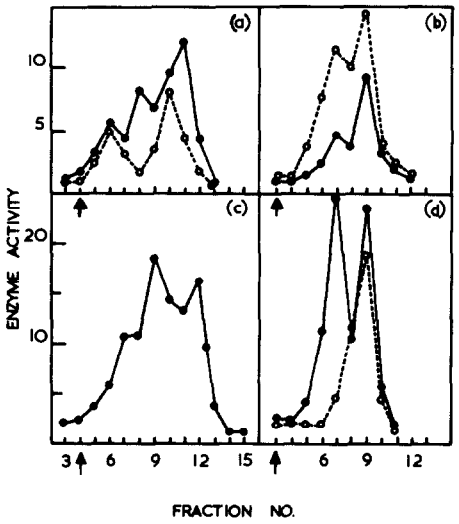

Fig. 2. Polyacrylamide gel electrophoresis of liver extracts from 2-day old rats after injection with the following compounds at the doses shown in the experimental section: (a) ●—● pyridoxine + hydrocortisone + adrenalin, ○---○ pyridoxine + adrenalin; (b) ●—● glucagon + actinomycin D, ○---○ glucagon; (c) animals were normal well-fed adults; (d) ●—● hydrocortisone + adrenalin, ○---○ adrenalin. Enzyme activities are μg *p*-hydroxyphenyl pyruvate per hour. Haemoglobin is indicated by the arrow.

The electrophoretic profile from the liver of a normal well-fed adult rat is shown in fig. 2c and each of the forms demonstrated in the neonatal rat in the previous figures can be recognized. There is a slight difference in electrophoretic mobility probably due to slight differences in the gel porosity and electrophoretic conditions.

The enzyme profile obtained with hydrocortisone is compared with that from adrenalin treatment in fig. 2d and both intermediate and fast forms are present. The duality of effects of hydrocortisone and glucagon may be due to a reciprocal stimulation of endogenous release of each hormone by the other, at the high dose-rates used in the intact neonatal animal, and further experiments to resolve this question are in progress.

All of the electrophoretic patterns have been obtained in 3–5 replicated experiments. Since comparisons of each paired hormonal experiment were made in the same gel slab it is unlikely that the enzyme profiles are artefact and the deletion experiments of figs. 1b and 2a make such a possibility even less likely.

Zonal ultracentrifugation of liver extracts from animals treated with the triple inducer mixture demonstrates a single sedimentating peak of transaminase activity in sucrose gradients containing the electrophoretic buffer. Polymerized forms of the enzyme are thus unlikely to be responsible for the electrophoretic patterns.

Previous immunological evidence for a single form of tyrosine aminotransferase, largely obtained with hydrocortisone induced enzyme [12,13] may now require re-examination. The apparent diversity of inducing agents for the enzyme might now be explained since the results above (see also [9]) indicate the existence of multiple forms of tyrosine aminotransferase, each of which may be under the inductive control of a different hormone.

Acknowledgements

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References

[1] E.C.C.Lin and W.E.Knox, Biochem. Biophys. Acta 26 (1957) 85.
[2] D.Holten, W.D.Wicks and F.T.Kenney, J. Biol. Chem. 242 (1967) 1053.
[3] D.Holten and F.T.Kenney, J. Biol. Chem. 242 (1967) 4372.
[4] O.Greengard and H.K.Dewey, J. Biol. Chem. 242 (1967) 2986.
[5] W.D.Wicks, Science 160 (1968) 997.
[6] C.B.Hager and F.T.Kenney, J. Biol. Chem. 243 (1968) 3296.
[7] P.G.Holt and I.T.Oliver, Biochem. J. 108 (1968) 333.
[8] P.G.Holt and I.T.Oliver, Biochemistry 8 (1969) 1429.
[9] J.E.Miller and G.Litwack, Fed. Proc. 28 (1969) 667.
[10] F.Sereni, F.T.Kenney and N.Kretchmer, J. Biol. Chem. 234 (1959) 609.
[11] S.Hayashi, D.K.Granner and G.M.Tomkins, J. Biol. Chem. 242 (1967) 3998.
[12] F.T.Kenney, J. Biol. Chem. 237 (1962) 1610.
[13] D.K.Granner, S.Hayashi, E.B.Thompson and G.M.Tomkins, J. Mol. Biol. 35 (1968) 291.