EVIDENCE FOR A NEW FORM OF ENOLASE IN RAT BRAIN

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SUMMARY. Rat brain enolase (2-phospho-D-glycerate hydrolyase, EC 4.2.1.11) is unaffected by an antiserum raised against rat muscle enolase (isoenzyme 3) and an antiserum raised against rat liver enolase (isoenzyme 1) affects only 70% of the total brain activity. By gradient elution of QAE-Sephadex at pH 8.5, brain enolase is separated into two major peaks. The first is chromatographically and immunochemically identical with isoenzyme 1 whilst the second more complex peak is apparently specific for brain. Gel filtration chromatography on G-150 Sephadex shows no significant difference in molecular weight between these two components.

INTRODUCTION

We have previously reported (1,2) electrophoretic, chromatographic and immunochemical evidence for the existence in rat tissues of three isoenzymes of enolase (2-phospho-D-glycerate hydrolyase, EC 4.2.1.11). Liver contains only enolase 1, whereas enolase 3 is the sole form found in skeletal muscle. Heart contains both these isoenzymes and in addition the hybrid, enolase 2. Further studies (2) indicate that these isoenzymes probably arise from two independent genetic loci, α and β, thus isoenzymes 1, 2 and 3 are dimers of two subunit types, $\alpha\alpha$, $\alpha\beta$ and $\beta\beta$ respectively. In the present paper we report that there is a component of rat brain enolase which is chromatographically and immunochemically distinct from the dimers of α and β subunits found in other tissues.

MATERIALS AND METHODS

Enolase activity assays were performed at 37°C on an LKB 8600 Reaction Rate Analyzer using the coupled assay system previously described (1) containing 67 mM KCl, 7.7 mM $MgSO_4$, 3.2 mM EDTA, 1.8 mM ADP, 0.16 mM NADH, 38 mM triethanolamine buffer (pH 7.5), 2 IU pyruvate kinase, 5 IU lactate dehydrogenase and 1 mM 2-phosphoglycerate in a final volume of 1.5 ml. Enzyme activities

are expressed in international units, IU (1 IU = amount of enzyme catalysing conversion of 1 μmole of substrate in 1 min at 37°C).

Immunological tests were carried out in tapered micro-test tubes (0.5 ml capacity, Beckman-RIIC, Croydon, U.K.) using antisera raised against partially purified rat liver and muscle enolases (isoenzymes 1 and 3 respectively) as previously described (1). Standard volumes, typically 20 μl, of crude tissue supernatants containing known activities of enolase were mixed with appropriate volumes of chicken serum, either anti-enolase serum, or control serum taken from an uninjected chicken. After standing for 15 minutes at room temperature the tubes were centrifuged for 15 min at 20,000 g and 5°C using polypropylene adaptors made to fit the 6 x 7 ml head of an MSE Mistral 6L centrifuge (MSE, London, U.K.)

Analytical ion-exchange chromatography was performed at 5°C on 1.5 x 25 cm columns of QAE-Sephadex A50 (Pharmacia, Uppsala, Sweden) equilibrated with 12.5 mM sodium diethylbarbiturate-HCl buffer, pH 8.5, containing 5 mM $MgSO_4$ and 0.1 mM EDTA. Tissue samples were homogenized in 4 volumes of this buffer and centrifuged at 12,500 g for 40 min at 5°C. The high speed supernatants were dialysed against 50 volumes of buffer for 2 hours at 5°C prior to application to the columns. After running through approximately 2 bed volumes of buffer, the columns were eluted with a linear concentration gradient of buffer containing in addition 0 - 0.6 M NaCl.

Molecular weight estimation was performed by the method of Andrews (3) on a 5 x 77 cm (1500 ml) G-150 Sephadex column as described previously (1). 10 ml fractions were collected.

RESULTS

Table 1 shows the effect of the anti-muscle and anti-liver enolase sera on the enolase activity in crude extracts of rat tissues. Except for adult brain and heart and foetal muscle total tissue activity is 95% susceptible to one or other of the antisera. In the case of adult heart and foetal muscle both antisera cross-react, and there is an apparent overlap of immunological

	% enolase activity susceptible to anti-muscle enolase serum	% enolase activity susceptible to anti-liver enolase serum
Adult Tissues		
Muscle	95	0
Liver	0	95
Heart	45	90
Spleen	0	95
Kidney	0	95
Testis	0	95
Brain	0	70
Foetal Tissues		
Muscle	50	80
Liver	0	95
Heart	0	95
Kidney	0	95
Brain	0	95

TABLE 1. EFFECT OF ANTI-ENOLASE SERA ON THE ENOLASE ACTIVITIES IN CRUDE EXTRACTS OF VARIOUS RAT TISSUES. All tissues were homogenized 1:9, except muscle 1:19, in 20mM Tris HCl buffer, pH 7.0. Samples of high speed supernatants, were tested with antisera as described in the Methods Section, using in each case at least a five-fold excess of antisera titre. The enolase activity remaining in the presence of the antisera is expressed as a percentage of that obtained with the same volume of control serum. Each percentage is the mean of at least duplicate determinations, and is expressed to the nearest 5%.

susceptibility indicating the presence of enolase 2, which being comprised of both α and β subunits is capable of reacting with both antisera. Only in adult brain is the sum of the immunological susceptibilities less than 95%. It would therefore appear that 30% of the total enolase activity of rat brain is unaffected by antisera directed against enolase α and β subunits.

This is further shown in Fig. 1. Increasing titres of anti-muscle eno-

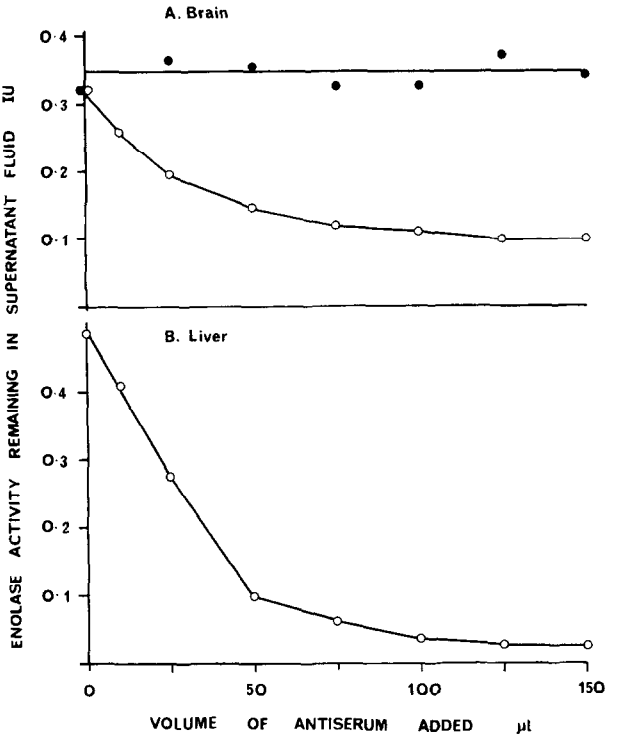

Fig. 1. Antibody titration curves for enolase activity in crude extracts of A, brain, and B, liver tested as described in the Methods Section with antisera raised in chicken against rat muscle (●) and rat liver (o) enolases. Throughout 20 µl of a high speed supernatant obtained from tissue homogenized in four volumes of 15 mM sodium phosphate buffer, pH 7.0, containing 5 mM $MgSO_4$ and 0.1 mM EDTA, was added to varying volumes of antiserum, and in each case the total serum volume raised to 150 µl by addition of control serum. The same batch of anti-liver enolase serum was used in A and B.

lase serum have no effect on the brain enolase activity, demonstrating the absence of β enolase subunits. The anti-liver enolase serum causes a considerable reduction in activity, however this activity loss reaches a maximum of 70%, compared with a maximum loss of 95% for liver enolase titrated against the same batch of anti-liver enolase serum (Fig. 1B). Thus although the presence of a substantial proportion of enolase α subunits in brain is indicated, the anti-liver enolase serum is not as effective against brain enolase as it is against the enolase activity of a tissue known to contain enolase α subunits exclusively.

Fig. 2 shows the chromatographic profiles of the enolase activities from crude extracts of rat brain and heart. The activity in heart is resolved into three peaks, A, B and C, which possess the characteristic chromatographic and immunochemical (Table 2) properties of enolases 3, 2 and 1 respectively (2). Brain enolase is resolved into a sharp peak, D, at 6 mmhos and a complex broad peak eluting between 25 and 55 mmhos. From its complete susceptibility to only the anti-liver enolase serum (Table 2) and its chromatographic elution,

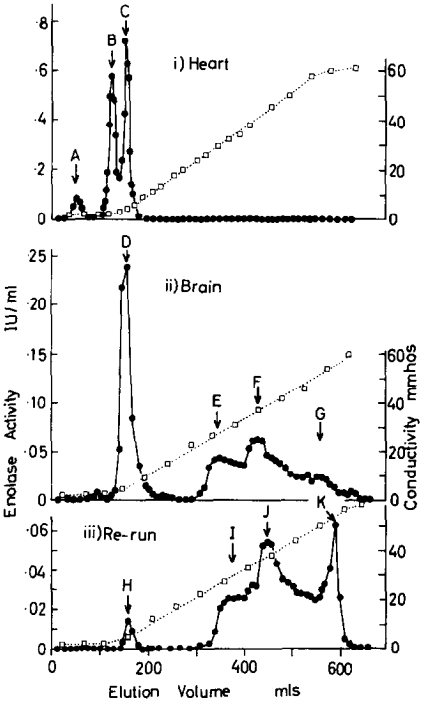

Fig. 2. Elution profiles for the enolase activity of crude tissue extracts from QAE-Sephadex. The columns, 45 ml in bed volume, were eluted with 12.5 mM sodium diethylbarbiturate-HCl buffer, pH 8.5, containing 5 mM $MgSO_4$ and 0.1 mM EDTA (conductivity 2 mmhos) prior to the application of linear gradients of buffer containing in addition increasing concentrations of NaCl up to 0.6 M (65 mmhos). i) and ii) were obtained using 2 ml of crude heart and brain extracts respectively. For the re-run, iii), the entire second peak E - G of a chromatographic run similar to that shown in ii) was dialysed against the starting buffer and applied to an identical column. In each case at least 90% of the initial enolase activity was recovered. The immunochemical characteristics of the applied samples, and various fractions indicated below, are given in Table 2. •, enolase activity, ◘ , conductivity.

	Percentage of enolase activity lost	
	Anti-muscle enolase serum	Anti-liver enolase serum
Heart		
Original sample	55	75
Peak A	85	12
Peak B	90	70
Peak C	3	97
Brain		
Original sample	5	55
Peak D	0	95
Peak E	0	45
Peak F	5	10
Peak G	5	10
Re-run		
Original sample	3	30
Peak H	0	90
Peak I	0	45
Peak J	5	5
Peak K	0	5

TABLE 2. IMMUNOCHEMICAL CHARACTERISTICS OF CHROMATOGRAPHIC PEAKS OBTAINED ON GRADIENT ELUTION OF RAT BRAIN AND HEART ENOLASE FROM QAE-SEPHADEX. The values shown are the percentages of the enolase activities susceptible to antiserum raised in chicken against partially purified rat muscle and liver enolases. The designation of the chromatographic peaks is that shown in Fig. 2.

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the first peak, D, would appear to be enolase 1. Although the latter peak shows some susceptibility to the anti-liver enolase serum, mostly confined to the initial fractions, and a marginal effect with the anti-muscle enolase

serum, it is largely devoid of immunochemical cross-reaction. A chromatographic re-run of this peak shows only a very small amount eluting as enolase 1, whilst the remainder shows the same elution pattern as before.

The enolase activity in a crude extract of rat brain eluted from a calibrated G-150 Sephadex column as a single, apparently symmetrical peak was not significantly different in elution volume or shape from that obtained with rat muscle enolase, indicating a molecular weight for both enzymes of 90,000.

DISCUSSION

The immunological and chromatographic results described present evidence for further molecular heterogeneity of rat enolase. The new form of enolase, as well as being chromatographically distinct from enolases 1, 2 and 3, is largely incapable of immunochemical cross-reaction with antisera directed against α and β subunits. This immunochemical distinction suggests that the new form differs from enolase 1, 2 and 3 by more than minor post-transcriptional modifications (4). However, the apparently identical molecular weight of brain and enolases 1, 2 and 3 suggests that major alterations in polyptptide chain length cannot have occurred. One possible explanation consistent with this data is that there is a third locus apparently active only in brain producing a third type of subunit, hereby designated γ. The chromatographic profiles of the brain and re-run (Fig.2) could be interpreted as showing the presence of $\alpha\gamma$ hybrids eluting at 20-30 mmhos, although this is not yet certain. Further studies on the nature and physiological significance of the new brain component are now being undertaken. However it is probable that enolase, like aldolase (5), possess a brain specific isoenzyme.

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