Heterogeneity of Rat Brain and Liver Mitochondrial Monoamine Oxidase: Subcellular Fractionation*

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Although biochemical (Johnston, 1968; Neff & Goridis, 1972) and electrophoretic (Youdim et al., 1969, 1970) studies have shown that monoamine oxidase (EC 1.4.3.4) may exist in more than one form, no adequate explanation has been put forward for the basis of multiplicity. The presence of monoamine oxidase 'isoenzymes' in different mitochondria has been suggested by Youdim (1972) and Hanker et al. (1973), and Kroon & Veldstra (1972) have partially separated subcellular mitochondrial fractions possessing various monoamine oxidase activities. Further, phospholipids have been implicated not only to play an important role in the activity but also in the heterogeneity of this enzyme (Tipton et al., 1972).

The different fractions of mitochondria separated by zonal centrifugation have different enzyme activities with regard to NADH dehydrogenase, succinate dehydrogenase and α-glycerophosphate dehydrogenase (Wilson & Cascarano, 1972). An attempt was therefore made in the present investigation to demonstrate biochemical differences in the liver and the brain mitochondrial monoamine oxidase as separated by zonal centrifugation. It was considered essential to maintain the prepared (Gray & Whittaker, 1962) brain and liver mitochondria in their physiological state, and to avoid artifacts that may influence the enzyme activity: therefore an iso-osmotic gradient of Ficoll–sucrose was used according to the method of Wilson & Cascarano (1972) to prevent changes caused by centrifugation. Fractionation of the brain and liver mitochondria were carried out in the zonal rotor of a MSE 65 Mk. II ultracentrifuge. After the run 40 fractions of volume 15 ml were collected and stored at −20°C for later assay of monoamine oxidase activity. The brain mitochondrial protein distribution (mg/ml) on the Ficoll–sucrose gradient was determined by the method of Lowry et al. (1951) and showed a single broad band; the results from three zonal centrifugation runs were very similar, spanning 34 fractions and reaching a maximum in fraction 21, with a shoulder at fraction 26.

Kynuramine (Kraml, 1965), [14C]benzylamine, [14C]dopamine, [14C]tryptamine and [14C]tyramine (Robinson et al., 1968) were used as substrates, and the monoamine oxidase activity exhibited significant differences in pattern of distribution for each substrate. Almost 85% of the original activity for kynuramine was recovered. When the activities were expressed as percentages of the highest specific activity the results showed the following: kynuramine monoamine oxidase activity remained fairly constant in all fractions (70–80%), and benzylamine monoamine oxidase activity was 90% in fraction 21 and 50% in fraction 32. A comparison with tyramine monoamine oxidase showed that in earlier fractions benzylamine and tyramine monoamine oxidase have similar activity; however, in later fractions (e.g. 32) there is twice as much activity for tyramine. The activity of dopamine monoamine oxidase showed three peaks and increased from 60% in fraction 8 to 93% in fraction 30. In this fraction there is a greater activity with dopamine than with tyramine. A very similar distribution of enzyme activity was observed with the liver mitochondrial preparation. Several attempts have been made to separate different forms of mitochondrial monoamine oxidase. These have included separation of different forms not only by gel electrophoresis of the soluble enzyme (Youdim et al., 1969) but also indirectly by the effect of several inhibitors on the activity of mitochondrial enzyme from a number of sources (Neff & Goridis, 1972). There have been a number of objections to results indicating the existence of different forms of monoamine oxidase, including the suggestion that they are caused by artifacts of the solubilization and purification procedure (Collins, 1972). In the light of criticism of previous reports special care was taken in the present investigation to ensure that the

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mitochondria were not altered or damaged. To maintain the mitochondria in their physiological state, the gradient consisted of iso-osmotic sucrose with Ficoll added to increase the density. Ficoll is a synthetic polymer of sucrose with negligible osmotic effect, since it has a molecular weight of approx. 400000. The observed differences in amine oxidase activities may reflect the fact that the brain and liver mitochondria are not completely homogeneous. It is possible that the heterogeneity obtained may result from microsomal contamination or fragmentation of mitochondria, but this is ruled out because no extramitochondrial monoamine oxidase was observed by electron-microscope studies (Boadle & Bloom, 1969). Other enzymes, including NADH dehydrogenase and succinate dehydrogenase, have been shown to have different distributions in mitochondria (Wilson & Cascarano, 1972) by rate-zonal centrifugation and histochemical studies.

The results of the present investigation demonstrate that both liver and brain mitochondria are heterogeneous with regard to monoamine oxidase; further, one can conclude that in rat brain and liver there is a monoamine oxidase that may preferentially deaminate dopamine and that is different from the tyramine-deaminating system. Kroon & Veldstra (1972) have presented strong evidence for the presence of a 'dopamine monoamine oxidase' in the synaptosomes that are different from noradrenaline-deaminating synaptosomes. The present studies provide further evidence to support the view that multiple forms of monoamine oxidase have a physiological function, and that deamination of non-methylated biogenic monoamines can take place in a definite type of neuron. The re-uptake mechanism has been thought to be acting as a primary system of defence against amine released at the receptor site (Iversen, 1967). However, with the present knowledge of the multiple forms of monoamine oxidase and their different substrate specificities, the nature of the back-up offered by monoamine oxidase obviously needs re-evaluation.


Isoenzymes of Enolase in Rat Tissues

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Despite much interest during the last decade in the heterogeneity of the glycolytic enzymes (Criss, 1971), little attention has been paid to the enzyme enolase (phosphoenolpyruvate hydratase; EC 4.2.1.11). An investigation was therefore undertaken of the electrophoretic, chromatographic and immunological properties of enolase extracted from rat tissues.

Cellulose acetate electrophoresis was carried out at pH 8.6 on supernatant fluids from