The Distribution of Monoamine Oxidase and α -Glycerophosphate Dehydrogenase in Pig Brain

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Activities of the enzymes monoamine oxidase (EC 1.4.3.4), α -glycerophosphate dehydrogenase (EC 1.1.99.5) and cytochrome oxidase (EC 1.9.3.1) were determined in homogenates and in the mitochondrial fraction prepared from individual regions of pig brain. The variation in the activity of α -glycerophosphate dehydrogenase paralleled that of cytochrome oxidase, but this was not the case with monoamine oxidase. The differences in the activities of the enzymes among homogenates of the various regions of the brain persisted in mitochondria prepared from these homogenates. The purification of these three enzymes paralleled each other when mitochondria were prepared, suggesting that the three enzymes are bound to the same particles.

Studies on the subcellular distribution of monoamine oxidase (EC 1.4.3.4) have shown that the enzyme is largely associated with the mitochondrial fraction of both liver (Cotzias & Dole, 1951; Baudhuin *et al.* 1963; Oswald & Strittmatter, 1963) and brain cells (Arnaiz & De Robertis, 1962; Marchbanks, 1966). The distribution of this enzyme in various regions of the brain has been studied in man (Bülbring, Philpot & Bosanquet, 1953; Birkhäuser, 1940), pigeon (Aprison, Takahashi & Folkerth, 1964), dog (Bogdanski, Weissbach & Udenfriend, 1957; Weiner, 1960) and cow (Weiner, 1960). These studies have shown that, though the enzyme activity does vary among individual regions of the brain, the degree of variation is usually small, being much less than that found for some other enzymes. Birkhäuser (1940), in a parallel study on the distribution of monoamine oxidase and cholinesterase in dog and cow brain, showed that cholinesterase activity varied over 35-fold among the various regions whereas that of monoamine oxidase varied less than threefold.

One of the α -glycerophosphate dehydrogenase enzymes (EC 1.1.99.5) has been shown to be mitochondrial in origin and to have particularly high activity in brain tissue (Green, 1936; Sacktor, Packer & Estabrook, 1959), but little work has been carried out on the distribution of this enzyme in the various regions of the brain. Histochemical studies on rabbit (Kul'tas, 1965), rat (Hess & Pearse, 1961) and guinea pig (Felgenhauer & Stammeler, 1962) have shown it to be widely dis-

tributed in different types of nerve cells and have indicated that its distribution roughly parallels that of cytochrome oxidase and succinate dehydrogenase. In view of the observation that succinate dehydrogenase activity tends to parallel that of cytochrome oxidase in a variety of tissues whereas α -glycerophosphate dehydrogenase activity does not (Pette, Klingenberg & Bücher, 1962), it was decided to test whether the α -glycerophosphate dehydrogenase/cytochrome oxidase activity ratio was constant in different regions of the brain. Pig brain was chosen for this study because it has been shown to be a rich source of α -glycerophosphate dehydrogenase (Ringler, 1961) as well as containing monoamine oxidase (Castelli & De Guiseppe, 1955), and our own studies (Tipton, 1968; A. P. Dawson, unpublished work) on the purification of α -glycerophosphate dehydrogenase and monoamine oxidase used pig brain as the starting material. In the present paper we report the distribution of these two enzymes both in homogenates and in mitochondria prepared from individual regions of pig brain.

MATERIALS AND METHODS

Preparation of mitochondria. Pig brains were obtained from an abattoir within 15 min. of the animals being killed and were kept in ice until dissection (approx. 60 min.). The brains were dissected at 5° and the portions weighed and homogenized by hand in 9 vol. (v/w) of 0.25 M-sucrose adjusted to pH 7.6 with 1.0 M-K₂HPO₄. The brain regions were homogenized in a Potter-Elvehjem homogenizer with an all-nylon pestle. Samples of the homogenates were removed and stored at -10° until assayed. Mitochondria

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were prepared from the remainder of the homogenates by a method that was essentially that of Brody & Bain (1952) (method I). The homogenate was centrifuged at 1500g for 20 min. at 4° and the supernatant was carefully decanted and centrifuged at 18000g for 20 min. at 4°. The resulting residue was resuspended in half the original volume of 0.25 M-sucrose-K₂HPO₄ medium, pH 7.6, and again centrifuged at 18000g for 20 min. at 4°. The residues were taken up in 0.02 M-sodium phosphate buffer, pH 7.6, and stored frozen until assayed. Mitochondria were prepared from the homogenates of all the brain regions except for the corpus callosum, where it was found that insufficient material could be dissected from a single brain to give a useful yield of mitochondria.

Enzyme assays. Enzyme activities were determined by measuring oxygen uptake with a Clark oxygen electrode (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio, U.S.A.) connected via a voltage divider to a Honeywell-Brown 1mv strip-chart recorder. The apparatus was similar to that described by Dixon & Kleppe (1965). All assays were carried out at 30° and the apparatus was standardized with distilled water saturated with air at 30°. Proportionality of reaction velocity and enzyme concentration was verified in each case. For convenience of comparison, the activities of all the enzymes are expressed as $\mu\text{g. atoms of oxygen consumed/min./mg. of protein}$. The activities are given as means $\pm$ S.E.M. Six samples were used in all cases unless otherwise stated.

(a) Monoamine oxidase. The oxidation of tyramine was followed by measuring the oxygen consumption by the manometric method of Creasey (1956), adapted for use with the oxygen electrode. The assay mixture contained in a total volume of 2.4 ml.: 200 μmoles of sodium phosphate buffer, pH 7.0, 50 units of catalase, 20 μmoles of semicarbazide, 2 μmoles of KCN and enzyme. The mixture was equilibrated with air at 30° and the reaction was started by the addition of 100 $\mu\text{l.}$ of 0.2 M-tyramine hydrochloride. Separate blanks were determined in the absence of enzyme.

(b) α -Glycerophosphate dehydrogenase. The oxidation of glycerophosphate was followed by an adaptation of the method of Ringler & Singer (1959). The assay mixture contained in a total volume of 3 ml.: 150 μmoles of sodium phosphate buffer, pH 7.6, 1.5 mg. of phenazine methosulphate, 3 μmoles of KCN and enzyme. The reaction was started by the addition of 150 μmoles of DL- α -glycerophosphate. The rate was linear after an initial lag period of about 90 sec.

(c) Cytochrome oxidase. The consumption of oxygen

was measured by the manometric method of Tolani & Talwar (1963), adapted for use with the oxygen electrode. The assay mixture contained in a total volume of 2.4 ml.: 200 μmoles of potassium phosphate buffer, pH 7.4, 1.65 μmoles of cytochrome *c* and enzyme. The reaction mixture was equilibrated with air at 30° and the reaction was started by the addition of 100 $\mu\text{l.}$ of 0.6 M-*p*-phenylene-diamine hydrochloride. Separate blanks were determined in the absence of enzyme.

Chemicals. Tris (Trizma base), phenazine methosulphate and glycerol 3-phosphate (46% of the L-isomer) were obtained from Sigma Chemical Co. (London) Ltd. (London, S.W. 6), bovine serum albumin was obtained from Armour Chemicals Ltd., Eastbourne, Sussex.) and Triton X-100 was obtained from Lennig Chemicals Ltd. (Jarrow, Co. Durham). All other chemicals were obtained from British Drug Houses Ltd. (Poole, Dorset) or Hopkin and Williams Ltd. (Chadwell Heath, Essex). The quality of all chemicals was the highest produced by the manufacturers.

General methods. Distilled water was passed through a Permutit mark 11 deionizer before use. Measurements of pH were made with a Radiometer PHM 22r pH-meter. Centrifugation was carried out at 4° in an MSE Major refrigerated centrifuge for low-speed spins and in an MSE Highspeed 18 refrigerated centrifuge for high-speed spins. Measurements of extinction were made in a Unicam SP.500 spectrophotometer with quartz-glass cells of light-path 1 cm. Protein concentrations were determined by the micro-biuret method (Goa, 1953). Each sample was made 0.3% with respect to the detergent Triton X-100 to solubilize the protein. The colour yields were related to a serum albumin standard.

RESULTS

The activities of monoamine oxidase, α -glycerophosphate dehydrogenase and cytochrome oxidase in homogenates prepared from individual regions of pig brain are shown in Table 1. The activities of all three enzymes were lowest in the corpus callosum; and for cytochrome oxidase and α -glycerophosphate dehydrogenase the activity was the highest in the cerebellum, though this was not true for monoamine oxidase.

The variations of the activities of the three enzymes in the mitochondrial fraction obtained

Table 1. *Specific activities of homogenates of individual regions of pig brain*

Activities are expressed as $\mu\text{g. atoms of oxygen consumed/min./mg. of protein}$. Results are expressed as means $\pm$ S.E.M. of six observations unless indicated otherwise (* five observations).

Region	Monoamine oxidase	α -Glycerophosphate dehydrogenase	Cytochrome oxidase
Corpus striatum	0.62 $\pm$ 0.016	17.7 $\pm$ 1.4	54 $\pm$ 3.3
Frontal cortex	0.64 $\pm$ 0.03*	20.2 $\pm$ 1.9*	40 $\pm$ 2.0*
Thalamus	0.57 $\pm$ 0.017	19.7 $\pm$ 1.2	50 $\pm$ 1.7
Cerebellum	0.50 $\pm$ 0.03	30.8 $\pm$ 3.1	59 $\pm$ 2.1
Hypothalamus	0.78 $\pm$ 0.06*	23.8 $\pm$ 1.9	37 $\pm$ 0.5
Medulla and pons	0.57 $\pm$ 0.017	13.9 $\pm$ 1.4	42.5 $\pm$ 5.6
Corpus callosum	0.16 $\pm$ 0.01	8.3 $\pm$ 0.56	11.2 $\pm$ 0.6

Table 2. *Specific activities of mitochondrial fractions prepared from individual regions of pig brain*

Activities are expressed as $\mu\text{g. atoms of oxygen consumed/min./mg. of mitochondrial protein}$. Results are expressed as means $\pm$ s.e.m. of six observations unless indicated otherwise (* five observations; † four observations).

Region	Monoamine oxidase	α -Glycerophosphate dehydrogenase	Cytochrome oxidase
Corpus striatum	2.66 ± 0.19	$120.0 \pm 7.5^\dagger$	335 ± 9.2
Frontal cortex	$2.43 \pm 0.26^*$	$67.9 \pm 3.2^*$	$212 \pm 8.0^*$
Thalamus	3.52 ± 0.07	72.0 ± 9.0	228 ± 10.0
Cerebellum	1.57 ± 0.01	81.7 ± 7.1	170 ± 10.0
Hypothalamus	3.49 ± 0.11	45.0 ± 12.0	94 ± 7.1
Medulla and pons	5.43 ± 0.23	87.0 ± 9.0	329 ± 19.3

Table 3. *Specific activities of enzymes in individual regions of pig brain expressed relative to the specific activity of cytochrome oxidase*

All activities were measured as $\mu\text{g. atoms of oxygen consumed/min./mg. of protein}$ and expressed relative to that of cytochrome oxidase. For convenience the monoamine oxidase/cytochrome oxidase ratios have been multiplied by 100.

Region	Homogenate		Mitochondria	
	Monoamine oxidase	α -Glycerophosphate dehydrogenase	Monoamine oxidase	α -Glycerophosphate dehydrogenase
Corpus striatum	1.13	0.32	0.80	0.37
Frontal cortex	1.6	0.50	1.2	0.32
Thalamus	1.15	0.40	1.46	0.31
Cerebellum	0.86	0.52	0.96	0.48
Hypothalamus	2.1	0.64	3.8	0.48
Medulla and pons	1.35	0.33	1.7	0.26
Corpus callosum	1.48	0.75	—	—

Table 4. *Degree of purification of enzymes in the preparation of mitochondria from homogenates of individual regions of pig brain*

Results are expressed as the ratio of the specific activity in mitochondria to that in the homogenate.

Region	Cytochrome oxidase	Monoamine oxidase	α -Glycerophosphate dehydrogenase
Corpus striatum	6.2	4.3	6.8
Frontal cortex	5.3	3.8	3.4
Thalamus	4.6	6.2	3.7
Cerebellum	2.9	3.1	2.7
Hypothalamus	2.5	4.5	1.9
Medulla and pons	7.8	9.4	6.3

from the brain region homogenates are given in Table 2. Table 2 shows that the differences in the activities of the three enzymes found in homogenates of the various brain fractions persist in the corresponding mitochondrial fractions. The differences observed in the orders of the activities between the various regions in the homogenates and the mitochondria, e.g. the relatively higher activities of all three enzymes in the homogenate compared with those in the mitochondrial fraction,

are apparent rather than real and vanish if one takes into account the purifications achieved with the mitochondria from the various regions, as shown in Table 4.

Table 3 shows the monoamine oxidase/cytochrome oxidase and α -glycerophosphate dehydrogenase/cytochrome oxidase activity ratios for both the homogenates and the mitochondria from the individual regions of pig brain. The mean activities given in Tables 1 and 2 were used in each case. The

monoamine oxidase activities for both the homogenates and the mitochondria are shown to vary independently of the cytochrome oxidase activities, whereas the α -glycerophosphate dehydrogenase/cytochrome oxidase activity ratios appear to be more or less constant, this being particularly striking in the mitochondrial preparations.

The purifications of the three enzymes attending the preparation of mitochondria, calculated by expressing the activity of the enzyme in the mitochondrial preparation as a fraction of that found in the homogenate, are given in Table 4. The mean activities given in Tables 1 and 2 were used in each case. Though the purifications obtained with different starting materials varied widely, the purification factor obtained on each of the three enzymes was roughly the same in each individual region. This marked variation in the degree of purification obtained with individual fractions shows that, unless the purification factors are worked out in each case, the regional variations found for the homogenates will be more meaningful than those obtained after the preparation of a mitochondrial fraction.

DISCUSSION

Though a great many studies have been made on the regional distribution of monoamine oxidase in the brains of different species, only one such series of studies, that of Weiner (1960), has investigated the distribution of the enzyme in brain mitochondria as well as in homogenates. This is important in that any variation found in homogenates from individual regions of the brain could be due either to a variation in the numbers of mitochondria present in these regions, or to variation in the amounts of monoamine oxidase in an essentially constant mitochondrial concentration. It is not, however, meaningful to discuss variations among the activities of mitochondria prepared from individual regions of the brain unless some measure can be made of the efficiency of preparation in each case. In the present experiments the cytochrome oxidase activity of the mitochondrial fractions was used as a measure of the purification obtained.

As shown in Table 4, the efficiency with which the mitochondria were purified from the various fractions varied widely. This could be due to several factors, e.g. a variable degree of contamination of the mitochondrial fractions with other structures having similar sedimentation characteristics, the presence of materials such as lipoproteins that bind to the mitochondria and alter their sedimentation characteristics, or different numbers of mitochondria in different brain regions. The finding that the purification of monoamine oxidase attending the preparation of the mitochondria

parallels that of cytochrome oxidase suggests that these enzymes are both associated with the same particle, whereas the large variations in the monoamine oxidase/cytochrome oxidase activity ratio among the regions suggest that variation of the concentration of mitochondria cannot solely account for the differences encountered between brain regions.

Few studies have been made on the distribution of cytochrome oxidase in the various regions of brain. The distribution of the enzyme has been studied in hamster brain by Mokrasch & Grady (1964), in monkey brain by Tolani & Talwar (1963) and in rabbit brain by Ridge (1967). The results obtained for monkey and rabbit brain agreed in the relative activities of the brain regions, but differed markedly from the results obtained with hamster brain. In general, the results reported in the present paper agree with those reported for rabbit and monkey brain.

α -Glycerophosphate dehydrogenase activity appears to parallel that of cytochrome oxidase in the individual regions of pig brain. Further, the enzyme is purified to the same degree as cytochrome oxidase when mitochondria are prepared, a behaviour that would be expected from a mitochondrial enzyme. In general the α -glycerophosphate dehydrogenase/cytochrome oxidase activity ratio is lower in the mitochondria than in the total homogenate, probably indicating a lower stability of α -glycerophosphate dehydrogenase, since loss of enzyme from the mitochondria, to which it is tightly bound (Ringler & Singer, 1959), can be ruled out.

The present results are in agreement with the histochemical studies of Kul'tas (1965), Hess & Pearce (1961) and Felgenhauer & Stämmli (1962), which indicated a wide distribution paralleling that of cytochrome oxidase and succinate dehydrogenase.

The parallel distribution of succinate dehydrogenase and cytochrome oxidase in various regions of brain has been reported by Shimizu & Morikawa (1957), and thus it appears that the three enzymes cytochrome oxidase, succinate dehydrogenase and α -glycerophosphate dehydrogenase may be part of the invariant mitochondrial enzyme content of the brain. In view of the variation of α -glycerophosphate dehydrogenase activity relative to that of cytochrome oxidase in other tissues (Pette *et al.* 1962), it is surprising that it parallels cytochrome oxidase activity so closely in the different regions of the brain. This close parallel with two important mitochondrial enzymes of energy metabolism suggests that the enzyme must have some important function in brain metabolism, a suggestion that was made by Sacktor *et al.* (1959). The nature of this role is, however, uncertain, though it is possible that the enzyme may be involved in the transfer of

reducing power into the mitochondrion, a role in which it has been implicated in other tissues (Boxer & Devlin, 1961).

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