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THE SUB-MITOCHONDRIAL LOCALIZATION OF MONOAMINE OXIDASE IN RAT LIVER AND BRAIN

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SUMMARY

The large-amplitude swelling and phospholipase digestion techniques have been used to separate the outer- and the inner-membrane fractions of rat liver and brain mitochondria, and the distribution of monoamine oxidase (EC 1.4.3.4) in the fractions obtained has been compared with those of three other enzymes. The liquid nitrogen-temperature difference spectra of the reduced *minus* oxidized membrane fractions were also determined. The outer membrane fractions of liver mitochondria were rich in a cytochrome component with α-band maxima at 553 and 558 mμ but no evidence for such a cytochrome component in the outer membranes of brain mitochondria could be found. The results indicated that monoamine oxidase was at least partially located in the space between the outer and inner mitochondrial membranes. Penetration experiments showed that the inner of the two mitochondrial membranes was impermeable to a number of monoamine oxidase substrates.

INTRODUCTION

The enzyme monoamine oxidase (EC 1.4.3.4) has been shown to be largely associated with mitochondria in both liver[1–5] and brain cells[6,7], and recently SCHNAITMAN, ERWIN AND GREENAWALT[8] have presented evidence that the enzyme is localized in the outer membrane fraction that can be separated from rat liver mitochondria after treatment with digitonin. Techniques for separating the outer membrane from mitochondria have recently been developed by PARSONS and co-workers[9–11], using large-amplitude swelling, and by BACHMANN, ALLMANN AND GREEN[12] using phospholipase digestion. The large-amplitude swelling method gave results in terms of enzyme distribution which were significantly different from those reported for the outer membrane fractions separated after treatment of the mitochondria with phospholipase[12–14].

The present study was undertaken with the aim of studying the distribution of monoamine oxidase in the sub-fractions of rat brain and liver mitochondria produced by both the large-amplitude swelling method and the phospholipase method.

The phospholipase method would appear to be less specific for separating the outer membrane fraction than the large-amplitude swelling method, but it was used in parallel experiments with the latter method since it has been reported that brain mitochondria cannot easily be induced to swell[15] and it would therefore be expected that the method of PARSONS and co-workers might not be as successful when applied to brain mitochondria as with liver mitochondria.

KLINGENBERG AND PFAFF[16] have recently adapted the method of WERKSHEISER AND BARTLEY[17] for measuring the penetration of various compounds into the mitochondrion and have presented data to show that the inner of the two mitochondrial membranes can be identified with the barrier that is impermeable to sucrose and to adenine nucleotides except by an exchange process which is inhibited by atractyloside. This inner membrane has also been shown to be impermeable to coenzyme A (see ref. 18) and L-carnitine[19]. The ability of a number of substrates of monoamine oxidase to penetrate the mitochondrion was studied by this method which indicated that the inner of the two mitochondrial membranes is impermeable to these compounds.

MATERIALS AND METHODS

Preparation of mitochondria

Brain mitochondria were prepared from the brains of Sprague–Dawley rats immediately after they had been killed by decapitation. The mitochondria were prepared by the Method I of BRODY AND BAIN[20] using 0.25 M sucrose which had been adjusted to pH 7.6 with 1.0 M K_2HPO_4 as the homogenising medium and homogenates were prepared by hand in a Potter–Elvehjem homogenizer with an all-nylon pestle. The mitochondria were washed twice by resuspending in 0.25 M sucrose (pH 7.6) and re-isolated by centrifuging once at 18 000 × *g* for 20 min and finally at 10 000 × *g* for 10 min. The supernatant and loosely packed light coloured material were discarded. The final mitochondrial pellet was kept at 0° and used within 4 h.

Liver mitochondria were prepared by the method of HOGEBOOM, SCHNEIDER AND PALADE[21] with 0.25 M sucrose in 5 mM Tris chloride buffer (pH 7.2) as the homogenizing medium and using the Potter–Elvehjem homogenizer with an all-nylon pestle. The mitochondria were washed twice by re-suspension and isolated by centrifugation at 8000 × *g* for 10 min and the pellet was stored at 0° for not more than 4 h before use.

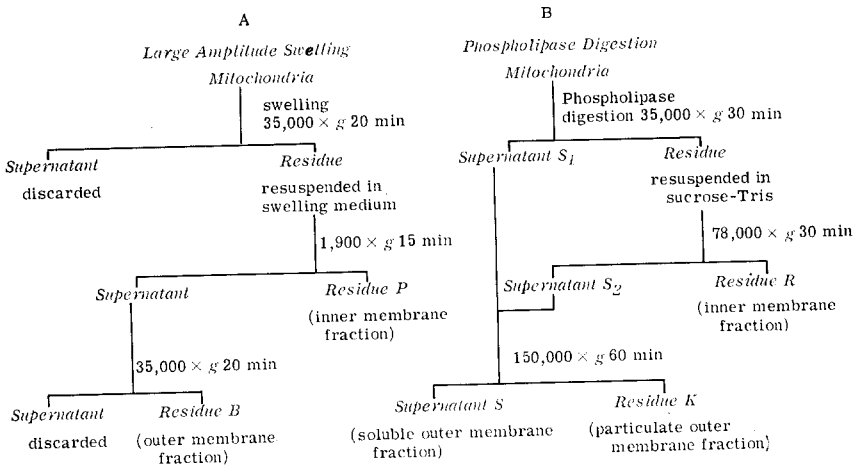
Preparation of mitochondrial membrane fractions

(*a*) *By large-amplitude swelling.* Liver and brain mitochondria were suspended in 0.28 M sucrose, 0.1 mM EDTA, 1.0 mM Tris chloride (pH 7.2) and the suspensions were centrifuged at 1500 × *g* for 10 min. The supernatants were carefully decanted and centrifuged at 9000 × *g* for 10 min. The supernatants were discarded along with the fluffy layer of broken mitochondria and microsomes. The mitochondrial pellets were then subjected to large-amplitude swelling in 20 mM phosphate buffer containing 0.02% bovine serum albumin followed by centrifugation by the method of PARSONS and co-workers[10,11]. This technique yielded 2 main fractions, the inner- and the outer-membrane fractions, and the supernatant fractions. A schematic diagram of the procedure is given in Scheme IA. The inner- and outer-membrane fractions

SCHEME I

THE PREPARATION OF MITOCHONDRIAL MEMBRANE FRACTIONS

The designations of the fractions used by the original workers are retained.



(P and B fractions respectively) were suspended in 10 mM phosphate buffer (pH 7.2) and stored at −10°. The membrane fractions were not further purified by the density-gradient fractionation procedure.

(*b*) *By phospholipase treatment.* Liver and brain mitochondria were re-suspended in 0.25 M sucrose, 10 mM Tris chloride (pH 7.8) and centrifuged at 15 000 × *g* for 10 min. The resultant pellets were dispersed in the sucrose–Tris medium and stored for 18 h at 4°. The homogenates were then treated by phospholipase digestion and differential centrifugation to yield 3 mitochondrial subfractions: the soluble component of the outer membrane or S fraction, the particulate fraction of the outer membrane or K fraction, and the inner membrane or R fraction. A schematic diagram of the procedure used is shown in Scheme IB. The fractions were suspended in 0.01 M phosphate buffer (pH 7.6) and were stored at −10°.

Determination of protein concentration

This was carried out by the micro-biuret method[22]. Each sample was made 1.5% with the detergent Triton X-100 to solublize the protein. The colour yield was related to a serum albumin standard.

Space measurements

The permeability of mitochondria to a number of monoamine oxidase substrates was studied using the method of YATES AND GARLAND[19]. The mitochondria were incubated at 1° for 20 min at a concentration of 12 mg mitochondrial protein per ml in a medium containing 20 mM Tris chloride (pH 7.2), 80 mM KCl, 1 mM EDTA, 20 mM sucrose and 70 μM iproniazid to inhibit the monoamine oxidase. An equal volume of the Tris chloride–KCl–EDTA–sucrose mixture was then added containing 10 mg blue dextran, 0.1 μC/ml tritiated water and 2 mM in the amine to be investigated. Incubation was continued for a further 10 min before the incu-

bation mixture was separated into a pellet and supernatant by centrifugation at 25 000 × *g* for 12 min. 5 different amines were used in the penetration studies in separate experiments: dopamine (3-hydroxytyramine), noradrenaline, serotonin (5-hydroxytryptamine), tyramine and *m*-*O*-methyladrenaline.

The pellet was extracted with 5% w/v perchloric acid and the extract and the supernatant were assayed for sucrose by the method of KULKA[23], tritiated water by liquid scintillation counting, and for blue dextran by measuring the absorbance at 610 mμ. Dopamine was assayed by the method of DRUJANS *et al.*[24], noradrenaline by the trihydroxyindole method of CROUT[25], serotonin by the method of BOGDANSKI *et al.*[26], tyramine by the method of OATES[27] and *m*-*O*-methyladrenaline by the method of AXELROD AND TOMCHICK[28]. Preliminary purification of extracts prior to dopamine and noradrenaline assay was carried out by the method of NEFF AND COSTA[29]. Reagent blanks and mitochondrial samples with internal standards were used in each assay.

Enzyme assays

Spectrophotometric assays were made with either a Beckman DK2A or a Unicam SP800 recording spectrophotometer, using quartz-glass cuvettes with a 1-cm light path. Measurements of oxygen uptake were carried out using a Clark oxygen electrode connected *via* a voltage divider to a Honeywell-Brown 1-mV strip-chart recorder, the apparatus used being similar to that described by DIXON AND KLEPPE[30]. All enzyme assays were performed at 30°. Units of enzyme activity are defined as μmoles of substrate used or product formed per min per mg protein.

Malate dehydrogenase. The reduction of NAD^+ was followed at 340 mμ by the method of SIEGEL AND ENGLARD[31]. The assay medium contained, in a total volume of 2.0 ml, 150 μmoles of glycine buffer (pH 10.0), 4 μmoles of NAD^+, 2 μg of antimycin A, 200 μmoles of L-malate, and enzyme. The reaction was started by the addition of L-malate. Rates were measured in a Beckman DK2A recording spectrophotometer. The molar extinction coefficient of NADH at 340 mμ was taken to be $6.22 \cdot 10^3$ $cm^{-1}M^{-1}$.

Succinate dehydrogenase. The reduction of indophenol to leucoindophenol was followed at 600 mμ by the method of GREEN, MII AND KOHOUT[32]. The assay medium contained, in a total volume of 2.0 ml, 20 μmoles of potassium phosphate buffer (pH 7.4), 1.0 mg of bovine serum albumin, 2 μmoles of KCN, 20 μmoles of succinate, 40 μg of 2,6-dichlorophenolindophenol, and enzyme. The reaction was started by the addition of substrate. Rates were measured in a Unicam SP800 recording spectrophotometer. The molar extinction coefficient of 2,6-dichlorophenolindophenol at 600 mμ was taken to be $16.1 \cdot 10^3$ $cm^{-1}M^{-1}$.

Cytochrome oxidase. The consumption of oxygen was measured by the manometric method of TOLANI AND TALWAR[33] adapted for use with an oxygen electrode. The assay medium 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 μl of 0.6 M *p*-phenylenediamine hydrochloride. Separate blanks in the absence of enzyme were determined.

Monoamine oxidase. The oxidation of tyramine was followed by measuring oxygen consumption by the manometric method of CREASEY[34] 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), 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 μl of 0.2 M tyramine hydrochloride.

Determination of spectra

The visible absorption spectra of the mitochondrial fractions were determined in a Johnson Foundation split-beam spectrophotometer (see *e.g.* ref. 35). The dithionite-reduced *minus* oxidized spectra were determined at liquid nitrogen temperatures using sample cuvettes with a 3-mm light path. The wavelength accuracy of the instrument was checked using a didymium filter before the spectra were determined.

Source of chemicals

All chemicals except those listed below were obtained from British Drug Houses Ltd. or Hopkin and Williams Ltd. Other chemicals were obtained from the following suppliers: Tris (Trizma base) and snake venom (*Naja Naja*) from the Sigma Chemical, London; bovine serum albumin from Armour Chemical; catalase and NAD^+ from Biochemica Boehringer, Mannheim, Germany; tritiated water from the Radiochemical Centre, Amersham; blue dextran from Pharmacia, London; Triton X-100 from Lennig Chemicals; and *m-O*-methyladrenaline from Koch–Light, Colnbrook, Bucks. Iproniazid (N^1-isonicotinyl-N^2-isopropyl hydrazide) was a gift from Roche Products Ltd. The quality of all chemicals was the highest produced by the manufacturer.

Other techniques

Distilled water was passed through a Permutit Mark 11 deionizer before use. Measurements of pH were made using a Radiometer type PH M22r pH meter. Centrifugation was carried out using a MSE 'Major' refrigerated centrifuge for low-speed spins (up to 1500 × *g*), a MSE 'High Speed 18' refrigerated centrifuge for high speeds (up to 35 000 × *g*) and a MSE Superspeed 50 refrigerated centrifuge for very high speeds. Measurements of absorbance were made in a Unicam SP500 spectrophotometer using quartz-glass cells with a path length of 1 cm. Liquid scintillation counting of tritium was done with a Nuclear-Chicago 720 series liquid scintillation counter using PPO and POPOP as the scintillant. Fluorescence measurements were made using an Aminco–Bowman spectrophotofluorimeter. Crude snake venom phospholipase (*Naja Naja*) was partially purified by the technique outlined by BACHMANN, ALLMANN AND GREEN[12].

RESULTS

Tables I and II show the total enzymic activities of the mitochondrial subfractions prepared by the large-amplitude swelling method and by the phospholipase digestion method respectively. 3 enzyme activities were used as markers for the inner and outer membrane fractions: succinate dehydrogenase, malate dehydrogenase and cytochrome oxidase. Succinate dehydrogenase has been reported to remain in the inner membrane fraction formed by either large-amplitude swelling or phospholipase digestion and the results shown in the tables indicate that almost all the

TABLE I

ENZYMIC ACTIVITIES OF MITOCHONDRIAL MEMBRANE FRACTIONS PREPARED BY LARGE-AMPLITUDE SWELLING

Methods of assay are given in the text. The units of activity are expressed as μatoms of oxygen consumed min^{-1} (mg protein)$^{-1}$ in the cases of cytochrome oxidase and monoamine oxidase, and as μmoles NAD^+ or dye reduced min^{-1} (mg protein)$^{-1}$ in the cases of malate dehydrogenase and succinate dehydrogenase.

Fraction	*Description*	*Source*	*Enzyme content (total units)*			
			Monoamine oxidase	*Cytochrome oxidase*	*Malate dehydrogenase*	*Succinate dehydrogenase*
A	Swollen mitochondria	Liver	1.8	80	17 000	180
		Brain	0.8	116	10 000	170
B	Outer membranes	Liver	1.07	8.55	267	8.2
		Brain	0.25	43.1	347	0
P	Inner membranes	Liver	0.347	66	16 600	205
		Brain	0.21	116	7 800	198

succinate dehydrogenase activity in both liver and brain mitochondria remains with the inner membrane fraction whichever of the two fractionation techniques was used. The situation regarding malate dehydrogenase is more difficult, however. PARSONS *et al.*[11] find that the enzyme is associated with the inner membrane fraction, whilst GREEN and his co-workers find that this enzyme and the other low molecular weight enzymes of the citric acid cycle were liberated into solution by their treatments[12,13] and thus concluded that these enzymes existed in the space between the inner and outer membrane of the mitochondrion. This difference between the results using the two treatments is illustrated by the results shown in Tables I and II. In mitochondrial

TABLE II

ENZYME ACTIVITIES OF MITOCHONDRIAL MEMBRANE FRACTIONS PREPARED BY PHOSPHOLIPASE TREATMENT

Methods of assay are given in the text. The units of activity are expressed as μatoms of oxygen consumed min^{-1} (mg protein)$^{-1}$ in the cases of cytochrome oxidase and monoamine oxidase, and as μmoles NAD^+ or dye reduced min^{-1} (mg protein)$^{-1}$ in the cases of malate dehydrogenase and succinate dehydrogenase.

Fraction	*Description*	*Source*	*Enzyme content (total units)*			
			Monoamine oxidase	*Cytochrome oxidase*	*Malate dehydrogenase*	*Succinate dehydrogenase*
S	Soluble portion of outer membranes	Liver	1.27	0	11 200	0
		Brain	0	0	356	0
K	Particulate portion of outer membranes	Liver	0	6.2	11.6	1.6
		Brain	0.158	1.2	15.4	0
R	Inner membranes	Liver	0.56	47.0	0	117
		Brain	0.206	12.1	0	27

subfractions prepared by the large-amplitude swelling method, almost all the malate dehydrogenase from liver mitochondria remains with the inner membrane fraction, only 1.2% of the total units being associated with the outer membrane fraction, whilst using brain mitochondria, the same treatment resulted in less than 5% of the total enzyme units appearing in the outer membrane fraction.

The third enzyme used as a marker was cytochrome oxidase which PARSONS, WILLIAMS AND CHANCE[10] showed to be largely a component of the inner mitochondrial membrane. Although GREEN and his co-workers did not use cytochrome oxidase as a marker in their assays, their finding that the enzymes of the respiratory chain are associated with the inner membrane implicates this enzyme as being a component of the inner membrane system. BROSMER, VOGELL AND BUCHER[36] have shown that this enzyme is associated with the inner mitochondrial membrane in developing insect mitochondria and SCHNAITMAN, ERWIN AND GREENAWALT[8] have used the enzyme as an inner membrane marker for rat liver mitochondria. Most of the cytochrome oxidase activity of both liver and brain mitochondria remains with the inner membrane fraction after the large-amplitude swelling technique, and similar results were obtained after phospholipase treatment.

In the case of monoamine oxidase, neither the large-amplitude swelling method nor phospholipase treatment accomplishes complete separation of enzyme into the outer membrane fraction. After large-amplitude swelling, approx. 75% of liver and more than 50% of brain mitochondrial monoamine oxidase activity appears in the outer membrane fraction. Phospholipase treatment was less successful at liberating monoamine oxidase than the large-amplitude swelling method. With liver mitochondria, about 70% of the monoamine oxidase activity was found to be liberated into solution in the S fraction, and no detectable amounts were found in the particulate fraction of the outer membrane. In the case of brain mitochondria, however, no monoamine oxidase activity could be detected in the S fraction, whilst about 43% of the monoamine activity was found in the K fraction.

As an additional marker for the membrane fractions, the liquid nitrogen-temperature difference spectra were determined. Figs. 1A and B show the dithionite-

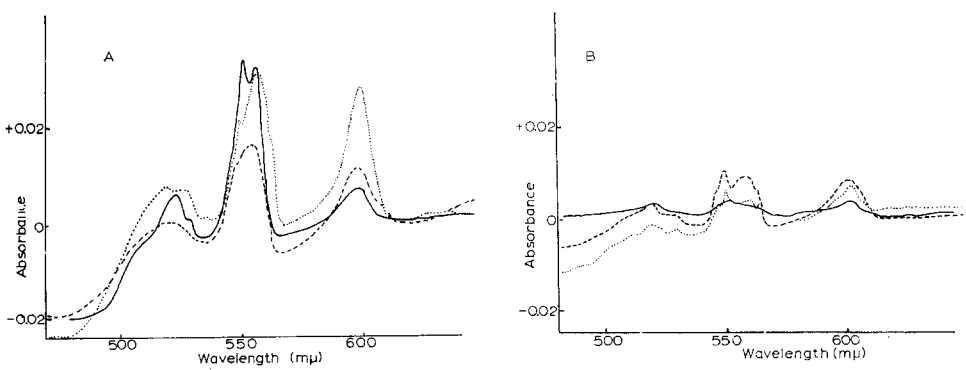


Fig. 1. The liquid nitrogen-temperature dithionite-reduced *minus* oxidized difference spectra of membrane fractions of liver and brain mitochondria produced by large-amplitude swelling. A. Fractions from liver mitochondria. All protein concns. 6 mg/ml. · · · · ·, inner membrane fraction; ——, outer membrane fraction; - - - - -, mitochondria after swelling. B. Fractions from brain mitochondria. All protein concns. 7 mg/ml. Legend as for A.

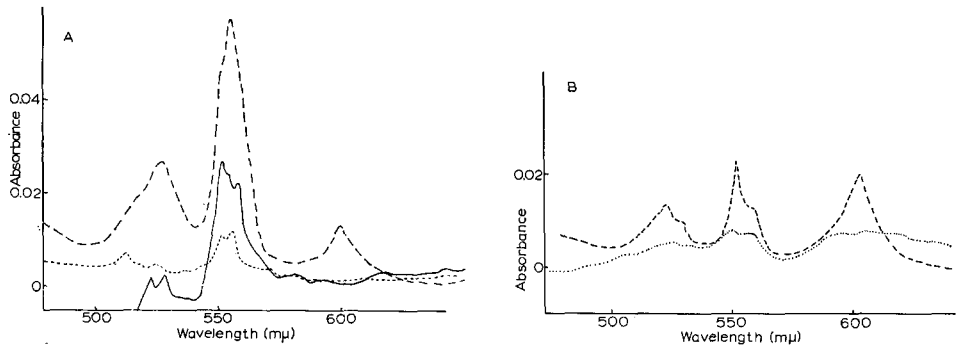


Fig. 2. The liquid nitrogen-temperature dithionite-reduced *minus* oxidized difference spectra of membrane fractions of liver and brain mitochondria produced by phospholipase A digestion. A. Fractions from liver mitochondria. All protein concns. 7 mg/ml; - - - - -, inner membrane (R) fraction; ———, soluble outer membrane (S) fraction; · · · · ·, particulate outer membrane (K) fraction. B. Fractions from brain mitochondria. Protein concns. 7 mg/ml. No difference spectrum was given by the soluble outer membrane (S) fraction. - - - - -, inner membrane (R) fraction; · · · · ·, particulate outer membrane (K) fraction.

reduced *minus* oxidized spectra for liver and brain mitochondrial membrane fractions respectively, prepared by the large-amplitude swelling method. Similar spectra for the fractions prepared by the phospholipase method are shown in Figs. 2A and B. As can be seen from Figs. 2A and B, the spectra of the inner membrane fractions of liver and brain mitochondria are similar to those of the whole mitochondria. The small cytochrome *c* content of these fractions is probably due to the fact that it is readily extracted from mitochondria by dilute phosphate buffers[35]. The spectrum of the liver outer membrane fraction shows the presence of little *a* and *c* cytochromes, but is dominated by a large absorption band with a split α maximum at 553 and 558 mμ. This cytochrome has been tentatively identified as cytochrome b_5 by PARSONS *et al.*[11] and its liquid nitrogen spectrum closely resembles the liquid nitrogen absorption spectrum reported for cytochrome b_5 (see refs. 37, 38). The spectra of the soluble (S) and particulate (K) fractions of the outer membranes of liver mitochondria produced by the phospholipase digestion method show that both contain the supposedly *b*-type cytochrome as their main cytochrome component.

The spectra for the membrane fractions obtained from rat brain mitochondria show a less clear picture. The inner membrane fractions resemble those obtained from liver mitochondria with the interesting exception that they are much richer in cytochrome *c*. This difference may reflect either a difference in the binding of the cytochrome in brain mitochondria, or a difference in the permeability of the brain mitochondrial membranes to cytochrome *c*. Differences in extractability of mitochondrial components between different organs and different species are not uncommon (see *e.g.* ref. 16).

The spectra of the outer membrane fractions of brain mitochondria prepared by either method contain only small amounts of cytochromes, and there is no evidence of an enhancement in the content of a *b*-type cytochrome as was observed with the liver mitochondria outer membrane fractions.

The results of the penetration experiments are shown in Table III. The finding that with liver mitochondria about 50–60% of the intramitochondrial water is

TABLE III

THE PENETRATION OF LIVER AND BRAIN MITOCHONDRIA BY VARIOUS SUBSTRATES OF MONOAMINE OXIDASE

For experimental details see text. The permeable space is given as a percentage of intramitochondrial water calculated from the uptake of tritiated water. The sucrose-permeable space was calculated to be 57 ± 6% for liver mitochondria and 18 ± 2% for brain mitochondria, from 6 separate determinations in each case.

Monoamine oxidase substrate	*Percentage permeable space*	
	Liver mitochondria	*Brain mitochondria*
Tyramine	51	14
Dopamine	50	16
Noradrenaline	55	11
Serotonin	49	20
m-O-Methyladrenaline	43	18

permeable to sucrose is in agreement with values previously reported for liver mitochondria[16,17]. The space values determined for the monoamine oxidase substrates are close to those of sucrose, implying that the inner of the two mitochondrial membranes is impermeable to these compounds. Similar results were obtained with rat brain mitochondria, in that the values determined for the amine space were close to those for the sucrose space, with the important difference that the sucrose-permeable space of these mitochondria was found to be less than 20% of the intramitochondrial water.

DISCUSSION

The results in the present paper support the conclusion of SCHNAITMAN, ERWIN AND GREENAWALT[8] that monoamine oxidase is localized outside the outer membrane of rat liver mitochondria, the enzyme being liberated with the outer membrane in fractions produced by either phospholipase treatment or large-amplitude swelling. This localization of monoamine oxidase is further supported by the finding that the inner mitochondrial membrane is impermeable to the substrates of this enzyme, as it is to sucrose.

The results obtained with the two techniques for preparing mitochondrial membrane fractions are similar to those found by PARSONS *et al.* and GREEN and his co-workers, although the technique used in this work differs from that of the latter group in that *Naja Naja* venom was used instead of the *Crotalus atrox* venom used by the originators of this technique. The results provide a useful comparison of the two techniques used in parallel. In the case of liver mitochondria, the compositions of the membrane fractions in terms of the marker enzymes and the cytochromes were similar whichever of the two methods was used, with the important exception of malate dehydrogenase, which appeared with the outer membrane fractions after phospholipase treatment, but remained with the inner membrane fraction after large-amplitude swelling. Since the large-amplitude swelling technique is a milder treatment than those of GREEN and his co-workers, it is possible that the outer membranes produced by phospholipase digestion may be heavily contaminated with

components of the inner matrix, although it is also possible that malate dehydrogenase and the other enzymes of the citric acid cycle are loosely associated with the outside of the inner mitochondrial membrane, and therefore represent components of the space between the inner and the outer membranes.

The results obtained with brain mitochondria appear to parallel those from liver mitochondria in terms of the distribution of the marker enzymes in the different membrane fractions. The separation of monoamine oxidase with the outer membrane fractions is, however, not as complete as that of liver. The phospholipase digestion method was surprisingly less successful than the large-amplitude swelling technique in this respect. However, since over 50% of the monoamine oxidase activity was liberated with the outer membrane fractions by large-amplitude swelling, and the penetration experiments showed that the inner mitochondrial membrane was impermeable to monoamine oxidase substrates, it can be concluded that some at least of the brain mitochondrial monoamine oxidase is associated with the outer mitochondrial membrane. The less successful extraction of monoamine oxidase in the membrane fractions of brain mitochondria by large-amplitude swelling can be ascribed to the resistance of brain mitochondria to swelling[15]. The relatively poor separation of monoamine oxidase obtained with the outer membrane fractions produced by the phospholipase technique could be due to the significantly different lipid content of brain, as opposed to liver, mitochondria[39].

The cytochrome contents of the brain mitochondrial membrane fractions differed principally from the liver mitochondria in that the washing procedure was less successful in extracting cytochrome *c* and that there was no evidence of the concentration of a *b*-type cytochrome in the outer membrane fractions. Studies on the cytochrome contents of rat brain mitochondria are in progress, which it is hoped will show whether cytochrome b_5 is a component of brain mitochondria.

The space measurements indicated that in neither liver nor brain mitochondria could the various amine substrates penetrate the mitochondria further than sucrose, indicating that the inner of the two mitochondrial membranes is impermeable to these substrates. The sucrose-permeable space of liver mitochondria has been shown to vary considerably according to the conditions[16], and it has been suggested that these variations can be correlated with swelling phenomena that can be observed in the electron microscope. In this context it is tempting to ascribe the much smaller penetrable space of brain mitochondria to their resistance to swelling, although this low value may be due in part to contamination of the brain mitochondrial preparation with other material, since it is known to be very difficult to prepare pure brain mitochondria (see *e.g.* ref. 18).

The possibility that the results obtained for the monoamine oxidase distributions in brain mitochondria is due to contamination with microsomal material can be discounted since monoamine oxidase has been shown to be absent from the microsomal fraction of this tissue[40]. Brain mitochondria are however contaminated by synaptosomes which have been shown to possess some monoamine oxidase activity[7] but this may be associated with synaptosomal mitochondria. In the case of liver monoamine oxidase activity has been found associated with the microsomal fraction[3,4] but SCHNAITMAN, ERWIN AND GREENAWALT[8] have suggested that this may be due to vesiculation of the mitochondrial outer membranes during their preparation.

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