COMMUNICATIONS

Existence of Cholinephosphotransferase in Mitochondria and Microsomes of Liver and Lung of Guinea Pig and Rat[1]

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We reported earlier on the occurrence of cholinephosphotransferase in the mitochondria of guinea pig lung. In order to determine whether organ and/or species specificities exist in regard to the cholinephosphotransferase activity in mitochondria, we have compared the subcellular distribution of the enzyme in the liver and lungs of rats and guinea pigs. Even though the activity of the enzyme was higher in microsomes than it was in mitochondria, the mitochondrial activity was authentic in both tissues of both species. The authenticity of mitochondrial activity was established by marker enzyme studies and ultrastructural examination of mitochondrial preparations.
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Cholinephosphotransferase, the terminal enzyme of the CDPcholine pathway, was generally presumed to be located exclusively in the endoplasmic reticulum (1). However, our laboratory had reported the presence of the enzyme in the mitochondria of guinea pig lung earlier (2,3). We further established that the enzyme is located in the outer mitochondrial membrane (3). Although the presence of cholinephosphotransferase has been observed by others in mitochondria of yeast (4), rat liver (5-7) and intestine (8), there are several reports on rat liver (9-12), rabbit lung (13) and fetal rat lung (14,15) which contradict these findings.

It is presently not known whether there is a species and/or tissue specificity as far as the presence of cholinephosphotransferase in mitochondria is concerned. We have therefore compared the distribution of cholinephosphotransferase in mitochondria and microsomes of the liver and lung of both rats and guinea pigs.

MATERIALS AND METHODS

Materials. All biochemicals were purchased from Sigma Chemical Company (St. Louis, MO). Radioactive cytidine diphosphocholine was purchased from New England Nuclear (Boston, MA). n-Butanol was purchased from Malinckrodt, Inc. (Paris, KY). Universol cocktail was purchased from ICN Radiochemicals (Irvine, CA).

Subcellular fractionation. Hartley strain male guinea pigs (350-400 g) and Wistar male rats (180-200 g) obtained from Camm Laboratory Animals (Wayne, NJ) were killed by decapitation. Liver and lung were quickly excised, washed in ice cold saline (0.9% NaCl), blotted dry and weighed. The tissues were then minced with scissors and homogenized in 4-6 volumes of 0.25 M sucrose/1 mM EDTA (pH 7.4), using a Potter Elvehjem homogenizer.

The nuclear fraction was prepared by centrifugation of whole homogenate at 600 × *g* for 10 min in a refrigerated Sorvall RC-5 centrifuge using a SS-34 rotor. The nuclear pellet was washed once with 0.25 M sucrose/1 mM EDTA (pH 7.4). The supernatants were combined and centrifuged at 10,000 × *g* for 10 min to obtain the mitochondrial fraction. In most cases, the supernatant was centrifuged in a Beckman L8-M ultracentrifuge at 105,000 × *g* for 60 min using a 70.1 Ti rotor to obtain the microsomal and cytosolic fractions. However, for guinea pig lung, the post-mitochondrial supernatant was first centrifuged at 20,000 × *g* for 10 min to collect an intermediary fraction. The post 20,000 × *g* supernatant was used to collect microsomes and cytosol. The crude mitochondrial fraction was purified by linear sucrose density gradient as described earlier (3). Protein content was determined by the method of Lowry *et al.* (16).

Biochemical assays. Cholinephosphotransferase activity was assayed by measuring the incorporation of radioactivity from cytidine diphospho-[*methyl*-^{14}C]-choline into phosphatidylcholine using dioleoylglycerol as a substrate, as described earlier (2). We have previously reported that dioleoylglycerol is the most preferred substrate for both guinea pig lung mitochondrial and microsomal cholinephosphotransferase (17).

Succinic INT-reductase, a mitochondrial marker, was assayed as described by Possmayer *et al.* (18). Rotenone-sensitive NADPH cytochrome c reductase (19), as well as glucose-6-phosphatase (20), were used as microsomal markers.

For the electron microscopic examination, mitochondrial pellets were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), followed by osmium tetroxide in the same buffer (21). The specimens were dehydrated through an upgraded ethanol series at room temperature. Preparations were embedded in Araldite-502. Post-staining was done in a saturated solution of uranyl acetate in 50% ethanol followed by Reynold's lead citrate. Sections were examined with a Phillips 300 transmission electron microscope.

RESULTS AND DISCUSSION

The subcellular distribution of cholinephosphotransferase and marker enzyme activity in the liver and lung of rats is shown in Table 1. The specific activity of cholinephosphotransferase in either mitochondria or microsomes is not significantly different between liver and lung. The microsomal activity is higher than mitochondrial activity in both tissues. As shown in Figure 1, the relative specific activity of microsomal

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TABLE 1

Specific Activity of Cholinephosphotransferase and Marker Enzymes in Subcellular Fractions of Rat Liver and Lung[a]

Fractions	Liver				Lung		
	A	B	C	D	A	B	C
Whole homogenate	0.5 (0.1)	44.6 (2.4)	14.5 (0.8)	25.9 (2.2)	0.3 (0.0)	3.2 (0.6)	2.4 (0.4)
Nucleus	0.5 (0.1)	40.0 (3.5)	12.2 (1.0)	23.4 (1.0)	0.1 (0.0)	2.6 (0.2)	1.7 (0.3)
Mitochondria	0.7 (0.1)	54.6 (1.5)	10.2 (1.0)	2.9 (0.7)	0.6 (0.2)	6.1 (0.4)	4.7 (0.9)
Microsomes	0.9 (0.1)	2.8 (0.5)	46.4 (2.2)	58.2 (1.3)	1.2 (0.2)	1.0 (0.4)	19.2 (3.2)
Cytosol	0.1 (0.0)	2.0 (0.4)	3.9 (0.3)	6.0 (0.2)	0.1 (0.0)	2.0 (0.6)	1.6 (0.3)

[a]The specific activities of enzymes are expressed in nmol/min per mg protein. A represents cholinephosphotransferase, B represents succinic INT reductase, C represents NADPH cytochrome c reductase, and D represents glucose 6-phosphatase. Values are mean ± S.E. of seven experiments.

cholinephosphotransferase was 1.25-fold and 2.17-fold higher than that of mitochondrial fraction in the liver and lung, respectively. Succinic INT-reductase activity was distributed in mitochondria almost 19-fold more than in microsomal fraction in liver and 6-fold more in the lung. NADPH cytochrome c reductase activity of microsomes was significantly higher than that of mitochondria in both liver (4.65-fold) and lung (3.98-fold), respectively.

The apparent occurrence of NADPH cytochrome c reductase in mitochondria may be explained by the presence of pyridine nucleotide transdehydrogenase in the mitochondria which, in conjunction with the mitochondrial NADH cytochrome c reductase and NAD^+, would catalyze the oxidation of NADPH by cytochrome c (19). Therefore, the presence of NADPH cytochrome c reductase activity in the mitochondrial fraction does not necessarily indicate the presence of microsomal vesicle in this fraction. To exclude the possibility of microsomal contamination of mitochondria, we have also measured another microsomal marker—glucose-6-phosphatase—in the liver. The relative specific activity was about 20-fold higher in microsomes than in mitochondria. Moreover, the ratio of relative specific activity of cholinephosphotransferase to that of succinic INT-reductase is higher than the ratio of relative specific activity of cholinephosphotransferase to that of NADPH cytochrome c reductase in mitochondria of both the lung and liver, suggesting a true cholinephosphotransferase activity in mitochondria in both tissues.

Table 2 presents the data on cholinephosphotransferase and marker enzymes in the guinea pig lung and liver. The distribution patterns of these enzymes are shown in Figure 2. In contrast to the rat, the specific activity of cholinephosphotransferase in guinea pig is higher in the lung than in the liver in all fractions studied, except the nucleus. The relative specific activity of the enzyme in microsomes was found to be 1.65-fold and 1.8-fold higher, as compared to that of mitochondria in liver and lung, respectively. Succinic INT-reductase activity is mainly enriched in mitochondria, approximately 8-fold and 7-fold more in mitochondria than in the microsomes of the liver and lung, respec-

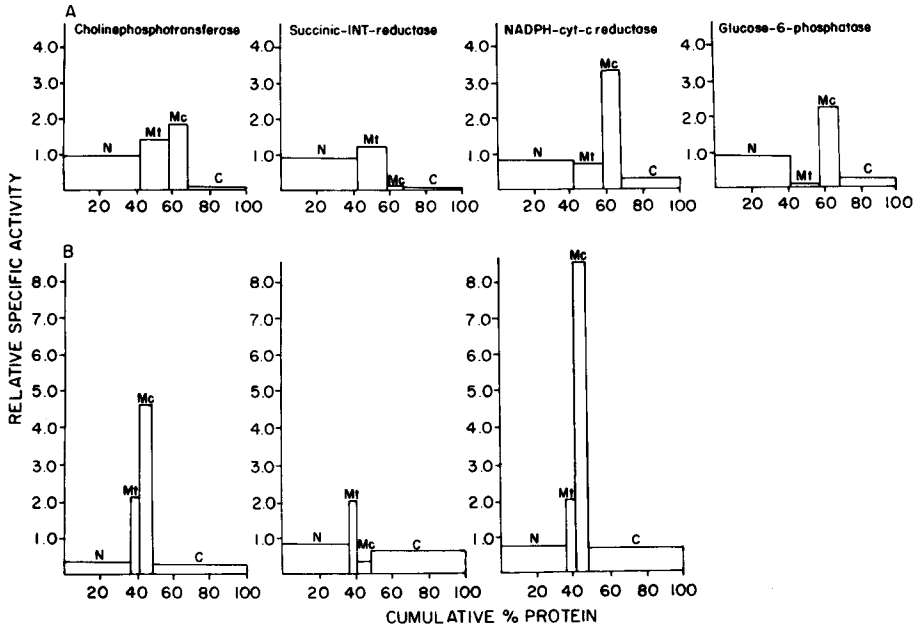

FIG. 1. Subcellular distribution of cholinephosphotransferase and marker enzyme activities of rat liver (A) and lung (B). Relative specific activity (the ratio of the specific activity of the respective fraction over that of the whole homogenate) is plotted as a function of cumulative percent protein. Relative specific activities were determined from the data in Table 1. N, nucleus; Mt, mitochondria; Mc, microsomes; and C, cytosol.

TABLE 2

Specific Activity of Cholinephosphotransferase and Marker Enzymes in Subcellular Fractions of Guinea Pig Liver and Lung[a]

Fractions	Liver			Lung		
	A	B	C	A	B	C
Whole homogenate	0.3 (0.1)	24.8 (2.2)	19.1 (2.6)	0.3 (0.1)	12.8 (0.3)	41.2 (1.2)
Nucleus	0.3 (0.1)	28.4 (6.7)	20.8 (3.4)	0.3 (0.1)	6.2 (0.2)	32.5 (1.2)
Mitochondria	0.5 (0.1)	99.9 (8.9)	26.6 (2.4)	1.1 (0.2)	89.2 (1.2)	42.1 (2.0)
20,000 × *g*				2.3 (0.1)	45.2 (2.0)	59.2 (2.0)
Microsomes	0.9 (0.2)	12.5 (3.2)	69.1 (5.9)	2.0 (0.4)	13.1 (0.7)	99.9 (4.5)
Cytosol	0.1 (0.0)	15.5 (4.8)	8.2 (1.2)	0.2 (0.1)	4.3 (0.2)	21.2 (2.3)

[a]The specific activities of enzymes are expressed in nmol/min/mg protein. A represents cholinephosphotransferase, B represents succinic INT reductase, and C represents NADPH cytochrome c reductase. Values are mean ± S.E. of eight experiments. For lung, the postmitochondrial fraction was centrifuged at 20,000 × *g* to collect an intermediary fraction.

tively. The relative specific activity of NADPH cytochrome c reductase was 2 to 2.5-fold higher in microsomes than mitochondria in both tissues. It should further be noted that the electron microscopic analysis of mitochondrial fraction from liver and lung of both species showed no contamination by endoplasmic reticulum. A typical electron micrograph of guinea pig liver mitochondria is shown in Figure 3.

There are some conflicting reports on the presence of cholinephosphotransferase in Golgi bodies. Although van Golde *et al.* (10) found no cholinephosphotransferase activity in Golgi bodies, others (5,22) reported considerable activity in the same organelle. Cholinephosphotransferase activity also has been detected in the plasma membrane of rat brain (23). Since the electron micrograph of the mitochondrial preparation of liver and lung of either guinea pig or rat did not show any contamination with Golgi bodies (Fig. 3), we did not study any marker for them. Furthermore, the mitochondrial preparation had negligible 5′-nucleotidase activity (marker for plasma membrane) (24). Therefore, the cholinephosphotransferase activity in mitochondria cannot be attributed to plasma membrane contamination.

In the present study, the activity of cholinephosphotransferase in the mitochondrial fraction is a true representation of the mitochondrial enzyme. This further confirms the previous observation on dual localization of the enzyme in the lung of guinea pigs (3) and rats (25), as well as in alveolar type II cells of fetal rat lung (26). The dual localization of the cholinephosphotransferase in both mitochondria and microsomes reported here is not unique. Glycerophosphate phosphatidyltransferase, a key enzyme in the synthe-

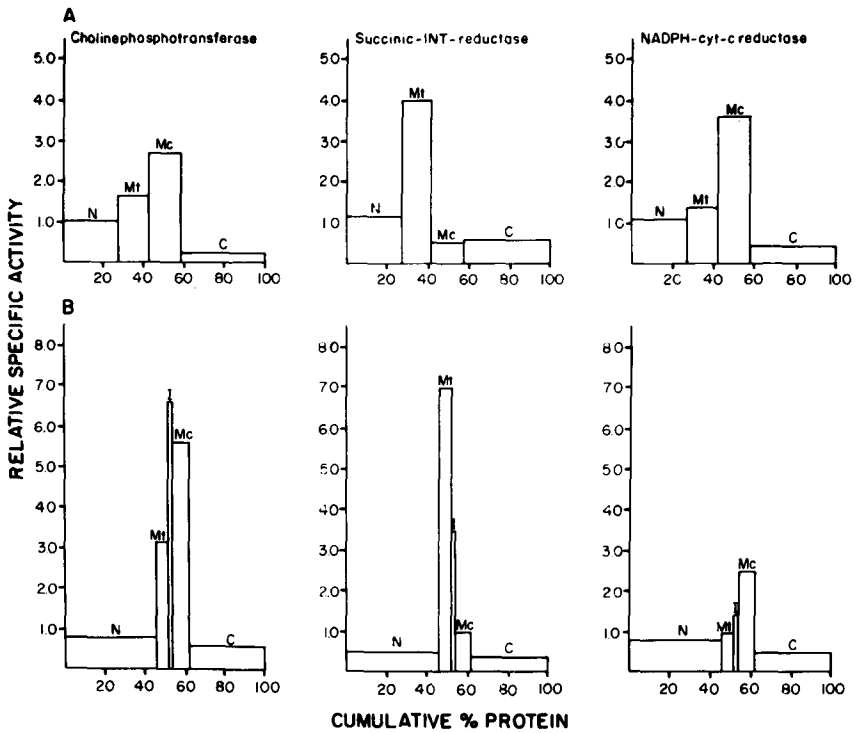

FIG. 2. Subcellular distribution of cholinephosphotransferase and marker enzyme activities of guinea pig liver (A) and lung (B). Relative specific activity (the ratio of the specific activity of the respective fraction over that of the whole homogenate) is plotted as a function of cumulative percent protein. Relative specific activities were determined from the data in Table 2. N, nucleus; Mt, mitochondria; I, 20,000 × *g* pellet; Mc, microsomes; and C, cytosol.

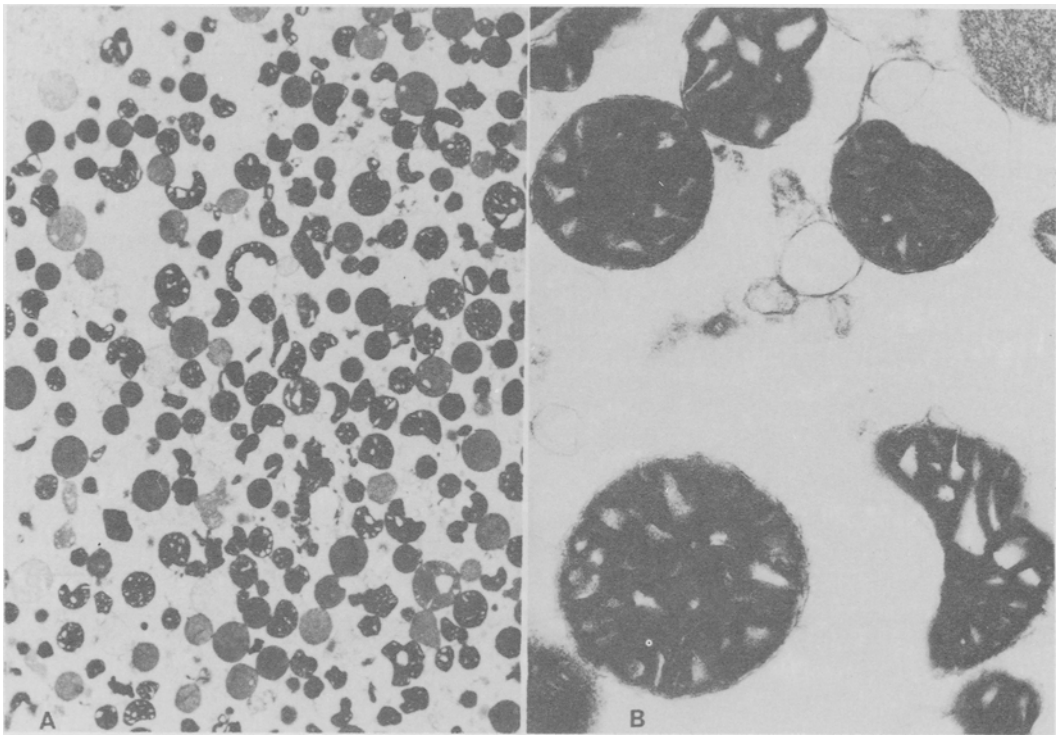

FIG. 3. A typical electron micrograph of guinea pig liver mitochondria (A at magnification × 5,440 and B at magnification × 34,850).

sis of phosphatidylglycerol, is present in both microsomes and mitochondria (10,26,27). CTP: cholinephosphate cytidyltransferase, which is thought to be located in microsomes and cytosol (28), has recently been reported to be present in human lung mitochondria (29). Hunt and Postle (29) reported that as much as 59% of human lung cytidyltransferase activity was associated with mitochondria, while the microsomal fraction accounted for only 4% of the activity. Stith and Das (2) reported a high cholinephosphotransferase activity in the mitochondrial fraction of the guinea pig lung between 45 and 60 days of gestation. The activity in fetal mitochondria was more than twice of that in fetal microsomes. Therefore, it is possible that mitochondrial cholinephosphotransferase may also be involved in phosphatidylcholine biosynthesis.

The results from the present study strongly suggest that the cholinephosphotransferase is localized in mitochondria, as well as in the endoplasmic reticulum in the liver and lungs of both species. However, there is some species and tissue specificity as far as the specific activity of cholinephosphotransferase is concerned. We have initiated studies to investigate the role of mitochondrial cholinephosphotransferase in phosphatidylcholine biosynthesis.

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