Plasmenylcholine (1-*O*-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphocholine) biosynthesis in guinea-pig heart and liver: cholinephosphotransferase is a bifunctional enzyme for the synthesis of phosphatidylcholine and plasmenylcholine

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Plasmenylcholine is present in significant proportion (32% of choline phosphoglycerides) in the guinea-pig heart but exists as a minor component (3% of choline phosphoglycerides) in the guinea-pig liver. In this study, the biosynthesis of plasmenylcholine in these two organs was examined. The organs were perfused with labelled choline for 15 min and chased with unlabelled choline for up to 7 h. The labelling of phosphatidylcholine was 6-fold higher than that of plasmenylcholine in the heart and about 60-fold higher in the liver. However, the same labelling ratio was maintained throughout the chase period in both organs. Alterations in the specific radioactivity of CDP-choline caused corresponding changes in the labelling of phosphatidylcholine and plasmenylcholine. Our results suggest that in guinea-pig heart and liver, CDP-choline is the immediate precursor of biosynthesis of phosphatidylcholine and plasmenylcholine. The biochemical cause for the difference in their rates of formation between the two organs was explored. The enzyme activities for the formation of both choline phosphoglycerides were determined. The two reactions share the same characteristics, and 1,2-diacylglycerol and 1-alk-1′-enyl-2-acylglcerol were found to be mutually inhibitory in a competitive fashion. The pool sizes of 1,2-diacylglycerol and 1-alk-1′-enyl-2-acylglycerol were determined, and their ratios were found to be 42 in the heart and 422 in the liver. We conclude that cholinephosphotransferase catalyses the formation of both phosphatidylcholine and plasmenylcholine in the guinea-pig tissues and the rate of plasmenylcholine biosynthesis is dependent on the availability of 1-alk-1′-enyl-2-acylglycerol. Plasmenylcholine biosynthesis is also subjected to modulation by the 1,2-diacylglycerol content of the tissue.

INTRODUCTION

Choline phosphoglyceride is the major phospholipid group in mammalian tissues [1]. It consists of a mixture of 1,2-diacyl-*sn*-glycero-3-phosphocholine (phosphatidylcholine), 1-*O*-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphocholine (plasmenylcholine) and 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (plasmanylcholine) [2]. In most mammalian tissues, phosphatidylcholine is the predominant form, whereas plasmenylcholine and plasmanylcholine usually constitute only small proportions of the total choline phosphoglyceride. In electrically active tissues, significant quantities of plasmenylcholine have been detected in several mammalian species [3]. The structural difference between phosphatidylcholine and plasmenylcholine implies there may be a divergence in their physiological functions. Although plasmenylcholine has been implicated in the pathogenesis of myocardial ischaemia [4] and protection against photo-oxidative stress [5], its exact role has not been defined.

Phosphatidylcholine biosynthesis in mammalian tissues has been well studied [6]. In mammalian liver, about 80% of phosphatidylcholine is synthesized *de novo* via the CDP-choline pathway and the remainder comes from the progressive methylation of phosphatidylethanolamine. In mammalian heart, the CDP-choline pathway accounts for over 95% of phosphatidylcholine formation [7]. In this pathway, choline is phosphorylated to phosphocholine by the action of choline kinase. The conversion of phosphocholine into CDP-choline, which is the rate-limiting step of the pathway, is catalysed by CTP–phosphocholine cytidylyltransferase [7,8]. Phosphatidylcholine is formed from condensation of diacylglycerol with CDP-choline by the action of CDP-choline-diacylgycerol cholinephosphotransferase.

Although the formation of the 1-*O*-alk-1′-enyl linkage in phosphoglycerides has been well studied using plasmenylethanolamine [9–12], the synthesis of plasmenycholine remains undefined. Plasmenylethanolamine is formed from plasmanylethanolamine by the action of an alkyl ether desaturase [9,10]. Attempts to demonstrate an analogous pathway for the formation of plasmenylcholine from plasmanylcholine have not been successful, as the alkyl ether desaturase appears to be specific for plasmanylethanolamine [2]. Alternatively, plasmenylcholine in the heart can be formed via the condensation of 1-alk-1′-enyl-2-acylglycerol with CDP-choline [13,14]. This reaction is corollary to the one catalysed by CDP-choline–1,2-diacylglycerol cholinephosphotransferase for the biosynthesis of phosphatidylcholine. However, it is not clear if the reaction is catalysed by the same or different enzymes. The importance of this pathway in the biosynthesis of plasmenylcholine has not been delineated.

In guinea-pig heart, a significant proportion of choline phosphoglyceride is in the plasmenylcholine form [13,15]. In contrast with the heart, plasmenylcholine constitutes only a minor portion of the total choline phosphoglyceride in guinea-pig liver [1]. The diversity in plasmenylcholine content between heart and liver may arise from dissimilar biosynthetic pathways, and hence these two organs are useful models for the study of

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plasmenylcholine formation. We report in this study that (a) phosphatidylcholine and plasmenylcholine are synthesized from the same choline-containing precursor in these two organs, and (b) cholinephosphotransferase is a bifunctional enzyme synthesizing both phosphatidylcholine and plasmenylcholine.

EXPERIMENTAL

Materials

CDP-choline, diacylglycerol (pig liver) and other lipid standards were purchased from Serdary Research Laboratory (London, Ont., Canada). [*methyl*-^{3}H]Choline chloride and [*methyl*-^{14}C]CDP-choline were the products of the Amersham International Limited (Oakville, Ont., Canada). T.l.c. plates (Sil-G25) were obtained from Brinkmann Instrument (Rexdale, Ont., Canada). Phospholipase C (*Bacillus cereus*), CMP and Tween 20 were purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of the highest available grade and were acquired from the Canlab Division of Travenol Canada Inc. (Winnipeg, Manitoba, Canada).

Ethanolamine phosphoglycerides were isolated from porcine heart lipid extract by silica-gel chromatography as previously described [16]. Phosphatidylethanolamine in the ethanolamine phosphoglycerides was preferentially hydrolysed by 0.35 M NaOH, and the plasmenylethanolamine in the fraction was repurified by silica-gel chromatography [16]. 1-Alk-1′-enyl-2-acylgycerol was obtained from plasmenylethanolamine by the hydrolytic action of phospholipase C [17].

Male albino guinea pigs, weighing 250–300 g, were obtained from the University of Manitoba Animal Care Unit. The animals were maintained on Purina chow and tap water *ad libitum* in a light- and temperature-controlled room.

Perfusion of isolated guinea-pig organs

The animal was decapitated under light ether anaesthesia and the heart excised and placed in Krebs–Henseleit buffer (pH 7.4). saturated with 95% O_2 and 5% CO_2. The aorta was cannulated and perfusion was carried out in the Langendorff mode at 37 °C with a coronary flow rate of 4 ml/min [7]. Each heart was initially perfused with Krebs–Henseleit buffer for 15 min in order to restore regular cardiac rhythm. The hearts were then perfused with the same buffer containing 0.5 μM radioactive choline for 15 min, followed by perfusion with 5.0 μM non-radioactive choline for the prescribed period of time. Subsequent to perfusion, 10 ml of air was forced through the cannula to remove the buffer from the vascular space of the organ. The heart was cut open and blotted dry for determination of wet weight before homogenization.

The liver was perfused *in situ* in Krebs–Henseleit buffer as previously described [18]. An inflow cannula was placed in the portal vein and an outflow cannula in the thoracic segment of the inferior vena cava. The hepatic artery and the inferior vena cava above the renal vein was ligated. Each liver was initially perfused with Krebs–Henseleit buffer for 15 min at a flow rate of 5 ml/min for stabilization purposes. The liver was then perfused with the same buffer containing 0.5 μM radioactive choline for 15 min, followed by perfusion with 5.0 μM non-radioactive choline for the prescribed period of time. Subsequent to perfusion, 15 ml of air was forced through the cannula to remove the buffer from the vascular space of the organ. The liver was blotted dry for determination of wet weight before homogenization.

Analyses of choline-containing metabolites

After perfusion the guinea-pig organ was homogenized in chloroform/methanol (1:1, v/v) to a 15% homogenate. The homogenate was centrifuged at 1000 *g* for 10 min and the resultant pellet washed twice with the same solution and recentrifuged under the same conditions. The supernatants were pooled and phase separation was achieved by adding chloroform and water. The diradylglycerophosphocholine (containing phosphatidylcholine, plasmenylcholine and plasmanylcholine) in the organic phase was separated from other phospholipids by t.l.c. with a solvent containing chloroform/methanol/acetic acid/water (35:15:1:2, by vol.) (solvent A) [7]. The diradylglycerophosphocholine fraction was eluted from the silica gel by three washes with chloroform/methanol/acetic acid/water (50:39:1:10, by vol.) and a portion was used for radioactivity determination. For the determination of plasmenylcholine, the diradylglycerophosphocholine eluted from the first chromatogram was applied to another t.l.c. plate. The plate was exposed to HCl vapours for 15 min [19] and developed in solvent A in order to separate the diradylglycerophosphocholine fraction (containing phosphatidylcholine and plasmanylcholine) from the 2-acylglycerophosphocholine fraction (the hydrolytic product of plasmenylcholine). Treatment of the diradylglycerophosphocholine with HCl vapour resulted in the hydrolysis of 92–95% of the vinyl ether group in plasmenylcholine but less than 1% of the acyl groups in phosphatidylcholine. The diradylglycerophosphocholine fraction was eluted and hydrolysis of the acyl groups in phosphatidylcholine and plasmanylcholine was achieved by incubation with 0.35 M NaOH for 60 min. Treatment with NaOH resulted in the hydrolysis of over 99% of the acyl groups. After NaOH treatment the sample was reapplied to a t.l.c. plate. The plate was developed in solvent A to obtain the lysoplasmanylcholine fraction which was formed by hydrolysis of plasmanylcholine. The phosphatidylcholine content was calculated from the amount of diradylglycerophosphocholine before t.l.c. and the lysoplasmanylcholine content after.

Determination of cholinephosphotransferase activity

The guinea-pig organ was homogenized in a buffer containing 0.25 M sucrose, 10 mM Tris/HCl and 1 mM EDTA (pH 7.4). The microsomal fraction was prepared by differential centrifugation as previously described [7]. The microsomes were washed once with the same buffer, and the resultant pellet was resuspended in 0.25 M sucrose buffer. Enzyme activity was determined in an assay mixture (1 ml) containing 40 mM Tris/HCl (pH 8), 10 mM $MgCl_2$, 1 mM EDTA, 0.2 mM [*methyl*-^{14}C]CDP-choline (2 μCi/μmol), 1 mM diacylglycerol or alkenylacylglycerol (in 0.015% Tween 20) and 0.05–0.1 mg of enzyme protein [20]. The reaction was initiated by the addition of the enzyme preparation and the mixture was incubated at 37 °C for 15 min. The reaction was terminated by the addition of 3 ml of chloroform/methanol (2:1, v/v). Water was added to the mixture to cause phase separation. The organic phase was removed and washed three times with 2 ml of 40% methanol. The radioactivity in phosphatidylcholine or plasmenylcholine was confirmed by t.l.c. analysis as described in the preceding section.

When the enzyme activity was assayed with 1,2-diacylglycerol in the presence of 1-alk-1′-enyl-2-acylglycerol or *vice versa*, the choline phosphoglyceride in the organic phase was separated from other phospholipids by t.l.c. The purified choline phosphoglyceride fraction was applied to another t.l.c. plate and the plate was exposed to HCl vapour in order to destroy the vinyl–ether bond. The plate was then developed in solvent A, and the

labelling of plasmenylcholine was estimated from the radioactivity in the lysophosphatidylcholine fraction.

Each experimental point in the kinetic study was calculated from two separate sets of experiments, with a duplicate assay for each set. The apparent K_m was obtained by linear-regression analysis of the data points.

Determination of radioactivity and pool size of CDP-choline

For radioactivity determination, CDP-choline was separated from the other water-soluble choline-containing metabolites by t.l.c. with a solvent containing methanol/0.66 M $NaCl/NH_4OH$ (50:50:5, by vol.) (solvent B) [7]. For pool-size studies, the CDP-choline fraction obtained from t.l.c. was applied to a Dowex AG-1 ion-exchange column, and the sample was eluted with 0.1 M ammonium acetate. The volume of the sample was reduced by lyophilization, reconstituted in 0.1 ml of Tris/HCl buffer (pH 8.5) and hydrolysed to choline with phosphodiesterase and alkaline phosphatase. The choline sample was further purified by t.l.c. with solvent B. Quantification of choline was carried out by the enzymic conversion of choline into [^{32}P]phosphocholine as previously described [7].

Determination of lipid and protein contents

Protein content was determined by the method of Lowry et al. [21]. Lipid phosphorus was determined by the procedure of Bartlett [22]. Vinyl ether groups in plasmenycholine and 1-alk-1′-enyl-2-acylglycerol were determined by the procedure of Gottfried and Rapport [23]. The diacylglycerol and 1-alk-1′-enyl-2-acylglcycerol pools were initially determined by estimating the total methyl ester of dimethylketal contents by g.l.c. The pool sizes of these lipids were confirmed by their acetylated products. Briefly, the diradylglycerols were acetylated with [^{3}H]acetic anhydride, and the acetylated products were separated by t.l.c. as previously described [24]. The amount of diradylglycerol was estimated from the radioactivity in each acetylated product.

RESULTS

Phosphatidylcholine and plasmenylcholine contents in guinea-pig heart and liver

Diradylglycerophosphocholine contents were quantified on the basis of lipid phosphorus determination of each subgroup (Table 1). In the guinea-pig heart, the distribution was 65% phosphatidylcholine, 32% plasmenylcholine and 3% plasmanylcholine. In the liver, the distribution was 95% phosphatidylcholine, 3% plasmenylcholine and 2% plasmanylcholine.

Labelling of choline phosphoglycerides in the isolated guinea-pig heart

The isolated heart was perfused with 0.5 μM labelled choline (10 μCi/nmol) in Krebs–Henseleit buffer for 15 min and then with 5 μM choline in the same buffer for 1–7 h. After perfusion, the heart was homogenized in chloroform/methanol (1:1, v/v) and the radioactivity associated with each choline phosphoglyceride group was determined. The results of the pulse–chase study are depicted in Figure 1. Labelling of phosphatidylcholine and plasmenylcholine shared the same pattern and linear increases in labelling of both phospholipids were maintained from 1 to 5 h of the chase. After this period, the increase in labelling was no longer linear for both phospholipids. Labelling of phosphatidylcholine was about 6-fold higher than that of plasmenylcholine at all chase time points. Labelling of plasmanylcholine was much lower than that of plasmenylcholine and did not appear to reach a maximum even at the end of the chase period.

Labelling of choline phosphoglycerides in guinea-pig liver

The liver was perfused with 0.5 μM labelled choline (10 μCi/nmol) in Krebs–Henseleit buffer for 15 min and then with 5 μM choline in the same buffer for 1–7 h. After perfusion, the radioactivity associated with the each choline phosphoglyceride group was determined (Figure 2). Similarly to the results for the perfused heart, labelling of phosphatidylcholine and plasmenylcholine in the liver shared the same pattern. Linear increases in labelling were maintained by both phospholipids from 1 to 5 h of the chase. However, labelling of phosphatidylcholine was about 60-fold higher than that of plasmenylcholine at all chase time points. Labelling of plasmanylcholine was low and did not bear any similarity to the labelling pattern of phosphotidylcholine or plasmenylcholine.

Labelling of choline phosphoglycerides in relation to the specific radioactivity of CDP-choline

The similarities between labelling patterns of phosphatidylcholine and plasmenylcholine in the heart and liver suggest that these two phospholipids might share a common precursor in both

Table 1 Choline phosphoglyceride contents in guinea-pig heart and liver

The guinea-pig organ was homogenized in chloroform/methanol (1:1, v/v) and the lipid extract was analysed for choline phosphoglyceride contents. Each set of values represents the mean ± S.D. of three separate determinations.

	Content (μmol of lipid P/g wet weight)	
Choline phosphoglyceride fraction	Heart	Liver
Phosphatidylcholine	9.15 ± 1.20 (65%)	15.65 ± 1.38 (95%)
Plasmenylcholine (plasmalogen)	4.48 ± 0.65 (32%)	0.48 ± 0.07 (3%)
Plasmanylcholine	0.38 ± 0.09 (3%)	0.35 ± 0.10 (2%)

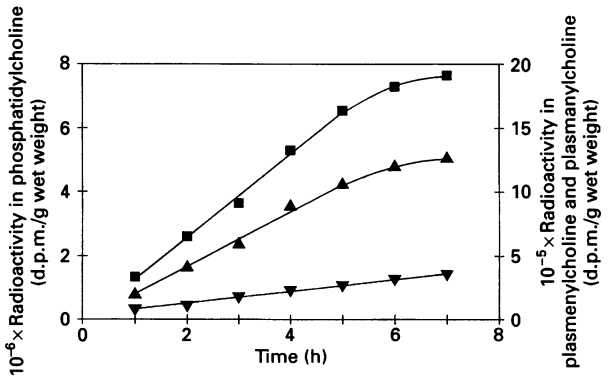

Figure 1 Time course for the labelling of choline phosphoglycerides in guinea-pig heart

Guinea-pig hearts were pulse-labelled with 0.5 μM [*methyl*-^{3}H]choline (10 μCi/nmol) for 15 min and chased with 5.0 μM choline for up to 7 h. They were homogenized after the chase, and radioactivity in the phosphatidylcholine (■), plasmenylcholine (▲) and plasmanylcholine (▼) fractions was determined. Each point is the mean of four separate experiments.

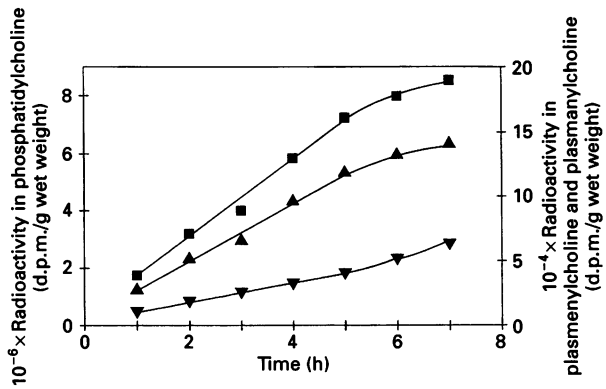

Figure 2 Time course for the labelling of choline phoshoglycerides in guinea-pig liver

Guinea-pig livers were pulse-labelled with 0.5 μM [*methyl*-^{3}H]choline (10 μCi/nmol) for 15 min and chased with 5.0 μM choline for up to 7 h. They were homogenized after the chase, and radioactivity in the choline phosphoglyceride fractions was determined. The symbols are the same as in Figure 1. Each point is the mean of three separate experiments.

Table 2 Specific radioactivity of CDP-choline in relation to the labelling of choline phosphoglycerides in guinea-pig organs

Guinea-pig organs were pulse-labelled with 0.5 μM [*methyl*-^{3}H]choline with a specific radioactivity of 10 μCi/nmol (experiment A) or 25 μCi/nmol (experiment B) for 15 min. Subsequently, the organs were chased with non-labelled choline for 5 h. The labelling of CDP-choline and choline phosphoglycerides as well as the pool size of CDP-choline were determined. Each set of experiments represents the mean ± S.D. of three separate determinations.

		10^{-6} × Radioactivity (d.p.m./g)	
	10^{-6} × Specific radioactivity of CDP-choline (d.p.m./μmol)	Phosphatidylcholine	Plasmenylcholine
Heart			
Expt. A	27.1 ± 6.6	6.8 ± 1.2	1.1 ± 0.3
Expt. B	62.8 ± 9.4	15.5 ± 3.8	2.5 ± 0.5
Liver			
Expt. A	3.7 ± 0.9	7.2 ± 2.1	0.12 ± 0.04
Expt. B	6.6 ± 1.2	12.9 ± 2.6	0.21 ± 0.05

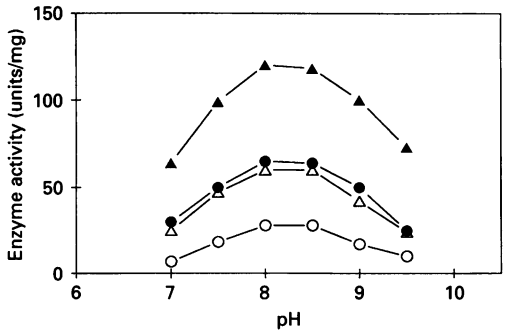

Figure 3 pH profiles of guinea-pig heart and liver CDP-choline–1,2-diradylglycerol cholinephosphotransferases

The activity of the enzyme was assayed at different pH values using 1,2-diacylglycerol (▲, △) or 1-alk-1′-enyl-2-acylglycerol (●, ○) as substrate. The open symbols represent the heart enzyme and the closed symbols the liver enzyme. One enzyme unit is defined as 1 nmol of product formed/15 min at 37 °C. Each point is the mean of two separate experiments.

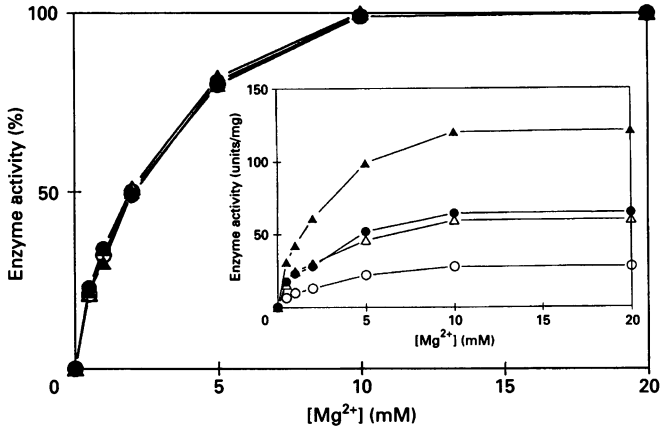

Figure 4 Effect of Mg^{2+} on guinea-pig heart and liver CDP-choline–1,2-diradylglycerol cholinephosphotransferase activities

The activity of the enzyme was assayed with 1,2-diacylglycerol or 1-alk-1′-enyl-2-acylglycerol as substrate in the presence of 0–20 mM Mg^{2+}. The activity obtained with 20 mM Mg^{2+} (inset) is regarded as 100%. The symbols are the same as in Figure 3 and each point is the mean of two separate experiments.

organs. If this were the case, changes in the specific radioactivity of the precursor would have direct and similar consequences on the labelling of phosphatidylcholine and plasmenylcholine. In this study, guinea-pig hearts and livers were pulse-labelled with 0.5 μM [*methyl*-^{3}H]choline at two different specific radioactivities (10 and 25 μCi/nmol). The organs were then chased with 5 μM unlabelled choline for 5 h. The pool size and radioactivity of CDP-choline as well as the labelling of phosphatidylcholine and plasmenylcholine fractions was determined. The pool sizes of CDP-choline in the heart (74 ± 8 nmol/g wet weight) and liver (35 ± 6 nmol/g wet weight) were found to be the same whether the organs were pulse-labelled with choline of low or high specific radioactivity. As shown in Table 2, labelling of phosphatidylcholine and plasmenylcholine in the heart and liver was in direct proportion to the specific radioactivity of CDP-choline. The ratios of labelling between phosphatidylcholine and plasmenylcholine were identical (6-fold) at low and high specific radioactivity of CDP-choline in guinea-pig hearts. In guinea-pig livers, the ratio of the labelling between these two phospholipids (about 60-fold) was also the same for low and high specific radioactivities of CDP-choline.

Formation of phosphatidylcholine and plasmenylcholine *in vitro*

The similarities between labelling patterns and ratios of phosphatidylcholine and plasmenylcholine in the heart and liver suggest that these two phospholipids might share a common precursor. Therefore the ability of both phospholipids to utilize CDP-choline for biosynthesis was explored. Condensation of CDP-choline with diacylglycerol to form phosphatidylcholine, catalysed by cholinesphophotransferase, has been demonstrated in microsomal fractions of guinea-pig heart and liver. In the present study, plasmenylcholine was also formed by condensation of CDP-choline and 1-alk-1′-enyl-2-acylglycerol under identical assay conditions, and the enzyme for this reaction was located exclusively in the microsomal fraction. The cholinephosphotransferase(s) for the two reactions in the heart and liver shared the same pH profile and optimum (8.0–8.5) (Figure 3). Both activities displayed an absolute requirement for Mg^{2+} (Figure 4) and were severely inhibited by Ca^{2+} (Figure 5). Under optimal

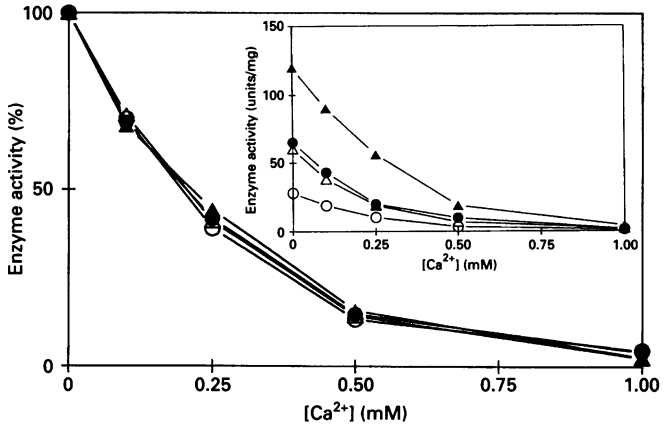

Figure 5 Effect of Ca^{2+} on guinea-pig heart and liver CDP-choline–1,2-diradylglycerol cholinephosphotransferase activities

The activity of the enzyme was assayed with 1,2-diacylglycerol or 1-alk-1′-enyl-2-acylglycerol as substrate in the presence of 10 mM Mg^{2+}. The activity obtained without Ca^{2+} (inset) is regarded as 100%. The symbols are the same as in Figure 3 and each point is the mean of two separate experiments.

conditions, both cholinephosphotransferases in the heart and liver were more active (about 2-fold) in the formation of phosphatidylcholine than plasmenycholine. However, the specific activities of the liver enzyme(s) were higher for both activities than those of the heart enzyme. Both enzyme activities in the liver and heart were inhibited by 1 mM argininosuccinate (results not shown).

Presence of diradylglycerol precursor

Formation of plasmenycholine via the cholinephosphotransferase reaction must be dependent on the availability of diradylglycerol precursors. Therefore, the pool sizes of 1-alk-1′-enyl-2-acylglycerol in guinea-pig heart and liver were determined. The pool sizes of 1,2-diacylglycerol in both organs were also determined for comparative purposes and the results are shown in Table 3. The ratio of 1,2-diacylglycerol to 1-alk-1′-enyl-2-acylglycerol was 42 in the heart and 422 in the liver.

Identity of CDP-choline–1-alkenyl-2-acyl-glycerol cholinephosphotransferase

The similarities in pH profile and response to bivalent metal cations between formation of phosphatidylcholine and plasmenylcholine from CDP-choline suggested that these two reactions might be catalysed by the same enzyme. In order to explore this possibility, both reactions were studied in the presence of 0–10 mM CMP. In previous studies, CMP was shown to be a potent inhibitor of CDP-choline–1,2-diacylgycerol cholinephosphotransferase [25]. As depicted in Figure 6, CDP-choline–1-alk-1′-enyl-2-acyl–glycerol cholinephosphotransferase activity was found to be inhibited by CMP in an identical manner. Further evidence for the identity of the two enzymes was obtained from a heat-stability study. Microsomal fractions from heart and liver were incubated at 55 °C for 0–10 min before assay of both enzyme activities (Figure 7). It is clear that both activities for the heart and liver enzymes were inactivated to the same extent at high temperature.

The identity of the cholinephosphotransferase that synthesizes plasmenylcholine was further examined by kinetic studies. Formation of phosphatidylcholine was assayed at 25–1000 μM 1,2-

Table 3 Diradylglycerol contents in guinea-pig heart and liver

The pool sizes of 1,2-diacylglycerol and 1-alk-1′-enyl-2-acylgycerol in guinea-pig heart and liver were determined. Each set of values represents the mean ± S.D. of three separate determinations.

	Content (μmol/g wet weight)	
	Heart	Liver
1,2-Diacylglycerol	1.40 ± 0.25	2.11 ± 0.15
1-Alkenyl-2-acylglycerol	0.033 ± 0.012	0.005 ± 0.001

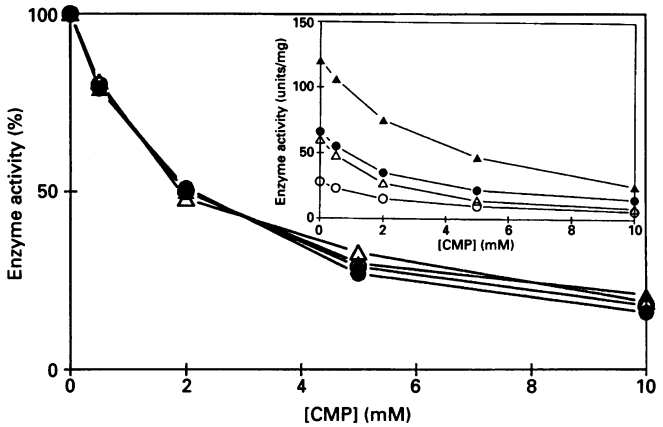

Figure 6 Effect of CMP on guinea-pig heart and liver CDP-choline–1,2-diradylglycerol cholinephosphotransferase activities

The activity of the enzyme was assayed with 1,2-diacylglycerol or 1-alk-1′-enyl-2-acylglycerol as substrate. The activity obtained without CMP (inset) is regarded as 100%. The symbols are the same as in Figure 3 and each point is the mean of two separate experiments.

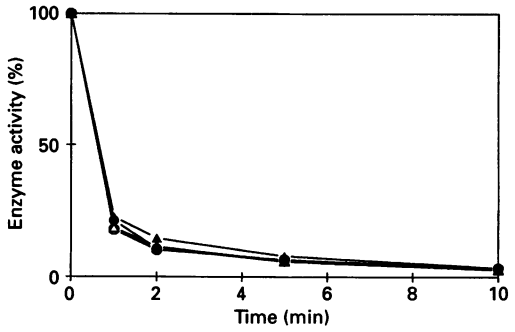

Figure 7 Effect of heat treatment on guinea-pig heart and liver CDP-choline–1,2-diradylglycerol cholinephosphotransferase activities

The enzyme in the microsomal fraction was incubated at 55 °C for 1–10 min. The activity of the enzyme after incubation was assayed with 1,2-diacylglycerol or 1-alk-1′-enyl-2-acylglycerol as substrate. Enzyme activity obtained at 37 °C is regarded as 100%. The symbols are the same as in Figure 3 and each point is the mean of two separate experiments.

diacylglycerol in the absence or presence of 1 mM 1-alk-1′-enyl-2-acylglycerol. By the use of a double-reciprocal plot, the apparent K_m for 1,2-diacylglycerol was estimated to be 450 μM and the reaction was inhibited by 1-alk-1′-enyl-2-acylglycerol (results not shown). Conversely, formation of plasmenylcholine in heart microsomes was assayed at 10–200 μM 1-alk-1′-enyl-2-acylglycerol in the absence or presence of 1 mM 1,2-diacylglycerol. The apparent K_m for 1-alk-1′-enyl-2-acylglycerol was estimated to be 68 μM, and as predicted, the reaction was inhibited by 1,2-diacylglycerol in a competitive manner.

Kinetic studies were also conducted on the enzyme from liver microsomes, as described above for the heart enzyme. Formation of phosphatidylcholine was assayed at 25–1000 μM 1,2-diacylglycerol. Analysis of the data by a double-reciprocal plot revealed that the apparent K_m for diacylglycerol (450 μM) was identical with that found for the heart enzyme. The reaction was inhibited by 1 mM 1-alk-1′-enyl-2-acylglycerol in a competitive manner. Formation of plasmenylcholine was also found to be competitively inhibited by 1 mM 1,2-diacylglycerol. The apparent K_m for 1-alk-1′-enyl-2-acylglycerol (63 μM) for the reaction was very similar to that obtained for the heart enzyme.

DISCUSSION

The present study was designed to answer two specific questions: (1) is CDP-choline the common precursor for biosynthesis of plasmenylcholine and phosphatidylcholine? If so, (2) are these two reactions catalysed by the same enzyme?

Guinea-pig heart and liver were employed as models because of their dissimilar plasmenylcholine contents, which might arise as the result of different modes of biosynthesis. Surprisingly, the ratio of labelled phosphatidylcholine to plasmenylcholine formation was the same as all time points of the chase in both organs, which suggests that the two choline phosphoglycerides have a common precursor. As the CDP-choline pathway has been shown to be the major pathway for phosphatidylcholine biosynthesis [6], CDP-choline may also be the precursor of plasmenylcholine synthesis. The ability of CDP-choline to condense with 1-alk-1′-enyl-2-acylglycerol to form plasmenylcholine in guinea-pig [13] and rabbit [14] heart lends further support to this supposition. The fact that an increase in the specific radioactivity of CDP-choline produced a corresponding increase in the labelling of phosphatidylcholine and plasmenylcholine provides us with compelling evidence that CDP-choline is the common precursor of these two phospholipids.

Despite the notion that these two choline phosphoglycerides share the same precursor, the ratio of phosphatidylcholine to plasmenylcholine labelling is 6:1 in the heart and about 60:1 in the liver. This is in contrast with the ratio of phosphatidylcholine to plasmenylcholine content in the heart (2:1) and liver (30:1). There is therefore a distinct discrepancy between the contents of these phospholipids and their rates of biosynthesis. One explanation is that, in these two organs, plasmenylcholine may also be synthesized by an yet unidentified pathway(s) which does not require choline as a precursor. The metabolic pathways that may contribute to plasmenylcholine biosynthesis have been documented by Ford and Gross [14]. However, a more plausible explanation is that the turnover of plasmenylcholine may be much slower than that of phosphatidylcholine. The turnover rate of plasmenylcholine in guinea-pig organs has not been reported, but the fact that plasmenylcholine is more resilient to phospholipase A [2] supports the notion that its turnover rate is different from that of phosphatidylcholine.

The reason for the relatively low rates of plasmenylcholine biosynthesis in the heart and liver was investigated. Analyses of cholinephosphotransferase in the heart and liver under optimal conditions revealed that the enzyme was twice as active in the biosynthesis of phosphatidylcholine than of plasmenylcholine. The selective utilization of diacylglycerol over 1-alk-1′-enyl-2-acylglycerol by the cardiac cholinephosphotransferase is similar to that reported in rabbit platelets [26], but different from that reported for rabbit heart ethanolaminephosphotransferase which displays a high degree of selectively for 1-alk-1′-enyl-2-acylglycerol [27]. We conclude that the difference in rates of formation of phosphatidylcholine and plasmenylcholine in the heart (6:1) and liver (60:1) could not be solely attributed to the selectivity of the cholinephosphotransferase. As formation of the phospholipid must also be dependent on the respective diradylglycerol concentrations, the pool sizes of 1,2-diacylglycerol and 1-alk-1′-enyl-2-acylglycerol were determined. The 1-alk-1′-enyl-2-acylglycerol content obtained in this study is very similar to that obtained in a previous study [13]. It is surprising to find that the ratio between 1,2-diacylglycerol and 1-alk-1′-enyl-2-acylgycerol is 42:1 in the heart and 422:1 in the liver. From the data obtained from kinetic studies, the apparent K_m of the enzyme for 1,2-diacylgycerol is over 6-fold higher than that for 1-alk-1′-enyl-2-acylglycerol. We postulate that the lower rate of plasmenylcholine biosynthesis in the heart and liver might result from a combination of factors, including the diradylglycerol contents of the tissue, the selectivity of cholinephosphotransferase and the affinity of the enzyme for the respective diradylglycerol. However, attempts to correlate directly the synthesis of choline phosphoglyceride with the diradylglycerol content are hampered by the fact that 1,2-diacylglycerol and 1-alk-1′-enyl-2-acylglycerol are mutually inhibitory for the respective formation of phosphatidylcholine and plasmenylcholine. Furthermore, the exact pool size of diradylglycerol available for choline phosphoglycerol synthesis cannot be estimated as diacylglycerol has been shown to exist in more than one pool in some tissues [28]. It is not clear which pool is the preferred one for choline phosphoglyceride formation.

The origin of 1-alk-1′-enyl-2-acylglycerol has been a subject of debate. It is generally accepted that hydrolysis of plasmenylethanolamine by a phospholipase C and the back-reaction of ethanolaminephosphotransferase are plausible pathways for its formation [13,14]. Some may also arise from plasmenylcholine by similar reactions. If the majority of 1-alk-1′-enyl-2-acylglycerol indeed originates from plasmenylethanolamine, its pool size should be a reflection of the plasmenylethanolamine content of the tissue. This does not appear to be the case in guinea-pig heart and liver as they have similar plasmenylethanolamine contents [1] but a 6-fold difference in 1-alk-1′-enyl-2-acylglycerol content. One facile explanation is that the rate of plasmenylethanolamine hydrolysis by phospholipase C is different in the two organs. It is also possible that some 1-alk-1′-enyl-2-acylglycerol is formed via other pathways.

A polar-head-group-remodelling cycle has been proposed by Ford et al. [27] which facilitates the efficient transfer of vinyl ether linkages in plasmenylethanolamine to the choline glycerophospholipid pool via known enzymic pathways. The essential features of the model include the generation of the vinyl ether linkage in plasmenylethanolamine by the alkyl ether desaturase, with the subsequent generation of 1-alk-1′-enyl-2-acylglycerol from plasmenylethanolamine. Plasmenylcholine is formed from the condensation of CDP-choline with the common pool of 1-alk-1′-enyl-2-acylglycerol. The notion that cholinephosphotransferase is a bifunctional enzyme which catalyses the formation of plasmenylcholine is in agreement with the polar-head-group-remodelling-cycle hypothesis [27]. In a previous study [18], we have shown that the synthesis of phosphatidylcholine via CDP-

choline is inhibited by arginosuccinate. The fact that plasmenylcholine synthesis in guinea-pig liver and heart is also inhibited by argininosuccinate lends further support to the bifunctional property of the cholinephosphotransferase. Our results show that the biosynthesis of phosphatidylcholine and plasmenylcholine are intimately related, and the rate of biosynthesis of each choline phosphoglyceride is subjected to modulation by the respective 1,2-diradylglycerol.

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