Phospholipids and Atherosclerosis

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Preface

The present volume includes a series of reviews, written by experts from different countries and scientific backgrounds, on the biochemistry and pharmacology of phospholipids, in particular on the role of phospholipids in modulating membrane properties and their pharmacological actions with respect to the development of atherosclerotic phenomena.

In our opinion, this book is timely because of the recent discoveries on the effect of phospholipids in cell regulation and the relation between phospholipids and hormonal actions as well as between phospholipids and plasma lipoproteins. The volume therefore should be of interest not only to laboratory investigators devoted to basic research on phospholipids and their relation to atherosclerotic phenomena, but also to clinicians interested in dietary and drug interactions with membrane phospholipids, and how these naturally occurring compounds can modulate membrane properties and enzyme action.

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Dynamic Aspects of Phosphatidylcholine in Biological Membranes

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Most membrane functions require a certain physical state of the membrane. For instance, membrane fluidity has been found to influence processes occurring at the membrane level (enzymic reactions and transport). Membrane fluidity depends on several factors, including the fatty acid composition of phospholipid. It increases when higher proportions of polyunsaturated molecular species are present.

The analyses of the phospholipid composition of membranes from different tissues and of the relative proportion of their subclasses and molecular species show evidence that their composition is maintained at a steady state typical for the tissue or subcellular component considered. On the other hand, membrane components, particularly phospholipids, are continuously renewed. Their renewal is due to catabolic and biosynthetic reactions, which underlie, as a final task, the compositional maintenance of the membrane and, consequently, its functional integrity.

The study of the metabolic processes connected with the turnover and rearrangement of membrane phospholipids is rather puzzling since, concurrently with the synthesis *ex novo* of phospholipid molecules, several reactions take place, leading to the interconversion of one molecule into another. This metabolic event appears quite evident since, in most cases, different portions of a phospholipid molecule turn over at different rates.

This chapter provides a brief outline of the problem connected with the renewal of membrane phospholipids. Particular attention will be given to phosphatidylcholine, which is the most abundant phospholipid in animal tissues, and to brain tissue, because the metabolism of phospholipids in the brain has been extensively studied in this laboratory.

DE NOVO SYNTHESIS OF CHOLINE PHOSPHOGLYCERIDES

As mentioned above, choline phosphoglycerides represent the major class of phospholipids in the membranes of most animal tissues (1). This class is rather heterogeneous in regard to the type of linkage between the hydrocarbon chain and

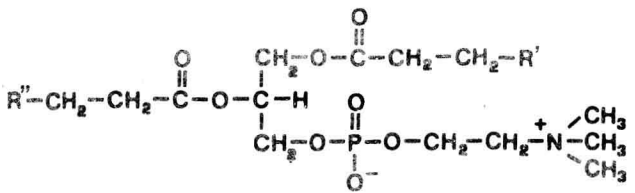
the C_1 of glycerol. On this basis, three subclasses can be distinguished: 1,2-diacyl-*sn*-glycero-3-phosphorylcholine (diacyl-GPC); 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholine (alkylacyl-GPC); and 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphorylcholine (alkenylacyl-GPC or choline plasmalogen). Their structures are indicated in Fig. 1. The relative concentration ratios of these subclasses vary noticeably in different tissues, but generally diacyl-GPC is present at much higher concentrations (44). Within each subclass, several molecular species have been found, depending on the different length and degree of unsaturation of the hydrocarbon chains bound to glycerol.

Diacyl-GPC is synthesized *ex novo* from choline and glycerol or dioxyacetone phosphate (Fig. 2) by the cytidine pathway (28,49). Choline is first converted into cytidine diphosphate (CDP)-choline by two consecutive reactions. The former is catalyzed by choline kinase (EC 2.7.13.2) and the latter by phosphorylcholine cytidylytransferase (EC 2.7.7.15). The second reaction is rate-limiting (37). Phosphorylcholine is then transferred from CDP-choline to diacylglycerol by cholinephosphotransferase (EC 2.7.8.2) producing diacyl-GPC and cytidine monophosphate (CMP). This reaction is reversible in several tissues (5,20,26,41,49). Diacylglycerol can be formed from glycerol phosphate or dioxyacetone phosphate, and phosphatidic acid is its direct precursor. This last reaction is catalyzed by phosphatidate phosphohydrolase (EC 3.1.3.4). The synthesis *ex novo* of one molecule of phosphatidylcholine requires a relatively high amount of energy since the cleavage of high-energy bonds is necessary if choline, glycerol, and two acyl-CoA are considered as precursors.

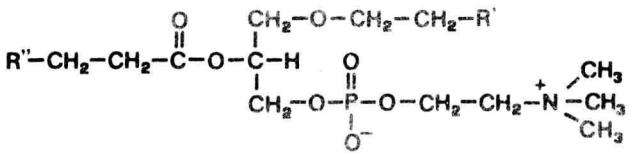
Since phosphatidylcholine is formed from diacylglycerol and phosphatidic acid, one would expect that the molecular species of these two lipids would be very similar. Actually this is not the case. Table 1 gives the composition of the molecular species of phosphatidylcholine, diacylglycerol, and phosphatidic acid of brain tissue. Phosphatidylcholine comprises monoenoic, tetraenoic, and saturated molecular species, and the diglycerides are mostly tetraenoics (39). The compositions of phosphatidic acid and diglyceride are also rather different, despite one being the metabolic precursor of the other.

In order to explain these apparent contradictions, other factors should be taken into consideration: the specificity of biosynthetic enzymes for different molecular species; the parallel occurrence of other reactions that produce or utilize phosphatidic acid, diglyceride, and phosphatidylcholine; the cell compartmentation of biosynthetic enzymes; and, in the case of membrane-bound enzymes, their relative localization. These last two factors are of particular interest since they could be connected with the availability of substrates to these enzymes and to the existence of metabolic pools of precursors and intermediates. All of these factors, in addition to the steady state composition of membrane lipids, contribute to the explanation of different molecular compositions.

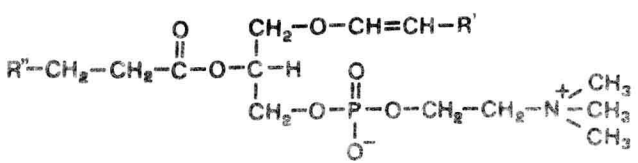
Regarding the specificity of the biosynthetic enzymes, phosphatidate phosphohydrolase and cholinephosphotransferase, several studies have been carried out using different precursors and different tissues (3,14,27). The distribution of labeled



diacyl-GPC



alkyl acyl – GPC



alkenyl acyl – GPC
(choline plasmalogen)

FIG. 1. Structures of cholinephosphoglycerides. Diacyl-GPC, 1,2-diacyl-*sn*-glycero-3-phosphorylcholine; alkylacyl-GPC, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphorylcholine; alkenylacyl-GPC, 1-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphorylcholine.

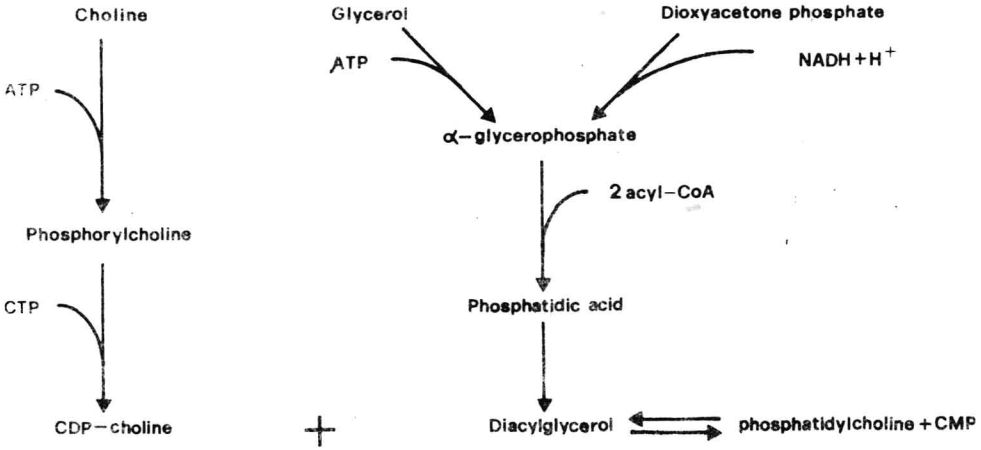


FIG. 2. Synthesis *ex novo* of phosphatidylcholine. ATP, adenosine triphosphate; CMP, cytidine monophosphate; CoA, coenzyme A; NAD, nicotinamide adenine dinucleotide (NADH, reduced form).

TABLE 1. *Composition of brain lipid molecular species*[a]

Fraction	Phosphatidylcholine	Phosphatidic acid	Diglyceride
Saturated	20.2	9.5	2.7
Monoenoic	33.7	14.2	5.1
Dienoic	0.5	13.7	5.0
Trienoic	1.2	4.5	0.9
Tetraenoic	32.5	13.2	71.2
Pentaenoic	3.5	10.1	
Hexaenoic	8.4	34.8	14.2

[a]Data expressed as percentage of the class.
From Porcellati and Binaglia (39), with permission.

choline among molecular species of brain phosphatidylcholine has been studied *in vivo* by Arienti et al. (2). Labeling was determined after very short time intervals from the injection of the radioactive precursor (10–300 sec). The results are shown in Table 2. After 10 sec from injection, monoenoic phosphatidylcholine possesses the highest specific radioactivity followed, in the order, by the tetraenoic and hexaenoic species. Rather low labeling was detected in the saturated molecular species. The time course of the incorporation of labeled choline indicated that monoenoic and tetraenoic phosphatidylcholine are preferentially synthesized, thus resembling the steady state composition (Table 1). If monoenoic diglyceride represents only 5% of the total, then it must have a certain specificity for these molecular species or be more readily available to synthesize enzymes. Another consideration is that choline can be incorporated into phosphatidylcholine by base exchange. This aspect will be discussed later.

TABLE 2. *Specific radioactivities of different phosphatidylcholines after the intracerebral injection of radioactive choline into rats*[a]

Fraction	Time after injection (sec)			
	10	30	60	300
Saturated	0.029	0.162	0.226	
Monoenoic[b]	0.831	6.370	6.560	84.960
Tetraenoic[c]	0.653	4.180	5.450	46.46
Hexaenoic	0.540	1.070	6.05	21.70

[a]The data are expressed as percent of the total recovered radioactivity incorporated in each fraction /μmole × 10^3 (mean values from 10 individual rats).
[b]Plus dienoic.
[c]Plus pentaenoic.
From Arienti et al. (2), with permission.

Other data on the synthesis of choline phosphoglycerides by cholinephosphotransferase have been reported by Roberti et al. (40). They labeled microsomal diglyceride from [^{14}C]glycerophosphate *in vitro* and incubated the labeled microsomes with CDP-choline for different time intervals. In this case, labeled diglycerides were formed from phosphatidic acid only, and the initial distribution of the label into phosphatidylcholine molecular species was very similar to that of diglycerides before incubation. However, a certain specificity for monoenoic diglycerides was also found (40).

All these data are consistent with the hypothesis that the composition of available diglycerides is as important as the specificity of biosynthetic enzymes, at least for the last metabolic step of phosphatidylcholine synthesis. In other words, cholinephosphotransferase can utilize only a certain pool of diglycerides which probably differ in molecular species composition from the total pool present in a particular membrane.

Binaglia et al. (4) have approached this problem by using different experimental models and have concluded that brain microsomal membranes have two pools of diglycerides. The first can be utilized very fast by cholinephosphotransferase since it is probably formed in the membrane area close to the enzyme. The second pool is utilized at a slower rate because it may need a diffusion process which is rate-limiting or may "flip-flop" from one side of the membrane to the other.

Roberti et al. (40) have also reported data on the incorporation of labeled diglycerides, produced *in vitro* from phosphatidic acid, into ethanolamine phosphoglycerides and particularly into their molecular species. In this case, a rather high specificity for polyenoic molecular species of diglycerides could be observed.

It is not known, however, whether the different behavior of choline- and ethanolaminephosphotransferases, concerning the specificity for molecular species of diglycerides, is due to the enzyme or to a differential availability of diglycerides

connected to their relative localization in the membrane. Furthermore, the pool size and composition of diglycerides depend also on the relative concentration of CDP-choline, CDP-ethanolamine, and CMP. Cholinephosphotransferase is competitively inhibited by CDP-ethanolamine; ethanolaminephosphotransferase, by CDP-choline. CMP, which is the product of the phosphotransferase reactions, inhibits the incorporation of CDP-choline and CDP-ethanolamine into the corresponding phosphoglycerides (8,15,16). The inhibition is probably connected with the reversibility of both phosphotransferases (5,20,26,41,49). Furthermore, microsomal membranes possess diglyceride lipase activity, which breaks down diglycerides; very little is known about their specificity and regulation (9,10,22). Diglyceride can be also converted to phosphatidic acid by diglyceride kinase.

From this brief discussion several factors, often interrelated, most likely influence the synthesis *ex novo* of phosphatidylcholine.

SYNTHESIS OF PHOSPHATIDYLCHOLINE BY INTERCONVERSION REACTIONS AT THE POLAR HEAD LEVEL

Several reactions that convert one phospholipid molecule into another take place in the cell. These reactions are extremely important since new phospholipid molecules are formed with low expenditures of energy. Furthermore, at least in some cases, this conversion occurs at the site where a new molecule is required.

One phospholipid can be converted into another by reactions that produce changes at the polar head level. These reactions are responsible for the interconversion of phospholipid classes. Conversion is performed either directly by a single reaction or indirectly by a certain number of reactions. Phosphatidylcholine can be formed from other phospholipids in this way. The most important reactions are summarized in Fig. 3.

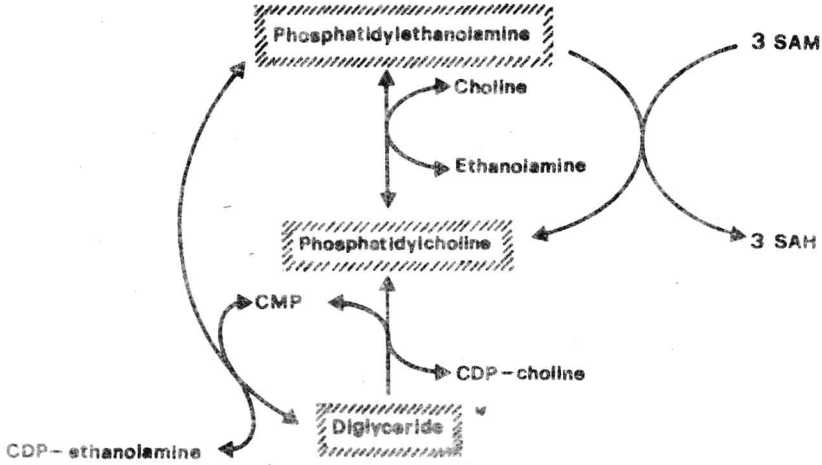


FIG. 3. Synthesis of phosphatidylcholine by interconversion reactions at the polar head level. SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; CMP, cytidine monophosphate.

As shown, phosphatidylcholine is produced from choline and another phospholipid molecule, mainly phosphatidylethanolamine by base-exchange or by *N*-methylation, with *S*-adenosylmethionine (SAM) being the methyl donor. Both mechanisms represent an example of direct conversion of a phospholipid molecule into another since a single reaction is required for the conversion. In the case of the *N*-methylation of phosphatidylethanolamine, three consecutive reactions are necessary; nevertheless, the mono- and dimethylated intermediates are also phospholipids.

Phosphatidylethanolamine can be converted into phosphatidylcholine by another mechanism, which can be considered an indirect conversion since the intermediate is not a phosphoglyceride but a diglyceride. More precisely, diglyceride can be produced from phosphatidylethanolamine by the reversal of the ethanolaminephosphotransferase reaction; then diglyceride can be utilized by cholinephosphotransferase for the synthesis of phosphatidylcholine. Diglyceride could be also formed by phospholipase C, but in this case its origin would be phosphatidylinositol for in animal tissue the enzymes involved show a specificity for this phospholipid class (30).

Base-Exchange Reactions

Base-exchange reactions, shown in Fig. 4, allow the rapid conversion of one phospholipid molecule into another at the membrane level without an apparent requirement of energy. Serine, ethanolamine, and choline can be used as exchanging bases even if the reaction rates are different, with ethanolamine and serine being better substrates than choline. Although other divalent cations are uneffective or strongly inhibitory, Ca^{2+} is required for enzyme activity. Regarding subcellular localization of the base-exchange enzymes, they are mainly localized in the microsomal fraction (38), but a relatively high activity has been found also in the neuronal plasma membrane (19).

A small pool of phospholipids is available for base-exchange. An estimation of the size of this pool has been provided by prelabeling brain microsomal phosphoglycerides with ethanolamine, serine, or choline by base-exchange and then measuring the extent of the displacement of the labeled bases after a short incubation with unlabeled or differently labeled bases (18). The results indicate that microsomal phosphatidylethanolamine can be a substrate for the incorporation of choline by base-exchange, but only 5 to 6% of the total ethanolamine phosphoglyceride is available for the exchange reaction. On the other hand, if microsomal phosphati-

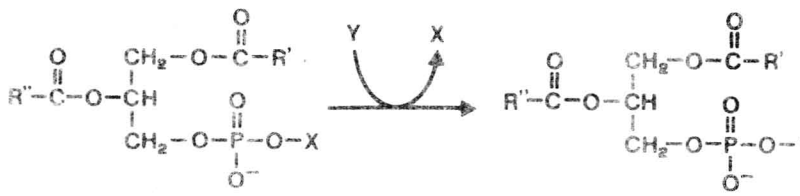


FIG. 4. Base-exchange reactions.

dylethanolamine is prelabeled *in vitro* by the cytidine pathway, choline cannot displace the labeled ethanolamine. Other studies have shown that choline is better incorporated into tetraenoic molecular species (12). There are several indications that base-exchange reactions can take place also *in vivo* (2,36,45), thus supporting the hypothesis that they might have a physiological role unknown at present.

Methylation Pathway

The *N*-methylation pathway for the synthesis of phosphatidylcholine was demonstrated in liver by Bremer and Greenberg in 1961 (6). Later, methyltransferase activities were found in other tissues (11,23,24,31,42,51). Particularly, Hirata and Axelrod (23) have shown that the conversion of phosphatidylethanolamine to phosphatidylcholine in erythrocyte membranes is carried out by two methyltransferases and that the methyl donor is *S*-adenosylmethionine. The reactions are shown in Fig. 5. Methyltransferase I requires Mg^{2+} and is localized on the cytoplasmic site of the membrane. Methyltransferase II, which catalyzes the other two methylations (Fig. 5), is present in the external surface.

According to these authors, this peculiar localization of the two enzymes could facilitate the transfer of phosphatidylcholine to the outer side of the membrane, thus contributing to the maintenance of the asymmetrical distribution of phospholipid in the erythrocyte membrane.

The existence of the *N*-methylation pathway in nervous tissue has been ruled out for several years, but recently it has been demonstrated in different laboratories (11,31,32,51). Mozzi and Porcellati (31,32) have reported that the methyl groups of *S*-adenosylmethionine can be incorporated into phosphatidylcholine of a rat brain

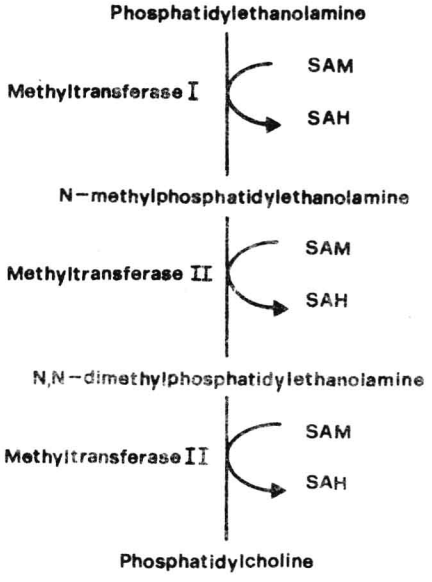


FIG. 5. Biosynthesis of phosphatidylcholine by *N*-methylation pathway. SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.