Review

Choline phospholipids: molecular mechanisms for human diseases: A meeting report

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Choline phospholipids have long been recognized as important structural components of membranes. There is a growing body of evidence that some choline phospholipids (phosphatidylcholine, sphingomyelin, plasmalogens, and their metabolites) also are important mediators and modulators of transmembrane signaling. Another choline phospholipid (platelet activating factor) is an important intercellular messenger acting on leukocytes, platelets, smooth muscle cells, liver, uterus, and the central nervous system. These functions may explain how choline phospholipids, such as lecithin, influence normal physiological processes as well as a diverse group of pathological processes, including cancer and Alzheimer's disease. Recent findings on the molecular actions of choline phospholipids were presented by leading experts in the field at a conference sponsored by the University of North Carolina and held in conjunction with the annual meeting of American Institute of Nutrition in April 1992. This review provides general background on choline and phospholipids in nutrition, metabolism, and signal transduction, and highlights the findings presented at this conference.

Keywords: Alzheimer's disease; cancer; choline; lecithin; phospholipids; plasmalogen; platelet activating factor; signal transduction; sphingomyelin; uterus

Introduction

First identified in mammalian tissues over 130 years ago, choline has long been recognized as an important nutrient involved in phospholipid and acetylcholine biosynthesis and in biological methylation reactions *(Figure 1)*.[1] It appears to be an essential nutrient for humans,[2,3] but no recommended daily allowance has been designated. Recently, choline phospholipids have been identified as precursors of important intracellular messengers (1,2-*sn*-diacylglycerol, ceramide, and sphingosine). These messengers are generated during transmembrane signal transduction, the cascade of reactions that translates a signal on the surface of a cell to the interior, influencing intracellular metabolism, cell divi-

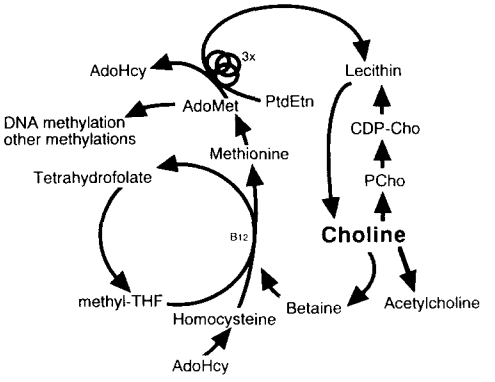

Figure 1 Metabolism of choline. Choline metabolism is inter-related with folate, B_{12}, and methionine metabolism. Abbreviations: PCho, phosphocholine; CDP, Cho-cytidine diphosphocholine; PtdEtn, phosphatidylethanolamine; AdoMet, *S*-adenosyl methionine; AdoHcy, *S*-adenosyl homocysteine; THF, tetrahydrofolate.

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sion, ion transport, and gene expression.[4,5] Plasmalogens, found in very high concentrations in myocardial tissue, are choline-phospholipids that generate arachidonic acid, a precursor for the synthesis of prostaglandins. Another choline phospholipid, platelet activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; PAF) is an important intercellular messenger.[6] Thus, choline phospholipids influence numerous physiologic and pathologic processes that are of interest to nutritional biochemists. A major international workshop on this subject ("Choline Phospholipids: Molecular Mechanisms for Human Diseases," San Diego California, April 3–5, 1992) discussed results from a broad range of recent studies by leading experts in the field. This review will summarize some of these recent findings and provide general background on choline phospholipids in nutrition and metabolism.

Choline in the diet

Choline, mostly in the form of lecithin, can be readily obtained in the diet from a wide variety of foods. Organ meats, eggs, soybeans, nuts, wheat germ, and spinach are particularly good sources.[7,8] Lecithin is also available over-the-counter as a nutritional supplement. Recently, one manufacturer began marketing a choline-supplemented sports drink, based on some suggestive evidence that it may improve endurance in runners.[9]

Choline is required in the diet of several animal species, including the dog, cat, rat, guinea pig[7] and humans.[2] At the Choline Phospholipid conference, Dennis Vance (University of Alberta, Alberta, Canada), discussed the mechanism responsible for fatty infiltration of the liver during choline deficiency.[10] There is an absolute requirement for lecithin as a component of very low density lipoproteins (VLDL) that are secreted by the liver.[11,12] Lecithin appears to allow the VLDL forming in the endoplasmic reticulum (ER) to "bud" and be released. Lecithin does not appear to be involved in the synthesis of high density lipoproteins (HDL).[13] Vance noted that in choline deficiency the ApoB-100 and ApoB-48 content of VLDL decreases. These apoproteins, synthesized in the ER, are ligands for certain cell receptors and are essential for normal VLDL metabolism. Other animal studies have shown choline deficiency results in decreased growth, infertility, abnormal kidney function, decreased red blood cell synthesis, high blood pressure, and liver cancer.[1]

Vance also described how the choline-deficient rat model had been used to characterize the regulation of lecithin biosynthesis. Choline deficiency results in decreased hepatocyte levels of lecithin and increased binding of CTP:phosphocholine cytidylyltransferase (CT), a key regulatory enzyme in lecithin synthesis, to the ER membrane. The active form of CT is membrane associated, while an inactive reservoir of the enzyme exists in the cytosol.[14] Claudia Kent (University of Michigan, Ann Arbor, MI) presented evidence that CT translocation is regulated by phosphorylation/dephosphorylation reactions.[15] This mechanism of inactivation/activation of CT has been identified in a variety of cell types. CT is down-regulated (i.e., decreased membrane binding) by intracellular lecithin, and is stimulated by 1; 2-*sn*-diacylglycerol (DAG). Although CT had always been thought primarily to be associated with the ER membrane (or in the cytosol in the inactivated form), Kent presented evidence that CT can translocate to the nuclear envelope, and that soluble CT can be identified in the nucleoplasm.[15,16] Thus, CT and lecithin biosynthesis may be involved in gene expression and signal transduction in the nucleus. These new observations require a reevaluation of existing dogma about CT's site of action within the cell.

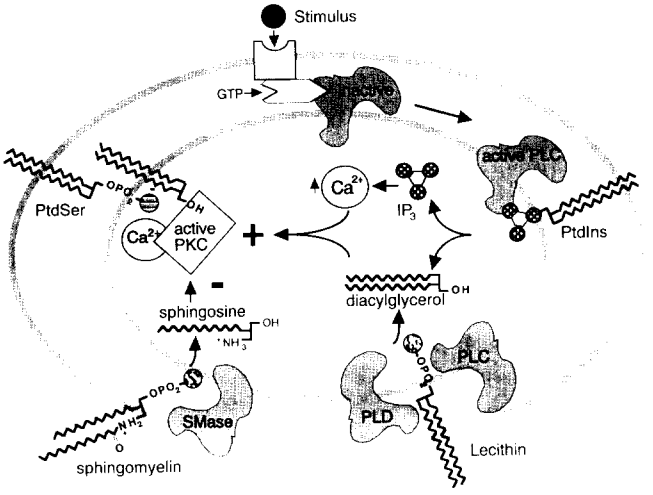

Figure 2 Simplified overview of signal transduction. When an appropriate receptor is stimulated by an agonist, there is a change in the conformation of a GTP binding protein (G-protein). This causes the activation of a phospholipase C (PLC) specific for phosphatidylinositol bis-phosphate (PtdIns). Activated PLC hydrolyzes PtdIns to form inositol-1,4,5-trisphosphate (IP_3). IP_3 acts to release calcium from intracellular storage sites. PtdIns hydrolysis also forms diacylglycerol. At the same time, receptor activation stimulates lecithin-specific PLC and phospholipase D (PLD) activity, which enhances diacylglycerol formation. Calcium, diacylglycerol, and phosphatidylserine (PtdSer) activate protein kinase C (PKC). Sphingomyelinase (SMase) activity forms sphingosine, which eventually inhibits PKC activity and terminates the signal.

Choline deficiency is also associated with changes in the lipid composition of membranes. In particular, the ratio of bilayer lipids (such as lecithin) to non-bilayer lipids (such as cholesterol and fatty acids) decreases. These membrane changes may affect signal transduction and other cell processes.

Lecithin and intracellular signal transduction

Signal transduction is an essential process that "translates" signals received from outside the cell into important activities inside the cell. The process begins with some extracellular stimulus—such as a hormone, growth factor, or neurotransmitter—that triggers a cascade of reactions inside the cell, culminating in some change in metabolism, ion or nutrient transport, growth, or gene expression *(Figure 2)*. Choline phospholipids in cell membranes play a role in generating various second messengers in the cascade.[5,17,18]

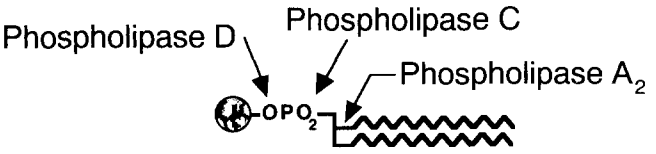

Figure 3 Sites of action of phospholipases. Phospholipase D forms choline and phosphatidic acid from lecithin. Phospholipase C forms diacylglycerol and phosphocholine. Phospholipase A_2 forms lysolecithin and free fatty acid.

As shown in *Figure 2,* the extracellular stimulus first binds to a specific receptor on the cell surface. Receptor-ligand interaction leads to altered conformation of the receptor so that it can activate a G-protein. In the inactive state, guanosine diphosphate (GDP) is bound to the G-protein. Activation triggers replacement of GDP with GTP and results in the subsequent activation of phospholipase C activity (PLC; generates 1,2-*sn*-diacylglycerol (DAG) and an aqueous soluble head group) and phospholipase D (PLD; generates phosphatidic acid and choline; *Figure 3*) within the plasma membrane. It is believed that specific receptors couple to specific phosphatidylinositol bis phosphate (PtdIns)-PLC isotypes.[19] In a similar manner, specific receptors appear to be linked to activation of specific lecithin-PLCs and PLDs.[5] Products generated by PtdIns-PLC include inositol-1,4,5-trisphosphate (IP_3) and DAG; lecithin-PLC generates DAG *(Figure 2).* IP_3 is a water soluble product, which acts to release calcium from stores in the endoplasmic reticulum. This increase in cytosolic calcium makes more calcium available for binding to PKC isotypes that are Ca^{2+} dependent. These phosphatide hydrolysis products (Ca^{2+} and DAG) serve as second messengers that act on a key regulatory enzyme in the cascade: protein kinase C (PKC). The object of a great deal of current research, this enzyme activates or inactivates a broad range of proteins downstream in the cascade, including many enzymes, membrane receptors, ion channel proteins, and contractile proteins.[20]

At the Choline Phospholipid conference, John Exton (Vanderbilt University, Nashville, TN USA) discussed how other phospholipases become involved in the signal transduction cascade.[21] Studies of the time course of DAG generation in a number of cell types show a prolonged phase of agonist-induced DAG production occurring later than the hydrolysis of phosphatidylinositides (the first phosphatides hydrolyzed during the signaling cascade). The acyl groups present in this DAG are consistent with its formation from lecithin catalyzed by PLC and PLD *(Figure 3).* The hydrolysis of lecithin occurs in response to a range of agonists, some of which activate lecithin-specific PLCs and PLDs via G-proteins.[5] The P2y-purinergic receptor (agonists: ATP and ADP) and the muscarinic receptor (agonist acetylcholine) operate via this mechanism.[5,22] Other agonists trigger PLD-mediated lecithin hydrolysis via activation of PKC. Phorbol esters activate PKC without triggering PtdIns breakdown, stimulate the release from lecithin of choline, DAG, phosphatidic acid (can be converted to DAG by phosphatidic acid phosphohydrolase), and arachidonic acid (formed by phospholipase A_2).[5] PLD can be directly activated by DAG and phorbols,[23] but there is reason to believe that usually PKC is involved as an intermediary. Inhibitors of PKC and down regulation of PKC attenuate lecithin hydrolysis. Exton suggested that the ability of PKC to activate phospholipase D may be mediated by a separate catalytic site that does not involve an ATP-dependent phosphorylation.[24]

One of the most challenging research areas in signal transduction relates to specificity. How can the vast number of different signals that impinge on cell surfaces work through a common reaction cascade, yet produce such varied effects on cell growth and function? At least part of the answer appears to lie in the diversity and specificity of various enzymes and second messengers in the reactions. Thus there appears to be not one, but a myriad of cascades. Addressing this question at the conference, I. Bernard Weinstein (Columbia University, New York, NY USA) noted that multiple isoforms of PKC have been identified.[25] Only some PKC isotypes are Ca^{2+} dependent (PKCα, $\beta_{1/2}$, and γ; PKC δ, ϵ, ζ, θ, and η lack the calcium binding C2-domain of PKC and therefore are not calcium dependent).[20] The various PKC isoforms also respond differently to DAG. Weinstein noted that different PKC isotypes have differing biologic effects, and the same isotype can have different effects when over expressed in different tissues. For example, over expression of $PKC_{\beta 1}$ in a colon cancer cell line inhibits cell growth, while in fibroblasts over expression of $PKC_{\beta 1}$ enhances growth.

Sphingomyelin and signal transduction

Whereas second messengers generated by hydrolysis of PtdIns and lecithin generally stimulate PKC and the signaling cascade, those arising from sphingomyelin (SM) have an inhibitory influence. SM is hydrolyzed by sphingomyelinase in response to some external cell stimulus, generating ceramide and phosphocholine. Ceramide attenuates cell proliferation and stimulates differentiation. Further hydrolysis of ceramide yields a fatty acid and sphingosine; the latter has been shown to inhibit PKC by blocking DAG stimulation, as well as inhibit cell differentiation and growth, and tumor growth.[26] At the conference, Alfred Merrill (Emory University, Atlanta, GA USA) presented recent studies showing that fumonisins, mycotoxins produced by *Fusarium moniliforme,* are potent inhibitors of *de novo* sphingolipid synthesis in hepatocytes, renal cells, and cerebellar neurons.[27] As a result, free sphinganine and sphingosine accumulate and chronically inhibit PKC. This disruption in the signaling cascade may underlie the toxicity of fumonisins, which have been shown to cause leucoencephalomalacia and hepatotoxicity in horses, pulmonary edema in pigs, liver cancer in rats, and have been associated with esophageal cancer in humans.

Platelet-activating factor

Stephen Prescott (University of Utah, Salt Lake City) described recent research on platelet-activating factor

(PAF), another choline-containing phospholipid similar in structure to lecithin. Once thought to be involved primarily in the activation of blood platelets, clotting, and inflammation, PAF has more recently been shown to play a role in such diverse functions as pulmonary regulation and allergic reactions.[28] PAF has thrombotic and inflammatory properties via its action on white blood cells, platelets, and smooth muscle cells. On endothelial cells, PAF facilitates neutrophil binding. Overproduction of PAF may result in a hyperresponsive state, as with asthma. Thus the regulation of PAF synthesis and breakdown, the focus of Prescott's studies, is a critical area of ongoing research.

Prescott reported that other oxidized phospholipids, similar in structure to PAF, may mimic some of its effects. PAF is synthesized either *de novo* or by the remodeling pathway. The latter is the most thoroughly studied and appears to be the most operative in inflammatory responses. PAF synthesis is initiated when a ligand binds to a receptor belonging to the G-protein family. This increases Ca^{2+} levels and PKC activity, which in turn stimulates phospholipase A_2(PLA_2) to hydrolyze phospholipid, generating lysophospholipid, which is then acetylated by a specific acetyltransferase, generating PAF. PAF is metabolized by PAF acetylhydrolase, a PLA_2 that is specific for phospholipids with a short acyl group in the *sn*-2 position. This enzyme is found in plasma, tissues, and in high-density and low-density lipoproteins. Prescott's group has shown that PAF can remain associated with surfaces of cells that synthesize it, where it can convey signals to adjacent cells.

Choline phospholipids and the molecular mechanisms underlying physiologic and disease processes

A number of presentations at the conference related abnormalities in choline phospholipids and signal transduction to physiologic and disease states, particularly pregnancy, cancer, Alzheimer's disease, and myocardial ischemia.

Pregnancy

Jack Johnston (University of Texas at Dallas, Dallas, TX USA) discussed the role of PAF in the implantation of a fertilized egg in the uterine wall, maturation of the fetus, and induction of labor.[29] He described how PAF levels in fetal lung tissue increase as the fetus matures. This stimulates glycogen breakdown. PAF is converted to ethanolamine plasmalogens in amnion derived cells. These plasmalogens are precursors for arachidonic acid formation, with subsequent production of prostaglandins. PAF stimulates prostaglandin formation (PGE_2) by fetal membranes and increases intracellular calcium concentrations in myometrial cells. At the same time that there is an increase in PAF biosynthesis within the fetus, maternal levels of the enzyme that inactivates PAF, PAF-acetylhydrolase, decrease due to the action of various steroid hormones (estrogens inhibit activity; glucocorticoids and progestins increase activity; progestins also stimulate the secretion of PAF from macrophages, the probable origin of plasma PAF). The net effect is an increase in PAF levels in maternal blood toward the end of pregnancy, inducing uterine contractions and the onset of labor.

Cancer

Choline deficiency has been associated with hepatocarcinogenesis in many animal studies.[30] Early signs of the disease include fatty infiltration of the liver, followed by cell death, fibrosis, cirrhosis and, eventually, carcinoma. Animals fed choline-free diets develop cancer spontaneously[31] and are markedly sensitized to carcinogens.[32] Dietary supplementation with choline, methionine, or both, reduced the incidence of liver cancer in animals exposed to a carcinogen.[33]

Several mechanisms have been proposed for the development of cancer during choline deficiency.[32] At the conference, Steven Zeisel (University of North Carolina at Chapel Hill, Chapel Hill, NC USA) presented data that suggest that perturbations in phospholipid-mediated signal transduction may be the critical events triggering carcinogenesis in choline deficiency.[34] In the study,[30] rats were fed either a choline-deficient or normal chow (control) diet. Compared with controls, those fed the deficient diet had increased liver plasma membrane levels of DAG and PKC activity. After 52 weeks, only 8% of the animals had normal livers, 69% had multiple atypical hepatic foci, and 23% had hepatocarcinomas. Control animals all had normal livers. Zeisel suggested that the increased levels of DAG and chronic stimulation of PKC were related to the liver abnormalities and the onset of liver cancer in these animals.

Weinstein speculated at the conference on the role of PKC in the development of colon cancer, the second most common form of cancer and one associated with the consumption of a diet high in fat. He cited results of one study in which colonic bacteria exposed to high levels of bile acids and phospholipid produced high levels of DAG.[35] He suggested that chronic high levels of DAG may over stimulate PKC and other downstream regulatory proteins, resulting in increased cell proliferation and tumor formation.

Alzheimer's disease

Alzheimer's disease (AD) is a degenerative disease involving a progressive loss of memory and mental function, eventually leading to profound dementia. The etiology of AD is unknown. Brain lesions in AD exhibit amyloid deposition, neurofibrillary tangles, and neural degeneration. Recent postmortem studies of brain samples from AD patients showed lower levels of acetylcholine (ACh) and membrane lecithin in cholinergic neurons.[36] Richard Wurtman (Massachusetts Institute of Technology, Cambridge, MA USA) speculated at the conference that these membrane abnormalities and the amyloid deposits may be related.[37,38] Amyloid precursor protein (APP), a normal brain constituent, can be degraded by the enzyme secretase, which destroys

the beta amyloid portion. Alternatively, APP can be processed by cellular lysosomes, which generate the beta amyloid fragments associated with AD. Although both pathways appear to operate in normal brain tissue, it is thought that APP is shunted more to the lysosomal pathway in AD.[39] Secretase requires activation by PKC, and PKC activity in defective neurons may be reduced.[39,40] At the conference, Wurtman described a theory of AD based on the "autocannabalism" of membrane lecithin.[37] In cholinergic neurons, lecithin serves as a structural component of the membrane, a reservoir for choline used in ACh synthesis, and a source for the generation of second messengers in signal transduction. This multiple role of lecithin may underlie the selective vulnerability of cholinergic neurons in AD. When these neurons are active, i.e. (secreting large amounts of ACh) they obtain additional choline by breaking down membrane lecithin. Further, the kinetics of the enzymes that convert choline to ACh (choline acetyltransferase, CAT) and lecithin (choline kinase, which generates phosphocholine) favor the formation of ACh instead of lecithin when the neurons are activated. This situation over time leads to a decrease in the quantity of membrane lecithin, a decrease in synaptic surface area, and ultimately jeopardizes the health of the neuron.[41] The depletion of membrane phospholipids may compromise membrane integrity, increasing lysosomal processing of APP and increasing production of beta amyloid.

The autocannabalism theory is supported by several lines of evidence. People dying of AD show significant reductions in cellular levels of lecithin and phosphatidylethanolamine and increased levels of the phospholipid metabolites glycerophosphocholine and glycerophosphoethanolamine.[38] Postmortem analyses show increased lecithin turnover and diminished choline availability. Nitsch et al.[42] showed that amyloid formation increased as a result of excessive activation of cholinergic receptors and appeared to be mediated by PKC. Thus, another line of evidence suggests that the abnormalities of AD may also involve disruption of phospholipid-mediated cell signal transduction. Additional research is needed to explore the relation between the decline in membrane lecithin and phospholipids, PKC-mediated signal transduction, amyloid formation, and the clinical manifestations of AD.

Myocardial infarction

Richard Gross (Washington University, St. Louis, MO) described recent studies of phospholipid breakdown during myocardial infarction.[43] The cell membrane of the heart muscle (sarcolemma) contains high levels of plasmalogen, a choline-containing phospholipid. Myocardial ischemia is accompanied by detrimental changes in ion transport across the sarcolemma. Membrane phospholipid content and structure can influence ion transport. Gross suggested that the adverse sequelae of acute myocardial ischemia may be a result of plasmalogen breakdown and the build-up of amphiphilic lipids during an ischemic attack. Support for this theory comes from recent studies demonstrating a new PLA_2 enzyme in the sarcolemma. Whereas most PLA_2 enzymes were thought to be calcium dependent, this new PLA_2, which hydrolyzes plasmalogen, is calcium independent and activated by ATP. Gross suggested that during myocardial ischemia, ATP levels drop and then increase, activating this PLA_2, which degrades plasmalogen and releases arachidonic acid, which in turn may alter membrane permeability and ion transfer. Future research may focus on development of drugs to inhibit the newly discovered plasmalogen specific PLA_2 enzyme.

Conclusion

Lecithin, sphingomyelin, and other choline-phospholipids provide important second messengers in transmembrane signaling. Abnormalities in signal transduction and/or phospholipid metabolism appear to be related to certain disease states including cancer and Alzheimer's disease. Platelet activating factor, another choline phospholipid, functions in the lung, uterus, liver, brain, and cardiovascular system. Excessive catabolism of plasmalogen, a choline-containing phospholipid found in the cardiac muscle membrane, appears to be related to the adverse sequelae of myocardial infarctions.

In the past phospholipids have been recognized as being biologically important without a complete understanding as to why. The new findings of the role of choline phospholipids in intra- and intercellular signaling provide molecular bases for the varied roles these substances play in health and disease states. Additional research is needed to further elucidate the critical controlling events in choline-phospholipid metabolism and to determine whether or how dietary or pharmacologic manipulations of these processes can be used to enhance health and modulate disease states.

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