Choline phospholipids: signal transduction and carcinogenesis[1]

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ABSTRACT **Phospholipids act as vital elements in transmembrane signaling. Agonist-induced hydrolysis of phosphatidylinositides has been established as a major mechanism for transmitting messages into the interior of cells via protein phosphorylation cascades, ultimately regulating gene transcription. There is a growing body of evidence that choline phospholipids (phosphatidylcholine, sphingomyelin, and their metabolites) also are important mediators and modulators of transmembrane signaling. These functions may explain how choline phospholipids influence normal physiological processes as well as a diverse group of pathological processes.—Zeisel, S. H. Choline phospholipids: signal transduction and carcinogenesis. *FASEB J.* 7: 551-557; 1993.**

Key Words: phosphatidylcholine • lysophosphatidylcholine • sphingomyelin • sphingosine • ceramide • diradylglycerol • protein kinase C • rat • hepatocarcinoma

CHOLINE PHOSPHOLIPIDS

Choline is a quaternary amine that is ubiquitously distributed in all cells, mostly in the form of the phospholipids: phosphatidylcholine (PtdCho), lysophosphatidylcholine (lysoPtdCho), choline plasmalogen, platelet-activating factor, and sphingomyelin (SM)—essential components of all membranes (1). Choline is an essential nutrient for humans (2, 3). The glycerol-based lipid, PtdCho, is the predominant phospholipid (> 50%) in most mammalian membranes. It is formed from diradylglycerol (DRG) and cytidine diphosphocholine (**Fig. 1**) or by the sequential methylation of phosphatidylethanolamine. LysoPtdCho is formed by a deacylation of PtdCho catalyzed by phospholipase A_2 (Fig. 1). SM is synthesized from ceramide (sphingosine with an amide-linked fatty acid) and a phosphocholine group (derived from PtdCho; Fig. 1). Approximately 5–20% of the phospholipid in tissues is SM.

Proteins, floating in a sea of these phospholipids, have traditionally been regarded as the key elements responsible for important membrane functions. During the last decade, we have begun to understand how phospholipids act as vital elements in transmembrane signaling. Most attention has been focused on the role of phosphatidylinositides [phosphatidylinositol (PtdIns) bisphosphate] in this process (4). There is a growing body of evidence that choline phospholipids (PtdCho, SM, and their metabolites) are important mediators and modulators of signal transduction.

CURRENT CONCEPTS OF SIGNAL TRANSDUCTION

Signals are transmitted across membranes via a highly interactive network of molecular events, which include positive and negative feedback loops. In the simplest form, an extracellular sensor detects a signal and transmits this message into the interior of the cell via a protein phosphorylation cascade to a final component that acts as a transcriptional regulator.

Messengers that induce phospholipid turnover include: acetylcholine (M1 receptor), norepinephrine (α1 receptor), epinephrine (α1 receptor), dopamine, histamine (H1 receptor), serotonin, vasopressin (V1 receptor), angiotensin II, cholecystekinin, gastrin, pancreozymin, substance P, bradykinin, thromboxane, thrombin, collagen, platelet-activating factor, secretagogues, growth factors, and mitogens. Cell-surface receptors fall into three main types: those that are GTP-binding protein (G-protein) linked, those with associated catalytic functions, and those that operate ion channels.

The G-protein linked receptors characteristically share a seven-pass transmembrane structure typified by the β-adrenergic receptor and rhodopsin. The extracellular domain is composed of transmembrane helices that form a ligand binding pocket; the intracellular domain contains regions that interact with the G-protein, as well as regions with several phosphorylation sites involved in receptor desensitization (5). Receptor-ligand interaction leads to altered conformation of the receptor so that it can activate the G-protein (**Fig. 2**). These G-proteins are a highly conserved family of membrane-associated heterotrimeric proteins composed of α, β, and γ subunits. In the inactive state, guanosine diphosphate (GDP) is bound to the complex. Activation triggers replacement of GDP with GTP, with subsequent dissociation of the α subunit from β and γ subunits. The activated GTP-α stimulates the next effector protein in the signal cascade. Then GTPase activity hydrolyzes GTP, forming GDP-α, which reassociates with the β and γ subunits (4). To permit the G-protein to pass back to the active state in

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[2]Abbreviations: lysoPtdCho, lysophosphatidylcholine; PtdCho, phosphatidylcholine; SM, sphingomyelin; DRG, diradylglycerol; PtdIns, phosphatidylinositol; G-protein; GTP-binding protein; GDP, guanosine diphosphate; PLC, phospholipase C; DAG, 1,2-sn-diacylglycerol; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; AAG, 1-alkyl-2-acyl glycerols; PLA_2, phospholipase A_2; GGT, γ-glutamyl transpeptidase; GST, glutathione-S-transferase; TG, triglyceride; NF1, neurofibromatosis type 1; GAP, GTPase-activating protein; PAF, platelet-activating factor; Ins-1,4,5-P_3, inositol-1,4,5-trisphosphate; SMase, sphingomyelinase; PLD, phospholipase D.

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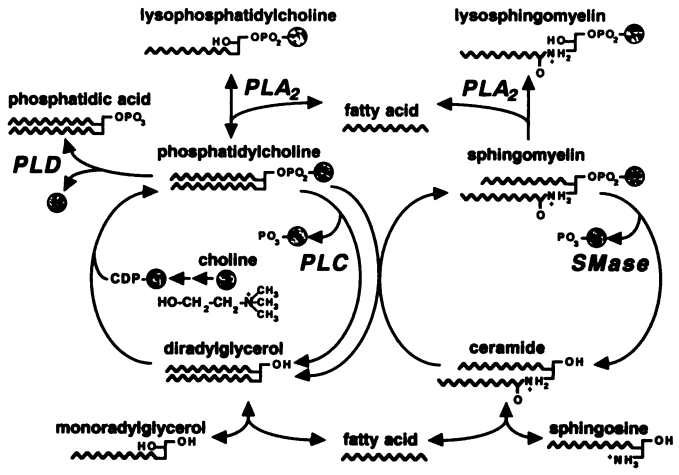

Figure 1. Pathways for metabolism of phosphatidylcholine and sphingomyelin. Phosphatidylcholine synthesis from choline, and its hydrolysis by phospholipase C (PLC), phospholipase D (PLD), and phospholipase A_2 (PLA_2), are depicted. Sphingomyelin synthesis from phosphatidylcholine and ceramide, as well as its hydrolysis by sphingomyelinase (SMase) and ceramidase, are also depicted.

response to a receptor activation, GDP must be released, allowing replacement with GTP (6). The intrinsic release of GDP is so slow that significant release would not occur without an exchanger activity associated with the receptor. At this time we do not know whether these exchangers are regulated by growth factors.

The activation of the G-protein results in the subsequent activation of phospholipase C (PLC) activity within the plasma membrane (Fig. 2). The PLCs are a family of phosphodiesterases that hydrolyze the glycerophosphate bond of intact phospholipids to generate 1,2-*sn*-diacylglycerol (DAG) and an aqueous soluble head group. Numerous PtdIns-specific PLCs exist, including proteins varying from 70 to 154 kDa in size, designated β through γ (4). All share two highly conserved domains that form the catalytic region. It is believed that specific receptors couple to specific PtdIns-PLC isotypes (4). In a similar manner, specific receptors appear to be linked to activation of specific PtdCho-PLCs (7) (see later discussion).

The action of PLC triggers the next event in the signal cascade, which is the activation of protein kinase C (PKC; serine/threonine kinase). The first step in PKC activation is the formation of a ternary enzyme + Ca^{2+} + phospholipid-membrane complex. Products generated by PtdIns-PLC include inositol-1,4,5-trisphosphate (Ins-1,4,5-P_3) and DAG (Fig. 2). Ins-1,4,5-P_3 is a water-soluble product that acts to release calcium from stores in the endoplasmic reticulum. This increase in cytosolic calcium makes more calcium available for binding to PKC isotypes, which 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) (8). Of the at least 10 isoforms of PKC encoded by distinct genes (**Fig. 3**) (9), only α, δ, and ζ isoforms have been identified in the liver (10). Calcium increases the tightness of association of these PKCs with the membrane, thereby increasing membrane occupancy. This facilitates binding of DAG, which as described earlier is the other product of PLC activity. The DAG-PKC complex approaches the membrane more closely, placing the kinase in a pocket of negatively charged phosphatidylserine head groups into which Ca^{2+} is attracted. Thus, DAG increases the affinity of PKC for calcium. Normally PKC is folded so that an endogenous "pseudosubstrate" region on the protein is bound to the catalytic site, thereby inhibiting activity (Fig. 3). The combination of DAG and Ca^{2+} causes a conformational change in PKC, causing flexing at a hinge region so as to withdraw the pseudosubstrate and unblock the PKC catalytic site. The appearance of DAG in membranes is usually transient, and therefore PKC is activated for only a short time after a receptor has been stimulated. The PKC-Ca^{2+}-DAG-membrane complex is only slowly dissociable by chelating calcium. This has been the basis for the commonly used "translocation assay" for assessment of PKC activity; more accurately, this assay measures the activated PKC, which is tightly bound to membrane, as compared to the inactive PKC, which is loosely associated with membranes (8).

The events that occur downstream from PKC are just beginning to be characterized. Serine-threonine kinases and tyrosine kinases catalyze phosphorylation events distal to PKC. These phosphorylation cascades serve to enhance amplification of the original signal. Clearly, PKC signals impinge on several known intracellular control circuits (8). The targets for phosphorylation by PKC include receptors for insulin, epidermal growth factor, and many proteins involved in control of gene expression, cell division, and differentiation (11, 12). The critical, carcinogenesis-triggering target for PKC remains to be identified, but several potentially important candidates have been studied. Activation of PKC results in site-specific dephosphorylation of the c-*jun* protein, which coincides with increased AP-1 protein binding activity and transactivation by c-*jun* (8). The dephosphorylated AP-1 complex has enhanced binding and transcriptional activity. NF-kB is a protein that can complex with DNA and be a transcriptional activator (8). It is ubiquitously distributed in cytosolic form, in association with an inhibitor protein (inhibitor-kB) forming an inactive complex. PKC phosphorylates the inhibitor protein and dissociates it, permitting movement of NF-kB from cytosol to DNA. Substrates for PKC phosphorylation also include the MARCKS protein

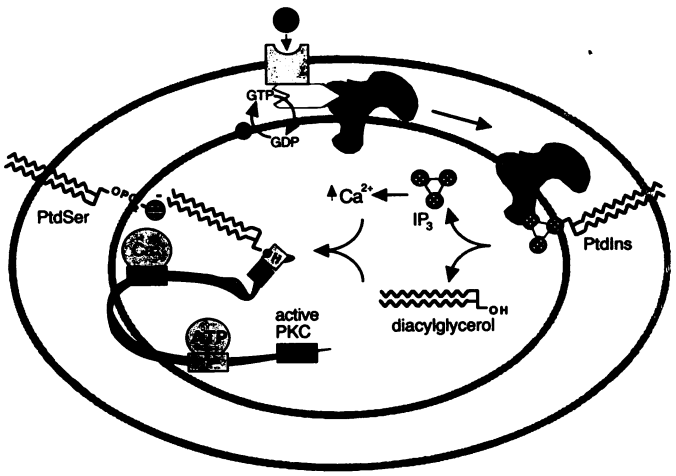

Figure 2. Phosphoinositide-mediated signal transduction. When an appropriate receptor is stimulated by an agonist, there is a change in the conformation of a GTP binding protein (G-protein). This causes the activation of a phospholipase C (PLC) specific for phosphatidylinositol bis-phosphate (PtdIns). The activation of the G-protein is terminated by conversion of bound GTP to GDP by a GTPase. Before sensitivity to a receptor can be restored, this GDP must be replaced with a GTP. Activated PLC hydrolyzes PtdIns bisphosphate to form inositol-1,4,5-trisphosphate (IP_3). IP_3 acts to release calcium from intracellular storage sites. PtdIns bisphosphate hydrolysis also forms diacylglycerol. Calcium, diacylglycerol, and phosphatidylserine (PtdSer) activate protein kinase C (PKC; see Fig. 3).

(13). A 40 to 47-kDa cytosolic protein (p47), tentatively identified as IP_3-5'-phosphomonoesterase, is also a substrate; one region of this protein closely resembles the pseudosubstrate sequence on PKC (8). It is likely that a plethora of PKC-targets will be studied in the next few years.

Signal transduction via PKC is carefully regulated by several other signaling pathways. As discussed earlier, some types of receptors have associated catalytic functions: phosphotyrosine phosphatase activity, guanylyl cyclase activity, tyrosine kinase activity; the latter type has been the most thoroughly described. Receptors that act via tyrosine kinase share a common mechanism for activation—ligand-induced dimerization or ligand-induced increased association between already dimerized units. Close apposition of two subunits results in cross-phosphorylation on tyrosines, activating the intracellular kinase domain. This category includes platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) -stimulated kinase. The PDGF receptor serves as a prototype for receptor tyrosine kinases. Certain enzymes, after associating with the receptor, are phosphorylated by activated PDGF kinase (a tyrosine kinase). These targets include PtdIns-PLC, PtdIns 3' kinase, GAP, the *raf* proto-oncogene product, and *src* and *src*-like tyrosine kinases (14). Thus, activation of receptors with tyrosine kinase activity modulates PKC signaling, and vice versa. The remainder of this review focuses on an alternative set of control mechanisms whereby PKC signal transduction is modulated by metabolites of choline-phospholipids.

CHOLINE PHOSPHOLIPIDS AND SIGNAL TRANSDUCTION

Studies of the time course of DRG generation in a number of cell types show a prolonged phase of agonist-induced DRG production occurring later than the hydrolysis of PtdIns. The acyl groups present in this DRG are consistent with its formation from PtdCho catalyzed by PLC and phospholipase D (PLD; generates phosphatidic acid and choline; Fig. 1). PtdCho can be converted to PtdIns by an exchange reaction (9). Occasionally such an exchange might occur prior to PtdIns hydrolysis, generating DRG with PtdCho-like fatty acid composition (9). The hydrolysis of PtdCho occurs in response to a range of agonists, some of which activate PtdCho-specific PLCs and PLDs via G-proteins (7). The P_{2y}-purinergic receptor (agonists: ATP and ADP) and the muscarinic receptor (agonist acetylcholine) operate via this mechanism (7, 15). Other agonists trigger PLD-mediated PtdCho hydrolysis via activation of PKC. Phorbol esters activate PKC without triggering PtdIns breakdown, stimulate the release from PtdCho of choline, DRG, phosphatidic acid (can be converted to DRG by phosphatidic acid phosphohydrolase), and arachidonic acid (formed by phospholipase A_2) from PtdCho (7). PLD can be activated, via PKC, by DAG and phorbols (9). Inhibitors of PKC and down-regulation of PKC attenuate PtdCho hydrolysis. The ability of PKC to activate PLD may be mediated by a separate catalytic site that does not involve ATP-dependent phosphorylation (16). Changes in intracellular calcium modulate PtdCho hydrolysis as phospholipases D and A_2 are stimulated by this ion. Also, it is likely that there is a direct link between activation of tyrosine kinases and PtdCho hydrolysis (7). Stimulation of PtdCho hydrolysis by EGF and PDGF results in prolonged (hours) elevation of intracellular DRG without triggering PtdIns hydrolysis.

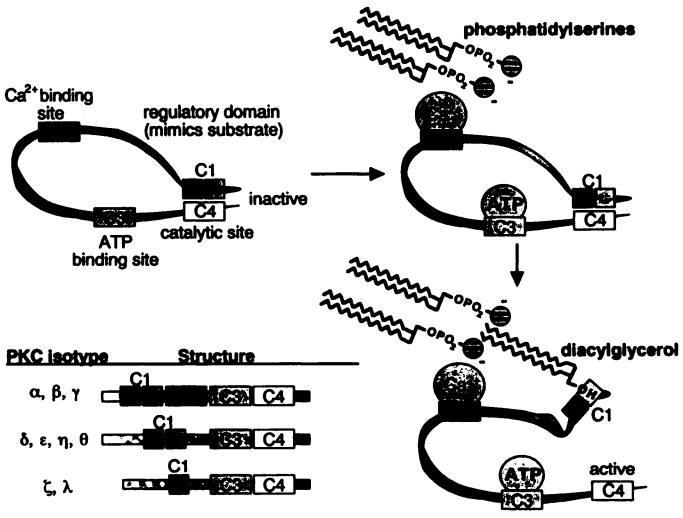

Figure 3. Isotypes of protein kinase C and their activation. There are multiple isotypes of protein kinase C (PKC) that share some sequence homology. All have a common ATP binding site (C3) and catalytic site (C4). Some isotypes also have a calcium binding site (C2) and/or a lipid binding site (C1). Normally, the catalytic site is occupied by a region of the enzyme that resembles the substrate (pseudosubstrate). When intracellular calcium concentration increases, PKC becomes more closely associated with membranes containing phosphatidylserine, making it possible for diacylglycerol to bind to PKC. When this occurs, the conformation of PKC changes, exposing the catalytic site and thereby activating it.

PtdCho hydrolysis can act to sustain a message that was initially transmitted via inositide breakdown. Sustained activation of PKC is essential for triggering cell differentiation and proliferation (9). PtdCho breakdown can generate second messengers independent of PtdIns breakdown. This is important, as several isotypes of PKC are activated by DAG in the absence of an increase in intracellular calcium (see earlier discussion; Fig. 3) (8). The fatty acid species in PtdCho are different from those in PtdIns, therefore the DRGs generated from each will differ. PtdCho is made up of different molecular species, including ester-, ether-, and vinyl-ether-linked species. Further diversity exists, as the acyl chain structure can vary greatly. Therefore, hydrolysis of PtdCho can generate multiple species of DRG. The predominant components of DRG in tissues are the ester-linked 1,2-diacyl species (DAG) and the 1-alkyl-2-acyl glycerols (AAG; ether-linked 1-*O*-alkyl-2-acyl and vinyl-ether-linked 1-*O*-alk-1'-enyl-2-acyl species). These subclasses of DRG may differ in their ability to activate PKC (17–22). DAG, a specific species of DRG, is the best-studied activator of PKC. Although AAG has been considered to be incapable of activating PKC (21), recent studies indicate that naturally occurring species of AAG activate PKC (17, 22). This activation may require free calcium concentrations found only in stimulated cells (17). The alkyl group in the 1-position diminishes the diglyceride's ability to activate PKC relative to the corresponding DAG (18), and AAGs with short chain fatty acid substituents appear to be better activators than are those with longer chain fatty acids (20). 1-*O*-alkyl-2-acetyl analogs of AAG inhibit PKC activation by DAG (19). This acetyl analog may differ from other AAGs in biologic activity; Rider et al. (23) have speculated that the biologic activity of this AAG may be dependent on conversion to alkylacetylglycerophosphocholine (platelet-activating factor). It is possible that DRGs generated from PtdCho are recognized by special isotypes of PKC that act in a different domain than does inositide-stimulated PKC, and this may provide a mechanism to maintain signal specificity.

Other products of PtdCho hydrolysis, such as phosphatidic acid, lysoPtdCho, and free fatty acids, also are second messengers (7, 24). Phosphatidic acid can act as a mitogen (25). LysoPtdCho stimulates PKC activity (9), but it is a membrane-lytic detergent with potential toxic effects. LysoPtdCho generation is important in chemotaxis, relaxation of smooth muscle, and activation of T-lymphocytes (9). Phospholipase A_2 (PLA_2) generates free fatty acids from PtdCho (Fig. 1). It is activated by many agonists, including tyrosine kinase activators (EGF and PDGF) and PKC activators (9). Arachidonic acid, generated by PLA_2, can be a precursor for lipoxygenase- or cyclooxygenase-generated products (8). Both phosphatidic acid and arachidonic acid can inhibit the activity of GAP (26). Physiologic activation of PKC may involve two mechanisms: the DAG-mediated process may activate membrane-associated PKC; and free fatty acids specifically may activate PKC that is not tightly associated with membranes. Oleic and arachidonic acids are able to activate soluble PKC but not membrane-bound PKC (27). This may be important for differential activation of the separate isotypes of PKC.

Whereas PtdCho hydrolysis generates a series of messengers that sustain the PKC phosphorylation cascade, SM is hydrolyzed to generate messengers that terminate the cascade (**Fig. 4**). The hydrolysis of sphingomyelin by sphingomyelinase (produces ceramide and phosphocholine from SM) is activated by multiple agonists, including 1-α, 25-dihydroxyvitamin D_3, tumor necrosis factor, and γ-interferon (28). SM, sphingomyelinase, and ceramidase (produces sphingosine and fatty acid from ceramide) are present in the outer leaflet of the membrane bilayer. Ceramide is a potent inhibitor of cell growth as well as a promoter of cell differentiation. Its metabolite, sphingosine, is a potent inhibitor of PKC that acts by blocking DAG activation (29). Sphingosine concentrations in cells are in the micromolar range, perhaps sufficient to inhibit PKC (30). Lysosphingomyelin also is formed during hydrolysis of SM (Fig. 1) and inhibits PKC (31). The synthesis of SM from ceramide and PtdCho generates DAG (Fig. 1), but we do not know whether this DAG is delivered to a subcellular location where it is available for PKC activation. At this time we suspect that a major function for receptor-mediated activation of sphingomyelinase is to generate products that stop the signaling cascade.

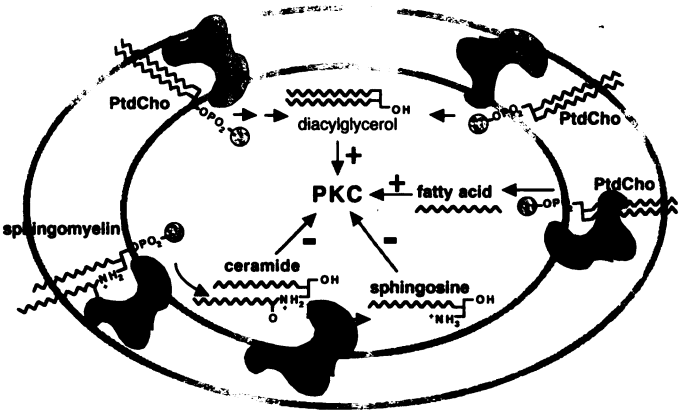

Figure 4. Metabolites formed from choline-phospholipids modulate PKC activity. As described in Fig. 1, phosphatidylcholine (PtdCho) can be metabolized to form several activators of PKC including diacylglycerol, lysophosphatidylcholine (LysoPtdCho), and *cis*-unsaturated fatty acids. The regulation of the phospholipases responsible for hydrolysis of PtdCho (phospholipase D [PLD]; phospholipase C [PLC], and phospholipase A_2 [PLA_2]) is described in the text. Sphingomyelin is metabolized by sphingomyelinase (SMase) to form several inhibitors of PKC (ceramide and sphingosine).

CHOLINE AND CARCINOGENESIS

Choline deficiency is an excellent example of how a nutrient can influence PKC signal transduction. Choline is the major dietary source for labile methyl groups, and its metabolism is interrelated with methionine and folate metabolism; choline deficiency depletes all of these methyl donors (1). Choline is the only single nutrient for which dietary deficiency is associated with development of foci of premalignant hepatocytes (which express γ-glutamyl transpeptidase (GGT) and the placental form of glutathione-S-transferase (GST) (32). Choline-deficient animals subsequently develop hepatocarcinomas in the absence of any known carcinogen (33).

The demand for choline as a methyl donor is probably the major factor that determines how rapidly a diet deficient in choline will induce pathology. The pathways of choline and 1-carbon metabolism intersect at the formation of methionine from homocysteine (1). Methionine is regenerated from homocysteine in a reaction catalyzed by betaine:homocysteine methyltransferase in which betaine, a metabolite of choline, serves as the methyl donor (34). Betaine concentrations in livers of choline-deficient rats are markedly diminished (35), as are total folate concentrations (36). The only alternative mechanism for regeneration of methionine is via a reaction catalyzed by 5-methyltetrahydrofolate:homocysteine methyltransferase (EC 2.1.1.13), which uses a methyl group generated de novo from the 1-carbon pool (37). Methionine is converted to S-adenosylmethionine in a reaction catalyzed by methionine adenosyl transferase. S-Adenosylmethionine is the active methylating agent for many enzymatic methylations.

Chronic ingestion of a diet deficient in choline has major consequences that include hepatic, renal, pancreatic, memory, and growth disorders. In the rat (38), hamster (39), guinea pig (40), pig (41, 42), dog (43–45), monkey (46), trout (47), quail (48), and chicken (49) choline deficiency results in liver dysfunction. During choline deficiency, extremely large amounts of lipid (mainly triglycerides) can accumulate in the liver, eventually filling the entire hepatocyte (38, 50–53). Secretion of triglyceride (TG) is inhibited due to the inability to form PtdCho, a constituent of the lipoprotein envelope. This causes TG and DAG to accumulate (54). In the plasma membrane, DAG reaches values higher than those occurring after stimulation of a receptor linked to phospholipase C activation (e.g., vasopressin receptor), and choline deficiency is associated with significant increases in PKC activity in hepatic plasma membranes. There is a stable activation of PKC and/or an increase in the total PKC pool in the cell (54) with changes in several PKC isotypes (at 6 wk of choline deficiency, amounts of PKCα and PKCδ are increased 2- and 10-fold, respectively). The accumulation of DAG and subsequent activation of PKC within the liver during choline deficiency may be the critical abnormality that eventually contributes to the development of hepatic cancer in these animals (54).

There are several other mechanisms that have been suggested for the cancer-promoting effect of a choline-devoid diet. In the choline-deficient liver there is a progressive increase in cell proliferation related to regeneration after parenchymal cell death (33, 55, 56). Cell proliferation, with associated increased rate of DNA synthesis, could be the

cause of greater sensitivity to chemical carcinogens (57). Other stimuli for increased DNA synthesis can be associated with carcinogenesis: hepatectomy and necrogenic chemicals are examples. However, Shinozuka and Lombardi (32) found that the overall rate of liver cell proliferation could be dissociated from the rate at which preneoplastic lesions formed during choline deficiency, suggesting that cell proliferation is not the sole condition acting as a promoter of liver cancer. Methylation of DNA is important for the regulation of expression of genetic information. It has been suggested that the under-methylation of DNA, observed during choline deficiency (despite adequate dietary methionine), is responsible for carcinogenesis (58, 59). Another proposed mechanism is based on the observation that when rats are fed a choline-deficient diet, increased lipid peroxidation occurs within the liver (presence of diene conjugates in lipids isolated from purified hepatic nuclei) (60). Lipid peroxides in the nucleus could be a source of free radicals that could modify DNA and cause carcinogenesis.

It is interesting that choline-deficient rats not only have a higher incidence of spontaneous hepatocarcinoma, but that they are markedly sensitized to the effects of administered carcinogens (33). These observations are consistent with the hypothesis that during a crucial period, choline deficiency can either initiate carcinogenesis, promote endogenously initiated cells, or make hepatocytes susceptible to initiation. Perturbed PKC signal transduction may lower the threshold dose of carcinogen needed to initiate the development of cancers.

PKC SIGNAL TRANSDUCTION AND CARCINOGENESIS

There are genes whose products generate positive signals controlling growth, and genes whose products are involved with inhibition of growth. Carcinogenesis must involve genetic changes in both control mechanisms. Several lines of evidence indicate that cancers might develop secondary to abnormalities in PKC-mediated signal transduction (12). First, many mitogens activate PKC (9). Second, several known tumor promoters mimic activation of PKC. The phorbol esters (DAG analogs) are potent mitogens and tumor promoters (11) whose effects may be explained by their interactions with PKC. Okadaic acid (an inhibitor of protein phosphatases whose net effect is to increase protein phosphorylation of PKC targets) is also a potent tumor promoter (61).

A major line of reasoning, which links carcinogenesis to perturbation of signal transduction, is based on the study of genes that are overexpressed in cancers. Oncogenes can be divided into four classes: growth factors (*sis, hist*), growth factor receptors (*erb*B, *fms, kit*), transducers of growth factors (*src, abl, ras, raf*), and transcription factors (*jun, fos, myc*). Anti-oncogenes, such as the neurofibromatosis type 1 (NF1) locus, also can act to modulate signal transduction. NF1 encodes a protein that is a GTPase-activating protein (GAP), which interacts with the $p21^{ras}$ product (61). Thus, the pathways responsible for start/stop signals during cell division and growth use intermediary steps mediated by the protein-products made by proto-oncogenes. The expression of oncogenes can perturb PKC-mediated signal transduction in a manner similar to that which we previously described for choline deficiency. In *erb*B-transformed fibroblasts DAG accumulates because it is not removed as rapidly by DAG kinase activity (62). Transformation by *ras* or *src* oncogenes is associated with permanent translocation of PKC to the cytoplasmic membrane (63). DAG is elevated in vivo in *ras*-transformed liver of neonatal transgenic mice (64). NIH 3T3 cells transformed with Ha-*ras*, Ki-*ras*, v-*src*, or v-*fms* oncogenes have elevated DAG levels as well as tonic activation and partial down-regulation of PKC (65). These elevated DAG levels are produced by activation of degradation of PtdCho (63, 66). Activated PKC, in turn, may induce expression of the *myc* oncogene (*myc* is thought to trigger hepatocytes to enter the cell division cycle) (67–69). Indeed, *myc* is overexpressed in liver and tumor tissue from choline-deficient rat liver (69, 70).

In no case has overexpression of wild-type PKC sequences and transient activation led to fully transformed phenotype (8). Transfection of fibroblasts with a gene for constantly active mutant forms of PKC (α or β) causes the cells to become transformed and tumorigenic (8, 71–73). Rat liver epithelial cells that constitutively overexpress $PKC_{\beta 1}$ are not transformed (note that the liver normally does not express $PKC_{\beta 1}$), but does overexpress *myc* (74). Thus, many observations suggest that chronically increased DAG concentration with subsequent sustained activation of PKC may be a perturbation of signal transduction that results in carcinogenesis.

SUMMARY

Choline phospholipids play major roles in cellular regulation in addition to their essential function as structural components of membranes and lipoproteins. As we begin to appreciate the complexity of control of transmembrane signal transduction, the unique functions of choline phospholipids as hormones (platelet-activating factor; 1-alkyl, 2-acetylphosphatidylcholine; PAF) and sources (phosphatidylcholine, sphingolipids) of second messengers (sphingosine, diacylglycerol, lysophospholipids, arachidonic acid and its metabolites) are of increasing interest. These functions may explain how choline phospholipids influence normal physiological processes as well as a diverse group of pathological processes, including carcinogenesis. FJ

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REFERENCES

1. Zeisel, S. H. (1990) Choline deficiency. *J. Nutr. Biochem.* **1**, 332–349
2. Zeisel, S. H., DaCosta, K.-A., Franklin, P. D., Alexander, E. A., Lamont, J. T., Sheard, N. F., and Beiser, A. (1991) Choline, an essential nutrient for humans. *FASEB J.* **5**, 2093–2098
3. Buchman, A. L., Dubin, M., Jenden, D., Moukarzel, A., Roch, M. H., Rice, K., Gornbein, J., Ament, M. E., and Eckhert, C. D. (1992) Lecithin increases plasma free choline and decreases hepatic steatosis in long-term total parenteral nutrition patients. *Gastroenterology* **102**, 1363–1370
4. Meldrum, E., Parker, P. J., and Carozzi, A. (1991) The Ptd-Ins-PLC superfamily and signal transduction. *Biochim. Biophys. Acta* **1092**, 49–71
5. Karin, M. (1992) Signal transduction from cell surface to nucleus in development and disease. *FASEB J.* **6**, 2581–2590
6. Marx, J. (1992) New genes may shed light on cell growth control. *Science* **257**, 484–485
7. Exton, J. H. (1990) Signaling through phosphatidylcholine breakdown. *J. Biol. Chem.* **265**, 1–4
8. Stabel, S., and Parker, P. J. (1991) Protein kinase C. *Pharmacol & Ther.* **51**, 71–95

9. Nishizuka, Y. (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* **258,** 607-614
10. Wetsel, W. C., Khan, W. A., Merchenthaler, I., Rivera, H., Halpern, A. E., Phung, H. M., Negro-Vilar, A., and Hannun, Y. (1992) Tissue and cellular distribution of the extended family of protein kinase C isozymes. *J. Cell Biol.* **117,** 121-133
11. Nishizuka, Y. (1986) Studies and perspectives of protein kinase C. *Science* **233,** 305-312
12. Weinstein, I. B. (1990) The role of protein kinase C in growth control and the concept of carcinogenesis as a progressive disorder in signal transduction. *Adv. Second Messenger Phosphoprotein Res.* **24,** 307-316
13. Lenox, R. H., Watson, D. G., Patel, J., and Ellis, J. (1992) Chronic lithium administration alters a prominent PKC substrate in rat hippocampus. *Brain Res.* **570,** 333-340
14. Aaronson, S. A. (1991) Growth factors and cancer. *Science* **254,** 1146-1153
15. Qian, Z., and Drewes, L. R. (1990) A novel mechanism for acetylcholine to generate diacylglycerol in brain. *J. Biol. Chem.* **265,** 3607-3610
16. Conricode, K. M., Brewer, K. A., and Exton, J. H. (1992) Activation of phospholipase D by protein kinase C. Evidence for a phosphorylation-independent mechanism. *J. Biol. Chem.* **267,** 7199-7202
17. Ford, D. A., Miyake, R., Glaser, P. E., and Gross, R. W. (1989) Activation of protein kinase C by naturally occurring ether-linked diglycerides. *J. Biol. Chem.* **264,** 13818-13824
18. Heymans, F., Da, S. C., Marrec, N., Godfroid, J. J., and Castagna, M. (1987) Alkyl analogs of diacylglycerol as activators of protein kinase C. *FEBS Lett.* **218,** 35-40
19. Daniel, L. W., Small, G. W., Schmitt, J. D., Marasco, C. J., Ishaq, K., and Piantadosi, C. (1988) Alkyl-linked diglycerides inhibit protein kinase C activation by diacylglycerols. *Biochem. Biophys. Res. Commun.* **151,** 291-297
20. Bass, D. A., McPhail, L. C., Schmitt, J. D., Morris-Natschke, S., McCall, C. E., and Wykle, R. L. (1989) Selective priming of rate and duration of the respiratory burst of neutrophils by 1,2-diacyl and 1-O-alkyl-2-acyl diglycerides. Possible relation to effects on protein kinase C. *J. Biol. Chem.* **263,** 19610-19617
21. Cabot, M. C., and Jaken, S. (1984) Structural and chemical specificity of diradylglycerols for protein kinase C activation. *Biochem. Biophys. Res. Commun.* **125,** 163-169
22. Dawson, W. D., and Cook, J. S. (1987) Parallel changes in amino acid transport and protein kinase C localization in LLC-PK1 cells treated with TPA or diradylglycerols. *J. Cell Physiol.* **132,** 104-110
23. Rider, L. G., Dougherty, R. W., and Niedel, J. E. (1988) Phorbol diesters and dioctanoylglycerol stimulate accumulation of both diacylglycerols and alkylacylglycerols in human neutrophils. *J. Immunol.* **140,** 200-207
24. Besterman, J. M., Duronio, V., and Cuatrecasas, P. (1986) Rapid formation of diacylglycerol from phosphatidylcholine: a pathway for generation of a second messenger. *Proc. Natl. Acad. Sci. USA* **83,** 6785-6789
25. Wakelam, M. J. O., Cook, S. J., Currie, S., Plamer, S., and Plevin, R. (1991) Regulation of the hydrolysis of phosphatidylcholine in Swiss 3T3 cells. *Biochem. Soc. Trans.* **19,** 321-324
26. Tsai, M. H., Yu, C. L., Wei, F. S., and Stacey, D. W. (1989) The effect of GTPase activating protein upon ras is inhibited by mitogenically responsive lipids. *Science* **243,** 522-526
27. Khan, W. A., Blobe, G. C., and Hannun, Y. A. (1992) Activation of protein kinase C by oleic acid. *J. Biol. Chem.* **267,** 3605-3612
28. Merrill, A. H. (1992) Ceramide: a new lipid "second messenger"? *Nutr. Rev.* **50,** 78-80
29. Merrill, A. H., and Stevens, V. L. (1989) Modulation of protein kinase C and diverse cell functions by sphingosine—a pharmacologically interesting compound linking sphingolipids and signal transduction. *Biochim. Biophys. Acta* **1010,** 131-139
30. Merrill, A. H., and Jones, D. D. (1990) An update of the enzymology and regulation of sphingomyelin metabolism. *Biochim. Biophys. Acta* **1044,** 1-12
31. Hannun, Y. A., and Bell, R. M. (1989) Regulation of protein kinase C by sphingosine and lysosphingolipids. *Clin. Chim. Acta* **185,** 333-345
32. Shinozuka, H., and Lombardi, B. (1980) Synergistic effect of a choline-devoid diet and phenobarbital in promoting the emergence of foci of gamma-glutamyltranspeptidase-positive hepatocytes in the liver of carcinogen-treated rats. *Cancer Res.* **40,** 3846-3849
33. Newberne, P. M., and Rogers, A. E. (1986) Labile methyl groups and the promotion of cancer. *Annu. Rev. Nutr.* **6,** 407-432
34. Finkelstein, J. D., Martin, J. J., Harris, B. J., and Kyle, W. E. (1982) Regulation of the betaine content of rat liver. *Arch. Biochem. Biophys.* **218,** 169-173
35. Pomfret, E. A., daCosta, K.-A., and Zeisel, S. H. (1990) Effects of choline deficiency and methotrexate treatment upon rat liver. *J. Nutr. Biochem.* **1,** 533-541
36. Selhub, J., Seyoum, E., Pomfret, E. A., and Zeisel, S. H. (1991) Effects of choline deficiency and methotrexate treatment upon liver folate content and distribution. *Cancer Res.* **51,** 16-21
37. Finkelstein, J. D., Martin, J. J., and Harris, B. J. (1988) Methionine metabolism in mammals. The methionine-sparing effect of cystine. *J. Biol. Chem.* **263,** 11750-11754
38. Lombardi, B. (1971) Effects of choline deficiency on rat hepatocytes. *Federation Proc.* **30,** 139-142
39. Handler, P., and Bernheim, F. (1949) Choline deficiency in the hamster. *Proc. Soc. Exp. Med.* **72,** 569
40. Tani, H., Suzuki, S., Kobayashi, M., and Kotake, Y. (1967) The physiological role of choline in guinea pigs. *J. Nutr.* **92,** 317-324
41. Fairbanks, B. W., and Krider, J. L. (1945) Significance of B vitamins in swine nutrition. *N. Am. Vet.* **26,** 18-23
42. Blair, R., and Newsome, F. (1985) Involvement of water-soluble vitamins in diseases of swine. *J. Anim. Sci.* **60,** 1508-1517
43. Best, C. H., and Huntsman, M. E. (1935) Effect of choline on liver fat of rats in various states of nutrition. *J. Physiol. (London)* **83,** 255-274
44. Hershey, J. M., and Soskin, S. (1931) Substitution of "lecithin" for raw pancreas in a diet of depancreatized dog. *Am. J. Physiol.* **93,** 657-658
45. Best, C. H., and Huntsman, M. E. (1932) The effects of the components of lecithin upon the deposition of fat in the liver. *J. Physiol. (London)* **75,** 405-412
46. Hoffbauer, F. W., and Zaki, F. G. (1965) Choline deficiency in the baboon and rat compared. *Arch. Pathol.* **79,** 364-369
47. Ketola, H. G. (1976) Choline metabolism and nutritional requirement of lake trout (Salvelinus namaycush). *J. Anim. Sci.* **43,** 474-477
48. Ketola, H. G., and Young, R. J. (1973) The need for dietary choline by young Japanese quail. *Poultry Sci.* **52,** 2362-2363
49. Ketola, H. G., and Nesheim, M. C. (1974) The influence of dietary protein and methionine levels on the requirement for choline by chickens. *J. Nutr.* **104,** 1484-1486
50. Lombardi, B., Pani, P., and Schlunk, F. F. (1968) Choline-deficiency fatty liver: impaired release of hepatic triglycerides. *J. Lipid Res.* **9,** 437-446
51. Yao, Z. M., and Vance, D. E. (1988) The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J. Biol. Chem.* **263,** 2998-3004
52. Yao, Z. M., and Vance, D. E. (1989) Head group specificity in the requirement of phosphatidylcholine biosynthesis for very low density lipoprotein secretion from cultured hepatocytes. *J. Biol. Chem.* **264,** 11373-11380
53. Blusztajn, J. K., and Zeisel, S. H. (1989) 1,2-sn-diacylglycerol accumulates in choline-deficient liver. A possible mechanism of hepatic carcinogenesis via alteration in protein kinase C activity? *FEBS Lett.* **243,** 267-270
54. daCosta, K. A., Cochary, E. F., Blusztajn, J. K., Garner, S. F., and Zeisel, S. H. (1993) Accumulation of 1,2-*sn*-diradylglycerol with increased membrane-associated protein kinase C may be the mechanism for spontaneous hepatocarcinogenesis in choline deficient rats. *J. Biol. Chem.* **268,** 2100-2105
55. Chandar, N., Amenta, J., Kandala, J. C., and Lombardi, B. (1987) Liver cell turnover in rats fed a choline-devoid diet. *Carcinogenesis* **8,** 669-673

56. Chandar, N., and Lombardi, B. (1988) Liver cell proliferation and incidence of hepatocellular carcinomas in rats fed consecutively a choline-devoid and a choline-supplemented diet. *Carcinogenesis* **9**, 259-263
57. Ghoshal, A. K., Ahluwalia, M., and Farber, E. (1983) The rapid induction of liver cell death in rats fed a choline-deficient methionine-low diet. *Am. J. Pathol.* **113**, 309-314
58. Locker, J., Reddy, T. V., and Lombardi, B. (1986) DNA methylation and hepatocarcinogenesis in rats fed a choline devoid diet. *Carcinogenesis* **7**, 1309-1312
59. Dizik, M., Christman, J. K., and Wainfan, E. (1991) Alterations in expression and methylation of specific genes in livers of rats fed a cancer promoting methyl-deficient diet. *Carcinogenesis* **12**, 1307-1312
60. Rushmore, T., Lim, Y., Farber, E., and Ghoshal, A. (1984) Rapid lipid peroxidation in the nuclear fraction of rat liver induced by a diet deficient in choline and methionine. *Cancer Lett.* **24**, 251-255
61. Hunter, T. (1991) Cooperation between oncogenes. *Cell* **64**, 249-270
62. Kato, M., Kawai, S., and Takenawa, T. (1989) Defect in phorbol acetate-induced translocation of diacylglycerol kinase in erbB-transformed fibroblast cells. *FEBS Lett.* **247**, 247-250
63. Diaz-Laviada, I., Larrodera, P., Diaz-Meco, M., Cornet, M. E., Guddal, P. H., Johansen, T., and Moscat, J. (1990) Evidence for a role of phosphatidylcholine-hydrolysing phospholipase C in the regulation of protein kinase C by ras and src oncogenes. *Embo J.* **9**, 3907-3912
64. Wilkison, W. O., Sandgren, E. P., Palmiter, R. D., Brinster, R. L., and Bell, R. M. (1989) Elevation of 1,2-diacylglycerol in ras-transformed neonatal liver and pancreas of transgenic mice. *Oncogene* **4**, 625-628
65. Wolfman, A., Wingrove, T. G., Blackshear, P. J., and Macara, I. G. (1987) Down-regulation of protein kinase C and of an endogenous 80-kDa substrate in transformed fibroblasts. *J. Biol. Chem.* **262**, 16546-16552
66. Price, B. D., Morris, J. D., Marshall, C. J., and Hall, A. (1989) Stimulation of phosphatidylcholine hydrolysis, diacylglycerol release, and arachidonic acid production by oncogenic ras is a consequence of protein kinase C activation. *J. Biol. Chem.* **264**, 16638-16643
67. Rozengurt, E. (1986) Early signals in the mitogenic response. *Science* **234**, 161-166
68. Kaibuchi, K., Tsuda, T., Kikuchi, A., Tanimoto, T., Yamashita, T., and Takai, Y. (1986) Possible involvement of protein kinase C and calcium ion in growth factor-induced expression of c-myc oncogene in Swiss 3T3 fibroblasts. *J. Biol. Chem.* **261**, 1187-1192
69. Hsieh, L. L., Wainfan, E., Hoshina, S., Dizik, M., and Weinstein, I. B. (1989) Altered expression of retrovirus-like sequences and cellular oncogenes in mice fed methyl-deficient diets. *Cancer Res.* **49**, 3795-3799
70. Chandar, N., Lombardi, B., and Locker, J. (1989) c-myc gene amplification during hepatocarcinogenesis by a choline-devoid diet. *Proc. Natl. Acad. Sci. USA* **86**, 2703-2707
71. Megidish, T., and Mazurek, N. (1989) A mutant protein kinase C that can transform fibroblasts. *Nature (London)* **342**, 807-811
72. Krauss, R., Housey, G., Johnson, M., and Weinstein, I. B. (1989) Disturbances in growth control and gene expression in a C3H/10T1/2 cell line that stably overproduces protein kinase C. *Oncogene* **4**, 991-998
73. Persons, D. A., Wilkison, W. O., Bell, R. M., and Finn, O. J. (1988) Altered growth regulation and enhanced tumorigenicity of NIH 3T3 fibroblasts transfected with protein kinase C-1 cDNA. *Cell* **52**, 447-458
74. Hsieh, L. L., Hoshina, S., and Weinstein, I. B. (1989) Phenotypic effects of overexpression of PKCβ1 in rat liver epithelial cells. *J. Cell Biochem.* **41**, 179-188