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LIPID MODULATION OF CELL FUNCTION

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INTRODUCTION

Lipids constitute the most structurally diverse class of nutrients. The major lipid classes (glycerolipids, sphingolipids, steroids, waxes, fat-soluble vitamins, etc) are composed of subgroupings within each class (the different phospholipids, for example), and within each subgrouping there is great structural heterogeneity (such as the compositional and positional variations in the fatty acids of phospholipids). It has been estimated that there are more than 1,000 distinct molecular species of lipids in eukaryotic membranes (100), and this is probably a conservative estimate. Does this imply that the exact structures are not important, or that they are so important that even subtle changes have biological consequences?

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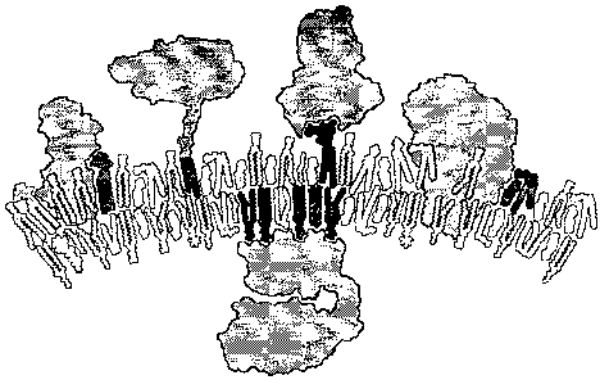
To answer this question it is helpful to envision lipids as they actually appear in biological membranes. Figure 1 depicts tracings of the space-filling models of the major lipids in rat liver plasma membranes. They are shown in the approximate amounts and distribution across the bilayer. When one looks closely at the difference in the shapes of the molecules, it is clear that they have different ways of interacting with each other and with membrane proteins. Despite the complexity of this diagram, it is an oversimplification because most lipids can assume a large number of conformations. Dipalmitoylphosphatidylcholine, for example, has been estimated to have 436,755 rotational isomeric configurations (94). Most membrane lipids also have a high degree of lateral mobility; in-plane exchange rates for phospholipids are on the order of 10^7 per second (33), although some lipids exist in separate, less fluid domains. Transbilayer movement is generally slow; however, this is not as true for the lipid hydrolysis products that are utilized as second messengers. Even phosphatidic acid crosses bilayers rather rapidly (with a half-time of 4.1 min) in the neutral (protonated) form (35). Despite



EXAMPLES OF LIPIDS AS MODULATORS OF CELL FUNCTION

Figure 1 Diagrammatic representation of tracings of the space-filling models of proteins and the major lipids in rat liver plasma membranes shown in their approximate amounts and distribution across the bilayer (2, 39, 46, 52, 72, 76, 125–127, 144). The highlighted lipids illustrate (from left to right): (*a*) noncovalent binding of lipids to cellular proteins to alter their behavior, as exemplified by the down-regulation of the EGF receptor by ganglioside G_{M3}; (*b*) covalent binding of lipids to proteins, as seen in attachment of some proteins to membranes via phosphatidylinositol glycan linkages; (*c*) dynamic interactions of lipids with enzymes to modulate their activities as part of signal transduction cascades, as in the activation of protein kinase C by diacylgylcerol and phosphatidylserine(s); (*d*) noncovalent interactions between cellular lipids and extracellular proteins, as exemplified by interactions between glycolipids and cytoskeletal proteins such as fibronectin; and, (*e*) association of some lipids with other lipids, as reflected in the apparent interaction of cholesterol and sphingomyelin.

these caveats, this model is a reasonable starting point to consider how lipids modulate cell behavior, particularly by affecting signal transduction.

PROPERTIES OF LIPIDS RELEVANT TO THEIR PARTICIPATION IN CELL REGULATION

Physiochemical Properties

Over a century ago, J. L. W. Thudichum (119) said, "The physical properties of lipids are, viewed from a teleological point of standing, eminently adapted to their functions." He was referring to the amphipathic nature of phospholipids: One domain is hydrophobic and tends to aggregate away from water, while another is hydrophilic and provides a more stable boundary at the water interface. These features allow lipids to form membrane bilayers, lipoproteins, pulmonary surfactant, and other biological structures, which Robert M. Bell has referred to as the "ordinary" functions of lipids. In comparison, Bell has termed the roles of lipids as second messengers and other bioactive compounds as the "extraordinary" functions of lipids.

One way that lipids modulate cell behavior is through subtle alterations in membrane "fluidity" (110). Lipid mobility allows vesicles to form and fuse with other membranes, enables cells to change shape during cell division and other processes, and provides a spontaneous mechanism that facilitates "self-sealing" when membranes are mechanically or biochemically disrupted. It also permits membrane-associated receptors, transporters, electron-transport enzymes, and other proteins to move as needed to perform their functions. Nonetheless, many changes in cell lipid composition and behavior do not correlate with fluidity changes (see Ref. 128), perhaps because cells tend to adjust their lipid composition to maintain a relatively constant overall fluidity. Many of the physiological changes attributed to alteration of the fatty acid composition of membrane lipids are probably due to alterations in discrete membrane domains or, as will be discussed later, to lipid-mediated signal transduction systems.

There are many different types of membrane domains. For example, simple neutral glycosphingolipids (such as glucosylceramide) tend to form patches of around 100 molecules. This causes the local concentration to become very high (1 to 2 M), which may facilitate binding by proteins (118). Another "cluster" is formed by lipids that tend to form nonbilayer structures (inverted micelles termed H_{II} micelles) (30). Lipids that form inverted micelles generally possess alkyl chains that occupy a relatively large area compared with that of the headgroup, such as dioleoylphosphatidylethanolamine. Some lipids can convert between bilayer and inverted micelle structures when there is a change in the effective area occupied by the headgroup; for example, when the

carboxyl group of phosphatidylserine is protonated or is chelated by a divalent cation (31, 74). Nonbilayer structures may facilitate transbilayer movement of lipids as well as membrane fusion.

Another type of domain can exist when there are preferred interactions between two different lipids, such as the interaction between cholesterol and sphingomyelin. This apparent association affects the interaction of cholesterol with cholesterol acyltransferase, the cholesterol side-chain cleavage enzyme, and other enzymes and influences cholesterol transport and metabolism in vivo (51, 86, 112). Cholesterol feeding alters sphingomyelin metabolism, as well (86). Even when lipids are in fluid membranes, the van der Waals attraction energies can be quite large and transient interactions that are analogous to the "flickering clusters" that occur between water molecules can take place. Van der Waals attraction energies have been calculated to be ~27 kcal/mol for a phospholipid with two saturated alkyl chains of 10-carbon atoms separated by a distance of 5 Å (108).

Interactions With Proteins, Membranes, and Other Structures

Many enzymes require lipids for activity and, while this requirement can sometimes be met by any hydrophobic environment, some proteins are highly specific for particular lipid classes (141). The mitochondrial enzymes cytochrome c oxidase (140) and the cholesterol side-chain cleavage enzyme, cytochrome P450scc (96), are stimulated by cardiolipin, the major phospholipid in mitochondria. In addition, some phospholipases not only utilize lipids as substrates but also require phospholipids as activators (32).

Another mode in which lipids can interact with proteins is as allosteric regulators. Ganglioside G_{M3} binds the epidermal growth factor receptor, resulting in down regulation of the protein kinase activity of the receptor (53). This has been proposed as a mechanism for cells to modulate their responsiveness to growth factors. Cell surface sphingolipid-protein interactions are also exemplified by the binding of cytoskeletal proteins to lipids on the external leaflet of cells (for example, fibronectin has binding sites for sulfatides), by the recognition of cell surface antigens by antibodies (some glycolipids are blood group antigens), and by microbial toxins (such as the binding of cholera toxin to ganglioside G_{M1}) (78).

Other lipid-protein interactions are covalent, such as the modification of proteins by fatty acids (47), more complex phospholipids (as in the phosphatidylinositol-glycan linked proteins)(41, 73), long-chain, thioether-linked isoprenyl groups (45, 106), and ceramides (41). The covalently attached lipid can be critical for binding of the protein to membranes (63), where they can become associated with other components of cellular signal transduction systems (115).

PROTEIN KINASE C AS A MODEL FOR LIPID REGULATION OF CELL FUNCTION

A recurring theme among lipid second messengers has been that the bioactive species are generally formed after cleavage of phospholipids by phospholipases (32, 43). These include the eicosanoids, platelet-activating factor, diacylclycerols, and diverse other lipid mediators. This review focuses primarily on one system, protein kinase C, as a prototype for a lipid-mediated signal transduction pathway that interacts with many different lipids that serve as activators and inhibitors (12). The major activators of protein kinase C and the pertinent phospholipases are shown in Figure 2, which is drawn from the perspective of protein kinase C as it approaches the membrane. Nearly all of the possible products of phospholipid cleavage (i.e. phospholipid headgroups, lysophospholipids, diacylglycerols, phosphatidic acid, fatty acids) have been implicated in the activation of protein kinase C. The reasons for the existence of multiple activators of protein kinase C are not entirely clear; they may provide synergistic activation of protein kinase C as is seen with diacylglycerol

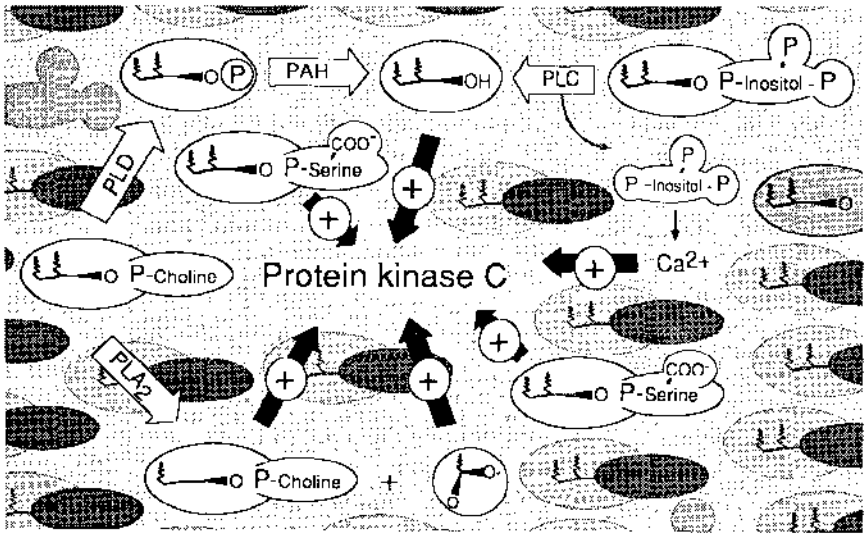


Figure 2 The turnover of membrane lipids to activate protein kinase C. This representation shows the hydrolysis of phosphatidylinositol 4,5-diphosphate by phospholipase C (PLC) to diacylglyerol and inositol triphosphate (IP_3, which stimulates release of calcium from intracellular stores), hydrolysis of phosphatidylcholine to phosphatidic acid by phospholipase D (PLD) (followed by the action of phosphatidic acid phosphohydrolase, PAH), and hydrolysis of phosphatidylcholine by phospholipase A_2 (PLA_2) to produce lysophosphatidylcholine and fatty acid. Also shown is the activation of protein kinase C by phosphatidylserine.

and fatty acids (87, 111). As shown in Figure 2, diacylglycerols can be derived from multiple phospholipids via different paths (i.e. cleavage by phospholipase C or by phospholipase D and phosphatidic acid phosphohydrolase). This seeming redundancy is thought to allow multi-phase or sustained activation (87); these two processes may be interrelated, because agonists can trigger the rapid release of diacylglycerol from phospholipids via phospholipase C to activate protein kinase C, which stimulates phospholipase D to generate more diacylglycerol (87, 124). Another consequence of utilization of different types of phospholipids is that the cleavage products can have different alkyl-chain compositions and can include both 1,2-diacylglycerols and 1-alkyl,2-acyl glycerols (from alkyl-ether lipids and plasmalogens) (104). Furthermore, utilization of phosphatidylinositol diphosphate in the first burst of diacylglycerol yields signals for both protein kinase C and other calcium activated systems, whereas the later mobilization of diacylglycerol from phosphatidylcholine will not necessarily involve intracellular calcium pools but may be dependent on extracellular calcium (6). These examples illustrate the extensive communication or "cross-talk" that occurs between different types of signalling systems (60).

Although it is not evident in the figure, multiple intracellular sites can be involved in these processes. For example, phospholipase C cleavage of inositol phospholipids in the plasma membrane generates the water-soluble second messenger inositol triphosphate, which triggers Ca^{2+} release from the endoplasmicreticulum. Ca^{2+}, in turn, facilitates the binding of some isozymes of protein kinase C to the plasma membrane.

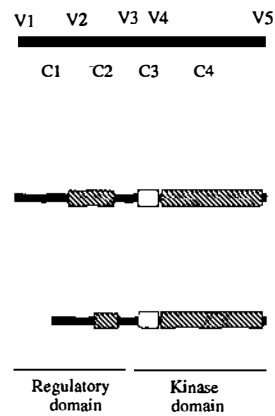
The Protein Kinase C Family

Protein kinase C was initially characterized as a phospholipid-dependent and diacylglycerol-activated protein kinase, and most subsequent studies have implicated lipids as important regulators of the activity in vivo (87). Protein kinase C is actually a family of enzymes (4, 12, 87, 136) with different characteristics and tissue distribution, some of which are summarized in Table 1. The common features of these isozymes are that they each have a regulatory domain and a catalytic domain; variations in these regions determine the lipid activators, protein substrates, requirement for calcium, and other characteristics of the individual isozymes. In addition to the features shown in Table 1, recent analyses of protein kinase C have revealed that approximately four zinc atoms are bound per molecule (99).

A large number of polypeptides are phosphorylated by this kinase in vitro and in intact cells. Acidic proteins in the molecular weight range of 65–87 kDa have been identified as major and specific substrates for protein kinase C in several cell types (21). Many of these proteins are alanine rich and myristoylated at the amino terminus, and one of note is referred to as the

Table 1 Characteristics of protein kinase C isozymes[a]

Subspecies	Known lipid activators	Tissues	Structural domains
Alpha (α)	PS,Ca^{2+},DG,FFA,LysoPC	Universal	cPKC
Beta (βI/βII)	PS,Ca^{2+},DG,FFA,LysoPC	Some tissues	
Gamma (γ)	PS,Ca^{2+},DG,FFA,LysoPC	Many tissues	
Delta (δ)	PS,DG	Brain only	nPKC
Epsilon (ε)	PS,DG,FFA	Universal	
Eta (η)	?	Brain & others	
Theta (θ)	?	Lung, skin & heart	
Zeta (ζ)	PS,FFA	Universal	aPKC
Lambda (λ)	?	Ovary, testis etc.	



[a]Shown are the known isozymes of protein kinase C and current information about their lipid activators and tissue localization. The overall structures of the three main groupings of the isozymes (cPKC, nPKC, and aPKC) are given diagrammatically with the variable domains (V1\–5) in solid and the conserved domains (C1\–4) in open (for the ATP-binding region) and partially filled blocks. Also indicated are the polypeptides that have the major regulatory (lipid-binding) domains and the protein kinase domains. Modified from Y. Nishizuka, 1992. Intracellular hydrolysis of phospholipids and activation of protein kinase C. Science 258:607-14.

MARCKS protein (myristoylated alanine-rich C-kinase substrate) (107). Selectivity for different protein substrates can be used to distinguish new isozymes (66).

Lipid Modulators of Protein Kinase C

The regulation of protein kinase C activation is generally thought to involve three lipid-mediated processes: association of the enzyme with membranes, activation of the enzyme by diacylglycerol and/or other lipids, and termination of the activation by removal of the lipids and/or proteolysis of protein kinase C to split the regulatory and kinase domains. Mindful of the inherent risks of oversimplification, one can summarize these events as follows.

MEMBRANE BINDING An early event in the activation of many of the isozymes of protein kinase C is association of the enzyme with membranes through its binding to phosphatidylserine and (for some isozymes) calcium (9, 113). The Ca^{2+}-binding site(s) for the calcium-requiring isozymes are thought to be generated at the interface between protein kinase C and the membrane (9), because this divalent cation complexes phosphatidylserine (83, 117). The binding of protein kinase C to membranes induces an increase in

surface pressure (113) that can be interpreted as a reorientation of the phospholipids and/or as possible insertion of a protein domain into the membrane.

There is little specificity toward the glycerol backbone of the phospholipid; 1,3-diacylphosphatidylserine activates protein kinase C despite considerable deviation from the usual 1,2-diacyl configuration (12, 69). Phosphatidylserines with unsaturated fatty acids are somewhat better activators than are the saturated phospholipids. However, this greater activation appears to be due to spacing of the anionic headgroups rather than to a specific interaction with the unsaturated fatty acid, because activation can be achieved with saturated phosphatidylserines when other phospholipids with unsaturated fatty acids are present or when cholesterol is added as a spacing group (17).

The nature of the phospholipid headgroup is more critical; the L-serine headgroup yields greater activity than do most other naturally occurring phospholipids or synthetic phospholipids made from serine analogs (12, 69). Activation can also be achieved by other acidic lipids (12, 70, 71). In some cases, such as with phosphatidylinositol 4,5-bisphosphate, phospholipids can reduce the concentration of phosphatidylserine required for activation (70). Whether the remainder of the phospholipid is phosphatidylethanolamine or phosphatidylcholine also influences the activation of protein kinase C (11, 23).

The exact stoichiometry of the interaction between protein kinase C and phosphatidylserine is not known; however, based on our understanding of the cooperativity of activation, at least four phosphatidylserines appear to be involved (56, 84). It has been suggested that electrostatic interactions with phospholipids promote the binding of protein kinase C to membranes, but cooperative interactions with phosphatidylserine provide the driving force for activation of the enzyme (93).

The requirement of a fairly large number of phosphatidylserines (and/or other acidic lipids) for activation has a number of structural implications. The ionization state of the carboxyl group of phosphatidylserine, and of many other ionizable groups in this environment [such as fatty acids (114) and phosphatidic acid (35)], is several units lower than the bulk pH (74); therefore, large proportions of negatively charged lipids will still be found on model membranes and plasma membranes in vivo. As a result, these membranes have a significant electrostatic potential that might affect second messenger systems (67, 77). Studies with annexin VI (annexins refer to the family of proteins, including protein kinase C, that bind to membranes in a calcium-dependent manner) have suggested that binding induces a clustering of the acidic phospholipids (10), and that the rate of clustering is slower with phosphatidylethanolamine than with phosphatidylcholine.

After binding phosphatidylserine (and calcium, for the relevant isozymes),

protein kinase C is thought to bind diacylglycerol (or phorbol esters) to become fully active (8). Phosphatidylserine alters the specificity of protein kinase C such that substrate phosphorylation is favored over autophosphorylation (85), presumably through reorientation of amino acid groups coupled to the catalytic site of the enzyme.

Depending on the conditions, lysophospholipids are able to both activate and inhibit protein kinase C (3, 90).

ACTIVATION BY DIACYLGLYCEROLS AND OTHER LIPIDS Protein kinase C is affected by numerous phospholipase cleavage products, which sometimes interact synergistically. The lipid-activated domain of protein kinase C is located in the amino terminal region, and binding of the lipid activator(s) is generally thought to induce a conformation change that displaces a pseudo-substrate polypeptide from the active site of the enzyme (87).

Activation by diacylglycerols requires fatty acids at positions 1 and 2 of *sn*-1,2-diacylglycerols (1,3- and 2,3- isomers are not active) and a free hydroxyl group at position 3. The nature of the alkyl chains is less important, and shorter- chain diacylglycerols will activate if they are sufficiently hydrophobic to be membrane bound (44). Alkyl-acyl diglycerides, as are found in plasmalogens, are also activators of protein kinase C (42). Studies of the structures of diacylglycerols, phorbol esters, and other modulators of the activity of protein kinase C have revealed functional groups that overlay well and probably define the structural requirements for activation (82, 102, 134). By reducing the alkyl chain length of phorbol esters (to phorbol dibutyrate), one can measure binding to protein kinase C directly, and K_d on the nanomolar order are typically obtained. A similar strategy led to the development of short-chain analogs of diacylglycerols (1-oleoyl,2-acetyl-glycerol and, the more useful, 1,2-dioctanoylglycerol) as more water-soluble activators of protein kinase C (44).

Unsaturated fatty acids (oleic, linoleic, linolenic, and docosahexanoic acids) are activators of some protein kinase C isozymes, both individually (36, 105) and in combination with diacylglycerols (142). This suggests that the receptor-mediated activation of multiple phospholipases (A_2, C, and D) leads to second messengers that can act in concert to activate protein kinase C. CoA-thioesters of long-chain fatty acids and ciprofibrate (a peroxisome proliferator) apparently can potentiate diacylglycerol-activated protein kinase C by decreasing the phosphatidylserine requirement (92).

Protein kinase C occurs in multiple intracellular sites so that the intracellular location of the lipid modulators is an important factor in the biological responses. Since the intracellular movement of lipids is very difficult to follow, this aspect of protein kinase C activation has not been evaluated thoroughly. Diacylglycerols are thought to be formed in the same membranes

as protein kinase C because they move very slowly between membranes; however, they could be generated on either leaflet because their rates of transbilayer movement appear to be fast ($t_{1/2}$ of ~10 ms) (54). Lysophospholipids and fatty acids can move more rapidly through the aqueous phase, and unsaturated fatty acids (36) and perhaps other lipids (19) may interact with cytosolic protein kinase C.

The location of the phorbol ester binding site in the regulatory domain of protein kinase C has been confirmed using deletion and truncation mutants expressed in the baculovirus-insect cell expression system (22). Mutation of the two cysteine-rich regions of the first conserved sequence (C1) revealed that each contains high-affinity binding sites for phorbol dibutyrate. Phorbol ester binding has been regarded as a marker for protein kinase C; however, one isozyme (zeta) does not appear to bind phorbol esters, although it is induced to translocate by phorbols (75). Protein kinase C eta binds phorbol esters, but it does not translocate to membranes in human neutrophils (75).

For many years protein kinase C was thought to be the only protein that binds phorbol esters tightly. n-Chimaerin, a neuron-specific protein, has considerable sequence homology with protein kinase C and binds phorbol dibutyrate with a nanomolar dissociation constant (1). These findings underscore the need to be cautious in using phorbol ester binding or translocation as proof (or disproof) that protein kinase C is involved in a cellular process. An additional word of warning: Biological responses to diacylglycerols do not necessarily mean that protein kinase C is involved. Diacylglycerols are thought to stimulate the respiratory burst of human neutrophils by both protein kinase C-dependent and -independent pathways (121); to increase actin polymerization by formation of actin polymerization sites (109); and to modulate the activity of the CTP:choline-phosphate cytidylyltransferase (123).

TRANSCRIPTIONAL ACTIVATION Treatment of HL-60 cells with 1-alpha,25-dihydroxyvitamin D_3 increases expression of the beta isozyme of protein kinase C by two- to threefold (89), primarily because of transcriptional activation. Phorbol esters also induce expression of this isozyme through transcriptional regulation of a promoter in the -111 to $+43$ region of the gene (88). Regulation of protein kinase C at this level may account for many instances in which increases in both the cytosolic and particulate activities are observed, rather than simply translocation of the enzyme.

INACTIVATION OF PROTEIN KINASE C Protein kinase C is inhibited by a number of compounds, but sphingosine, the long-chain base backbone of sphingolipids, is of particular interest. Initially, sphingosine and other long-chain bases were shown to inhibit protein kinase C in vitro (57) and cellular responses to protein kinase C activators in platelets (57), neutrophils

(137), and HL-60 cells (80). Unlike most other inhibitors, which act as substrate analogs, sphingosine is competitive with diacylglycerol, phorbol dibutyrate (PDB), and Ca^{2+}; sphingosine also blocks protein kinase C activation by unsaturated fatty acids and other lipids (90, 137). Sphingosine has now been shown to inhibit protein kinase C-dependent processes in over one hundred different cell types (13, 55, 116). Therefore, cells may utilize the backbones of glycerolipids (diacylglycerols, lysophospholipids, and fatty acids) as activators of protein kinase C and the backbones of sphingolipids (sphingosine and related long-chain bases) as inhibitors. The mechanism of protein kinase C inhibition by sphingosine is not known; however, since acidic lipids (e.g. phosphatidylserine) are required for maximal activity of protein kinase C, positively charged long-chain bases may localize in the same region of the membrane and block binding and/or activity (7, 57, 79, 101). Care must be taken in the handling of these and other inhibitors of protein kinase C because it is easy to introduce artifacts and/or overinterpret the specificity of the inhibitor. These factors have been analyzed recently (64).

In contrast to fatty acids, which activate protein kinase C, the CoA thioesters can either increase (by substituting for phosphatidylserine) (92) or inhibit (9, 75, 123) activity. The inhibition appears to be selective for only some protein kinase C isozymes (in neutrophils, nPKA is inhibited by micromolar fatty acyl-CoA but beta-PKC is not) (75). The chemotactic peptide formyl-methionyl-leucyl-phenylalanine triggers a transient doubling of the amounts of fatty acyl-CoA's in neutrophils; therefore, this inhibition may play a role in the regulation of nPKC during the activation sequence for these cells. Fatty acyl-CoA's play a central role in diverse metabolic pathways (129), and we believe that this facet of protein kinase C regulation warrants further investigation.

Alpha-tocopherol reportedly (19) stimulates phorbol dibutyrate binding by smooth muscle cells and inhibits the translocation, activation, and phorbol ester-induced down-regulation of protein kinase C. Alpha-tocopherol may interact with the cytosolic form of protein kinase C, and this may account for some of the effects of vitamin E on cell proliferation.

There are also protein inhibitors for protein kinase C. Pearson et al (95) have characterized a 13,390 dalton polypeptide that inhibits protein kinase C. The polypeptide has been detected in bovine, murine, avian, and human tissues. In the cow, the inhibitor is most concentrated in secretory tissues and striated muscle and is lowest in smooth muscle. Another protein kinase C inhibitor with a molecular mass of 41 kDa has been isolated from human neutrophils (5). A protein activator of protein kinase C has also been isolated (48); therefore, polypeptides may play a more important role in the regulation of protein kinase C than has heretofore been suspected.

OTHER CONSIDERATIONS Some discrepancies in the abilities of diacylglycerols liberated from various phosphoglycerolipids to activate protein kinase C may reflect intracellular compartmentation (68). One should keep in mind that there is both intracellular compartmentation and extensive movement of lipids (59). Furthermore, the asymmetry of phosphatidylserine in plasma membranes appears to be maintained by ATP-dependent "aminophospholipid translocase(s)" (33). These issues are especially pertinent to protein kinase C because many of the biological processes regulated by this enzyme involve some form of membrane movement and remodelling.

PERSPECTIVES FOR CELL REGULATION BY LIPIDS

Dietary Lipids and Protein Kinase C

The involvement of protein kinase C in diverse aspects of cell growth, differentiation, and function and its implication as a causal or contributing factor for numerous diseases makes it a major target for the development of new strategies to prevent and treat disease. This is of particular interest to the field of nutrition, given the roles of lipids in the regulation of protein kinase C and the many associations of dietary fat with chronic disease.

Perhaps the most obvious way that diet might alter the activity of protein kinase C would be through release of activators (and/or inhibitors) in the intestine during digestion and absorption of fat. This has been explored primarily in the colon because activation of protein kinase C would be suspected to stimulate cell proliferation, thereby promoting tumor formation (62). Craven & DeRubertis (27) found that intracolonic instillation of arachidonic, linoleic, or oleic acids (but not palmitic) induced the translocation of protein kinase C to membranes and increased the incorporation of [^{3}H]thymidine into DNA, which suggests that luminal fatty acids may serve as tumor promotors via activation of protein kinase C. Diacylglycerols, apparently formed by intestinal microflora, have been found in human feces (81) and may be able to activate protein kinase C in colonic epithelial cells. Bile acids additionally affect protein kinase C activity in the colon (29). Protein kinase C activity is altered in colonic cells that are in a preneoplastic state (20) and in colon cancers (28, 49, 130); the particulate activity is elevated in colonic mucosa in ulcerative colitis (103), a disease that is similar in some respects to cancer because it is believed to begin with mucosal hyperproliferation and subsequent inflammation. Therefore, modulation of the activity of this enzyme may not only be involved in the occurrence of cancer but also may provide a target for chemotherapy.

Diet may affect protein kinase C in other parts of the body as well (14). High fat diets have been shown to increase the level of diacylglycerol and

the amount of membrane-associated protein kinase C activity in epidermal cells (14, 15, 24, 34), a model often utilized for two-stage tumorigenesis. Additionally, protein kinase C may be involved in lipotroph-deficient diet-induced hepatocarcinogenesis (16).

As inhibitors of protein kinase C, sphingosine and other long-chain bases have potential as antitumor agents (57), especially since sphingolipids are widespread in nature (although rarely found in prokaryotes). Sphingosine blocked the induction of ornithine decarboxylase by phorból esters in mouse skin (38, 50), one biochemical marker of tumor promotion; however, it did not reduce the number of tumors in a longer term study (37). In a cell culture model of transformation (mouse C3H10T1/2 cells) (18), sphingosine and sphinganine reduced cell transformation in response to gamma irradiation and phorbol esters. In addition, a recent carcinogenesis study using mice treated with N,N-dimethylhydrazine (DMH) to induce colon tumors (D. L. Dillehay et al, unpublished observations) showed that including milk sphingomyelin in the diet reduced the number of aberrant colonic crypts in short-term studies and decreased the tumor incidence by approximately one third. Apparently, the effects of sphingosine depend on the cell type because low concentrations of long-chain bases are mitogenic for Swiss 3T3 cells (143).

Diacylglycerol levels can be affected by other factors, such as increasing the concentration of glucose (25, 26, 40, 98, 131, 138, 139), and the implications for these changes with respect to activation of protein kinase C have been explored. Studies with isolated glomeruli (26) observed that increasing the glucose concentration from 5 to 30 mM increased the mass of diacylglycerol within 5 to 15 min and activated protein kinase C as assessed by translocation of the activity from the soluble to particulate fraction. The authors suggest that this may be a stimulus for kidney growth in diabetes and may mediate glomerular hypertrophy in this disorder (26). Glucose also increased de novo synthesis of diacylglycerol in rat skin, which resulted in marked increases in vascular clearance of albumin and blood flow (139). These changes appear to be mediated via protein kinase C because they could be mimicked by phorbol esters and were attenuated by a protein kinase C inhibitor. Hepatocytes treated with insulin show both increased synthesis of diacylglycerol and higher cytosolic and particulate protein kinase C activities (25). A number of factors can affect the diacylglycerol levels of liver (120). Cytosolic protein kinase C activity is decreased in the epithelial cells from the small intestine of rats with streptozotocin-induced diabetes, but the particulate activity was unchanged despite an elevated diacylglycerol mass (131). Administration of insulin increased the levels of diacylglycerol further, and cytosolic protein kinase C returned to normal levels. These findings illustrate the complex nature of protein kinase C regulation.

Various leukocytes (neutrophils, monocytes, macrophages, etc) and plate-

lets are regularly exposed to unsaturated fatty acids and other activators of protein kinase C, for example, during the release of fatty acids from circulating lipoproteins by lipoprotein lipase. While much is known about the utilization of endogenous lipids as second messengers in the activation of these cells (97, 135), there is less information about the effects of plasma lipids on the behavior of these cells. Uhlinger et al (122) have observed that neutrophils isolated from subjects who had consumed a lipid-rich meal exhibit characteristics of "priming"—that is, the cells are not yet activated but have progressed to a state that is more readily activated. Such priming can occur when neutrophils are treated with low concentrations of protein kinase C activators. Consumption of dietary fat (58) and phospholipids (61) also affects the behavior of platelets and neutrophils. These findings have been interpreted as a reflection of changes in eicosanoid metabolism, although the results could be attributable to effects of unsaturated fatty acids on protein kinase C.

Implications for Nutritional Toxicology

If one considers the role of protein kinase C in the activation of leukocytes, there may be instances in which dietary fat causes inflammation of the gastrointestinal tract by activating local neutrophils or macrophages. This may account for the elevated particulate protein kinase C activity in colonic mucosa in ulcerative colitis (103). Activation of leukocytes results in the generation of superoxide anions, hydroxyl radicals, and other reactive oxygen species as well as the secretion of proteases and other bacteriostatic enzymes. These toxic species have been implicated in atherosclerosis, cancer, and inflammatory diseases.

At the other end of the spectrum, recent studies have discovered a marked elevation in the amounts of two inhibitors of protein kinase C, sphinganine and sphingosine, when animals were fed fumonisins (133), mycotoxins produced by *Fusarium moniliforme* and related molds (for additional information, consult the chapter in this volume by Riley, Norred & Bacon). These long-chain bases accumulate because fumonisins are potent inhibitors of ceramide synthase (132). Consumption of feed contaminated with *F. moniliforme,* the prevalent mold on corn worldwide, is known to result in several agricultural diseases (such as equine leukoencephalomalacia and porcine pulmonary syndrome) and has been correlated with human esophageal cancer (see chapter in this volume by Riley, Norred & Bacon). The mechanisms whereby accumulation of sphinganine and sphingosine produces these diseases are not known; however, these compounds are toxic to cells at high concentrations, seemingly because of inhibition of protein kinase C (79).

As more is learned about the pathways of cell regulation by lipids, a better understanding of some of the health effects of many other dietary constituents will emerge.

Perspectives for the Function of Other Bioactive Lipids

That so many new compounds—and new functions for old compounds—are still being discovered today is somewhat astonishing. In part, this is due to the development of better methods for analyzing lipids that are present in small amounts as second messengers. However, it is probably equally attributable to the general acceptance of a new paradigm that lipids function in diverse ways as modulators of cell function.

Our current knowledge about protein kinase C and other bioactive lipids (the eicosanoids, steroid hormones, etc) gives a glimpse of the complexity of these systems. Much is known about how phospholipases act on glycerolipids to activate protein kinase C, but we have probably only uncovered a few of the factors that regulate this system. Studies are just beginning to explore how the metabolism of an even more diverse class of lipids, the sphingolipids, participates in cell regulation. The hydrolysis of sphingomyelin to ceramides has already been implicated in some of the cellular responses to 1-alpha,25-dihydroxyvitamin D_3, tumor necrosis factor, and gamma-interferon (55, 65, 91). Other hydrolysis products of sphingolipids (e.g. sphingosine and some lysosphingolipids) are potent inhibitors of protein kinase C, as described above, and also have been found to inhibit a number of other regulatory enzymes (including the Na^+,K^+-ATPase and phosphatidic acid phosphohydrolase) and to activate the epidermal growth factor receptor (78). When one considers the iterative fine-tuning that nucleic acids and proteins have undergone over the course of evolution, one should not be surprised by the evolution of sophisticated properties in lipids.

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