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Overview

Regulation of Protein Kinase C Activity by Various Lipids

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Protein kinase C has recently attracted considerable attention because of its importance in the control of cell division, cell differentiation, and signal transduction across the cell membrane. The activity of this enzyme is altered by several lipids such as diacylglycerol, free fatty acids, lipoxins, gangliosides, and sulfatides. These lipids may interact with protein kinase C either directly or through calcium ions and produce their regulatory effect (activation or inhibition) on the activities of the enzymes phosphorylated by this kinase. These processes widen our perspective of the regulation of intercellular and intracelluular communication.

KEY WORDS: Protein kinase C; diacylglycerols; free fatty acids; lipoxins; gangliosides; sulfatides; phorbol esters; protein phosphorylation.

I. INTRODUCTION

Many cellular processes are regulated by the binding of agonists (hormones, neurotransmitters, and growth promoting factors) to specific receptors located in the plasma membrane (1, 2). The process of information transfer from an agonist in the extracellular compartment to the regulation of cell function by enzymes inside the cell is achieved by a number of different signaling mechanisms. A well understood example is the β-adrenergic stimulation of adenylate cyclase which causes an increased production of cyclic adenosine 3′-5′-monophosphate (cAMP) and subsequent activation of cAMP-dependent protein kinase (cAMP-PK). The phosphorylation of various target enzymes by this enzyme within the cell results in alteration of specific cell functions (2, 3). The modulation of cell function can also be achieved by the alteration in concentration of Ca^{2+} in the cytosol (4–6). Several studies have indicated that changes in Ca^{2+} concentration markedly affect the activities of various protein kinases and phosphatases (1, 2, 3) either directly or through calmodulin (7, 8). Many Ca^{2+} mobilizing agonists (hormones or neurotransmitters) express their biological response by activating a phoshodiesterase called "phospholipase C", which catalyzes the degradation of inositol-containing phospholipids (9–11). The breakdown of phoshatidylinositol 4,5-bisphosphate results in formation of $InsP_3$, and DAG. Both metabolites act as intracellular second messengers (Figure 1). $InsP_3$ is responsible for mobilizing intracellular Ca^{2+}, and DAG activates a phospholipid dependent protein kinase, termed protein kinase C (PK-C) (12). Thus hormones and neurotransmitters that affect the metabolism of inositol phospholipids not only mobilize calcium with subsequent phosphorylation of protein by Ca^{2+} dependent protein kinases or dephosphorylation by Ca^{2+}-dependent protein phosphatases, but also produce increased phosphorylation of a va-

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Abbreviations used: Protein kinase C (PK-C), cAMP dependent protein kinase (cAMP-PK), diacylglycerol (DAG), phosphatidylserine (PtdSer), inositol 1,4,5-trisphosphate ($InsP_3$), inositol 4,5 bisphosphate (PtdIns 4,5-P_2), free fatty acid (FFA), myelin basic protein (MBP), adenosine triphosphate (ATP), guanine triphosphate (GTP), 12-tetradecanoylphorbol-13-acetate (TPA), epidermal growth factor (EGF), platelet derived growth factor (PDGF), and N-acetylneuraminic acid (NeuNAc).

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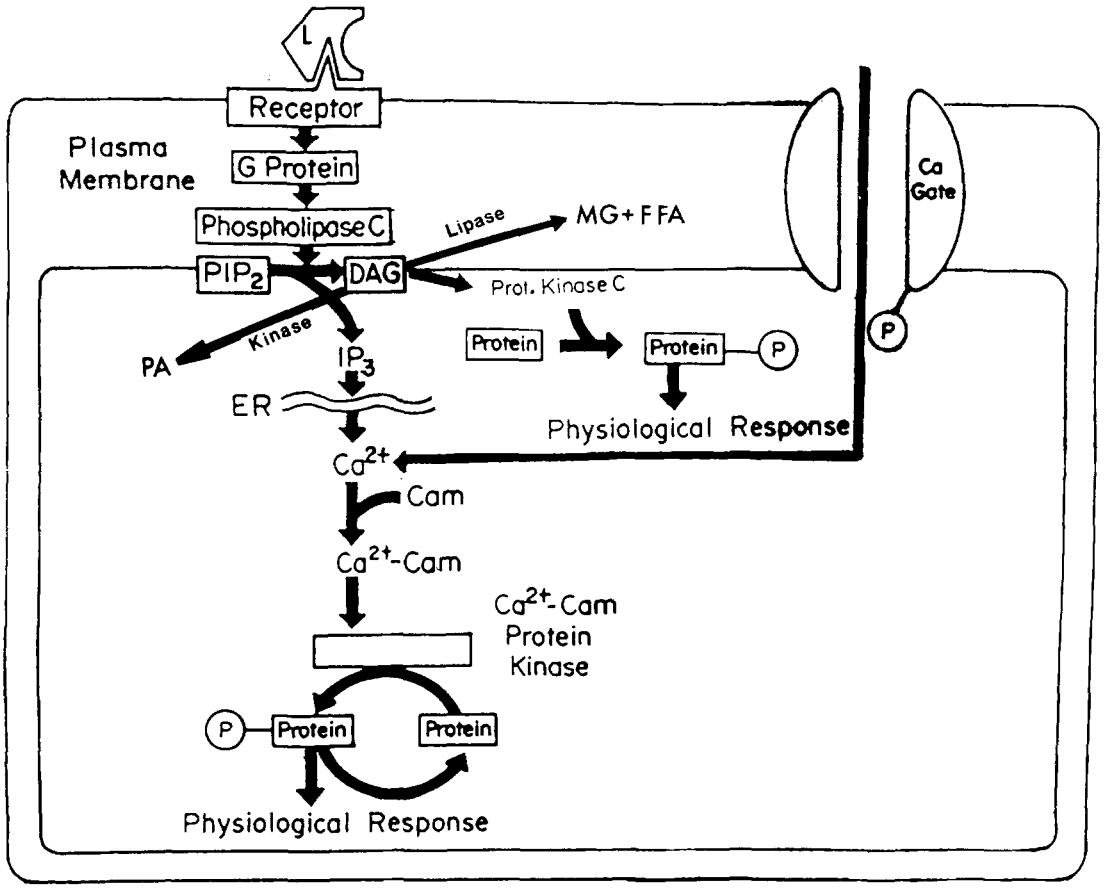

Fig. 1. Proposed model for receptor mediated breakdown of PIP_2 and stimulation of protein kinases by diacylglycerol and calcium ions. Abbreviations: PIP_2, phosphatidylinositol 4,5-bisphosphate; $InsP_3$, myoinositol 1,4,5-trisphosphate; DAG, 1,2-diacylglycerol, Cam kinase, Ca^{2+}-calmodulin protein kinase; ER, endoplasmic reticulum, MG, monoacylglycerol; FFA, free fatty acid; and PA, phosphatidic acid; Modified from Exton (128).

riety of intracellular proteins by the activation of PK-C (12, 13).

Several reviews have been published recently on the metabolism of polyphosphoinositides and their role in signal transduction (9–18), but these reviews deal only with the activation of PK-C by diacylglycerols and phorbol esters. Recently there have been significant developments in studies on the activation of PK-C by other lipids such as unsaturated free fatty acids, gangliosides, and sulfatides. The purpose of this review is to discuss and critically evaluate the recent developments on the activation of PK-C in order to understand the regulation of this enzyme at the molecular level.

II. PROPERTIES OF PK-C

PK-C was discovered and purified by Nishizuka and his associates (19, 20) from rat brain. The enzyme can be activated in vitro by a Ca^{2+}-dependent protease also present in brain, but it is not known whether this event occurs in vivo. Without proteolysis, the activation of PK-C requires calcium, phosphatidylserine, and diacylglycerol for optimal activity. Studies on subcellular localization of PK-C indicate that in guinea pig or rat heart the enzyme is mostly localized in the cytosolic fraction, whereas in spleen and brain it is present nearly equally in the cytosolic and particulate fractions (21, 22). In human neutrophils and leukemic cells PK-C is exclusively localized in the particulate fraction (23). Brain PK-C is a slightly acidic monomeric protein of Mr 70,000–90,000 (Table I). It contains a hydrophobic domain, which interacts with Ca^{2+} and phospholipids, and a hydrophilic domain which contains the active site (19). The two domains are cleaved by Ca^{2+}-dependent neutral proteases to produce a Ca^{2+}- and phospholipid-independent active enzyme fragment (12, 13). Diacylglycerol activates PK-C by increasing its affinity for Ca^{2+} and

Table I. Physicochemical and Kinetic Properties of PK-C

Property	Value
Mol. wt.	70,000–80,000
Isoelectric point (pH)	5.2
Nature	Non-globular
Frictional ratio (f/fo)	1.4
Ca^{2+}	stimulation
Phosphatidylserine	stimulation
K_m value ATP	4–8 μM
K_m value Troponin I	3.4 μM
pH optimum	7.5
Vmax (μmol/min/mg protein)	2.6

Summarized from Kuo et al. (97).

phosphatidylserine (19, 20). In the presence of diacylglycerol, this enzyme is activated at the physiological intracellular concentration of Ca^{2+} (10^{-7} M). Thus the processes which increase the concentration of diacylglycerol in plasma membrane markedly stimulate PK-C activity (15, 16) without mobilization of Ca^{2+} from intracellular stores (see below).

ATP is an efficient phosphate donor for PK-C catalyzed protein phosphorylation with a K_m value of about 4–8 μM (19, 24). GTP cannot replace ATP as a phosphorylating agent (24). Parker et al. (25) have recently determined the complete primary structure of bovine brain PK-C. The amino acid sequence data indicate that PK-C, at the amino terminal end, contains a cysteine-rich domain, a putative calcium-binding domain, and a carboxyl terminal kinase domain which shows substantial homology, but not identity with sequences of other protein kinases. It must be noted here that this enzyme does not show homology to any oncogenes (26) that have been described to date. Coussens et al. (27) have reported the occurrence of multiple distinct forms of bovine and human PK-C, designated as α, β, γ. These authors have identified a family of genes that encode polypeptides closely related to PK-C. Huang et al. (28) have separated the multiple forms of rat brain enzyme by hydroxylapatite column chromatography. These forms were designated as type I, II, and III. Polyclonal antibodies raised against a mixture of three types of the kinase preferentially inhibit type I and type II enzymes. Monoclonal antibodies against type I and type II kinase only recognize their respective enzymes but not the type III enzyme. The amino acid sequences of bovine and human brain PK-C multiple forms (α, β and γ) closely resemble rat brain isozymes (I, II and III) respectively. This observation recommends the use of one nomenclature in future studies.

PK-C has a broad protein substrate specificity which is different from cyclic nucleotide-dependent and calmodulin-dependent protein kinases (20, 24). PK-C catalyzes the transfer of phosphate groups to threonyl and seryl residues, which distinguishes it from tyrosine protein kinases (19). This enzyme can phosphorylate histone H_1, troponin T, smooth muscle myosin light chain, myelin basic protein, and many others (12). The enzymes phosphorylated by PK-C are shown in Table II. It is interesting to note that in most cases phosphorylation markedly increased the activity of substrate enzymes. However, in a few cases inhibition of enzyme activity has also been reported. Two important questions remain to be answered. (a) Is there an overlap in phosphorylation of enzyme proteins by various kinases, i.e. is a single enzyme protein phosphorylated by PK-C as well as by nucleotide dependent protein kinase? (b) What effect does this phosphorylation have on cellular response? The existence of multiple forms of PK-C suggests that cellular responses may be affected by the activation of one or more multiple forms of PK-C. Effects of various activators (see below) on the multiple forms of PK-C may be major determinants of the specificity of this phosphorylation activity.

Stimulation of PK-C by Various Lipids. PK-C activity is regulated by phospholipids, neutral lipids,

Table II. Phosphorylation of Various Enzyme Proteins by PK-C

Enzyme	Effect on activity	Reference
Glycogen phosphorylase kinase	Increased	(98)
Glycogen synthase	Decreased	(41, 123)
Phosphofructokinase	Increased	(99, 100, 101)
Adenylate cyclase	Increased	(102)
Myosin light chain kinase	Increased	(103, 104)
β-Hydroxy-β-methylglutaryl	Decreased	(105)
-Coenzyme A reductase		(106)
Tyrosine hydroxylase	Increased	(107)
NADPH oxidase	Increased	(108, 109)
Cytochrome P450	Increased	(110)
Guanylate cyclase	Increased	(111)
DNA methylase	Increased	(112)
Inositol trisphosphate		
5′-phosphomonoesterase	Increased	(113)
Ca^{2+}-Activated neutral protease	Increased	(114)
Protein phosphatase	Increased	(115)
Ca^{2+}-ATPase	Increased	(116)
Na^+, K^+-ATPase	Increased	(117)
Phospholipid methyltransferase	Increased	(118)
Phospholipase C	Increased	(119, 120)

glycolipids, and unsaturated free fatty acids (Table III). Phospholipids, sulfatides, and gangliosides interact with PK-C through Ca^{2+} whereas unsaturated free fatty acids and lipoxin A do not require Ca^{2+} for this activation. In the following section we discuss the regulation of PK-C activity by various lipids.

a. Stimulation by Diacylglycerol. In the resting cell, PK-C is found mainly in the cytosol, presumably in an inactive state. When an appropriate agonist interacts with the cell, a rapid activation of inositol specific phospholipase C through a G protein (16, 18) results in a marked increase in transient levels of diacylglycerol from PtdIns 4,5-P_2 in plasma membrane. PK-C and Ca^{2+} interact with the phospholipid (phosphatidylserine) head groups of the membrane. The interaction of PK-C with diacylglycerol shifts the Ca^{2+}-dependence of this enzyme from a high concentration (10^{-5} to 10^{-4} M) to a low concentration (10^{-7} M), thereby activating it (29). It has been suggested that a change in PK-C distribution by agonists probably serves as a regulatory mechanism to alter the endogenous protein substrates readily available for phosphorylation by this enzyme. Kishimoto et al. (30) studied the structural requirement of diacylglycerol for the activation of PK-C and found that the activation required the presence of an unsaturated fatty acid within the DAG molecule. Thus 1,2-diacylglycerols containing two long-chain saturated fatty acids were less effective than diolein. Cabot and Jaken (31) and Lapetina et al. (32) on the other hand, indicated that some saturated diacylglycerols (1,2-didecanoylglycerol and 1-palmitoyl-2-butyrylglycerol) were capable of activating PK-C activity. During their studies on the nature of PK-C activation by physically defined phospholipid vesicle and DAG, Boni and Rando (33) found that the specificity of PK-C activation is directed at the glycerol backbone and the position but not the nature of fatty acid side chains. These studies indicated that only 1,2-*sn*-diolein, but not its 2,3-*sn*-enantiomer or its 1,3-isomer, was capable of activating PK-C. Thus it appears that, although an unsaturated fatty acid is not required in DAG for PK-C activation, the acyl ester must satisfy certain balanced physical properties for water/lipid solubility or orientation so that it can partition favorably into a bilayer for an effective access to PK-C (31–34). Brockerhoff (35) proposes, on the basis of space-filling molecular model studies, a 3-point fixation model for the attachment of DAG with PK-C. According to this model, *sn*-1,2-DAG donates a hydrogen bond from the *sn*-3 hydroxyl to the enzyme and it receives two hydrogen bonds in the *sn*-1 and *sn*-2 ester C = O groups from the enzyme. Ca^{2+} does not appear to bind to PK-C directly. Nevertheless, it interacts with vesicles of phosphatidylserine (15,18).

Using mixed micelles of Triton X-100, phosphatidylserine, calcium, and DAG, Hannun et al. (36, 37) studied the stoichiometry of PK-C activation. They indicated that: 1) PK-C is fully activated by mixed micelles containing 8 mol% phosphatidylserine and 2.5 mol% DAG; 2) activation does not require a phospholipid bilayer; 3) activation by phosphatidylserine is highly cooperative and requires four or more molecules of phosphatidylserine; 4) activation by DAG is noncooperative and requires only a single molecule of this neutral lipid and 5) PK-C monomers bind to mixed micelles in a phosphatidylserine and calcium-dependent manner and are inactive in the absence of DAG.

A number of other enzymes are also regulated by DAG (Table IV). The stimulation of phospholipases A_1 and A_2 is of particular interest. According to Dawson et al. (39), this stimulation is caused by the ability of DAG to change the organization or orientation of the phospholipid bilayer. Michell et al. (40) also indicated that high concentrations of DAG are detrimental to normal bilayer membrane structures.

The disruption of bilayer membrane structures also results in inhibition of glycogen synthetase (41) and Na^+,K^+-ATPase (42). Kido et al. (43) and Verma et al. (44) have recently reported that DAG

Table III. Activators of PK-C

Name	Reference
Diacylglycerol	(20, 30)
Unsaturated fatty acids	(53–56, 72, 121, 130)
Gangliosides	(60, 62, 63, 67)
Sulfatides	(70)
Eicosanoids	(72)
Lipoxin A	(72)
Lipopolysaccharides	(75)
γ-Interferon	(90)
Bryostatin	(122)
Insulin	(129)
Tumor promotors*	
Phorbol esters	(48)
Chloroform	(76)
Benzene	(77)
Toluene	(77)

* Not physiological

Table IV. Direct Effects of Diacylglycerols on Activities of Various Enzymes

Enzyme	Effect on activity	Reference
PK-C	Stimulated	(13, 78)
Phospholipase A_2	Stimulated	(39, 124, 125)
Glycogen synthetase	Inhibited	(41, 126)
Tyrosine aminotransferase	Stimulated	(43)
Ornithine decarboxylase	Stimulated	(43)
Na^+,K^+-ATPase	Inhibited	(42)
β-Glucosaminidase	Stimulated	(127)
Cytidylyltransferase	Stimulated	(131)

specifically enhances the induction of tyrosine aminotransferase and ornithine decarboxylase by dexamethasone phosphate in a dose-dependent manner. The level of DAG in the membrane is controlled by two important enzymes. Diacylglycerol kinase rapidly phosphorylates DAG to phosphatidic acid (45) and diacylglycerol lipase hydrolyzes this neutral lipid to monoacylglycerol and free fatty acid (46, 47). Both diacylglycerol kinase and lipase have been recently purified to homogeneity.

b. Stimulation by Phorbol Esters. Castagna et al. (48) were the first to report that TPA mimicked the action of DAG on a partially purified preparation of PK-C. The activation of PK-C by phorbol esters requires phospholipids. The catalytic properties of calcium- and DAG activated protein kinase have been compared with PK-C which was activated by TPA without calcium, and the K_m value for histone H was found to be 4–5 fold higher for the TPA activated enzyme (49). Nevertheless, it is not clear whether the lower K_m is due to calcium or some other factor.

Brasseur et al. (50) studied the specific structural parameters of DAG required for the binding of this lipid with PK-C and compared them with phorbol ester (Figure 2). These authors suggested that the hydrophobic domain of DAG (acyl chains at C_1 and C_2) and phorbol ester (acyl chain at C_{12}) is required for relatively unspecific interactions with the adjacent lipid microenvironment and that, in addition, highly specific electrophilic interactions involving the CH_2OH group (at C_3 of DAG and C_{20} of phorbol esters) are essential for the binding to the PK-C-phosphatidylserine-calcium complex and subsequent enzyme activation. However, it should be noted here that the apparent affinity of 1,2 diolein for activating PK-C is 2 to 3 orders of magnitude less than that of phorbol ester (48) suggesting that additional interactions may occur in the vicinity of the CH_2OH group in C20 which may confer to the phorbol ester molecule a higher stereospecificity. This hypothesis of Brasseuer et al. (50) is consistent with a recently reported stereochemical model which indicates that the region of the phorbol molecule containing the 3-keto, 4-OH, and 20-CH_2OH residues is critical for biological activity of phorbol esters (51).

DAG competitively inhibits phorbol ester binding to a brain cytosolic phorbol ester aporeceptor (52). The regulation of PK-C involves binding of the cytosolic enzyme to the plasma membrane. Similar to the above process, phorbol esters also cause a rapid decrease in cytosolic PK-C and a corresponding increase in the amount bound to the plasma membrane (12, 13). Transfer of this enzyme from the cytosol to the plasma membrane which brings it into contact with DAG and phospholipids has been extensively used as a selective and sensitive probe of the DAG activated PK-C pathway in intact cells.

c. Stimulation by Unsaturated Fatty Acids. McPhail et al. (53) were the first to show that arachidonate and other unsaturated FFA acids markedly stimulate human neutrophil PK-C (Table IV). This activation by FFA required Ca^{2+} and was enhanced by DAG (diolein) but not by phosphatidylserine. Using a purified preparation of rat brain PK-C, Murakami and Routtenberg (54) also found that oleic and arachidonic acids activate PK-C but independently of phospholipid and Ca^{2+}. They also compared the effect of *cis* and *trans* FFA on PK-C and reported that oleic acid showed much higher potency as an activator than elaidic acid, although both were required at very high concentration. The physiological implications of *cis*-unsaturated FFA-induced activation of PK-C are not clear at present. It is likely that other signaling mechanisms may exist for PK-C activation, in addition to phospholipase C/phosphatidylinositol turnover signaling, possibly via the liberation of *cis*-FFA either by the Ca^{2+}-dependent phospholipase A_2 system or by plasma membrane diacylglycerol lipase reaction. Murakami et al. (55) have recently found that *cis*-linoleic acid also activates PK-C in vitro in a dose dependent manner. Full activation can be obtained with *cis*-fatty acids in the absence of Ca^{2+}. However, the effect of Ca^{2+} becomes significant when lower concentrations of fatty acids are used. Sekiguchi et al. (56) have recently indicated that differ-

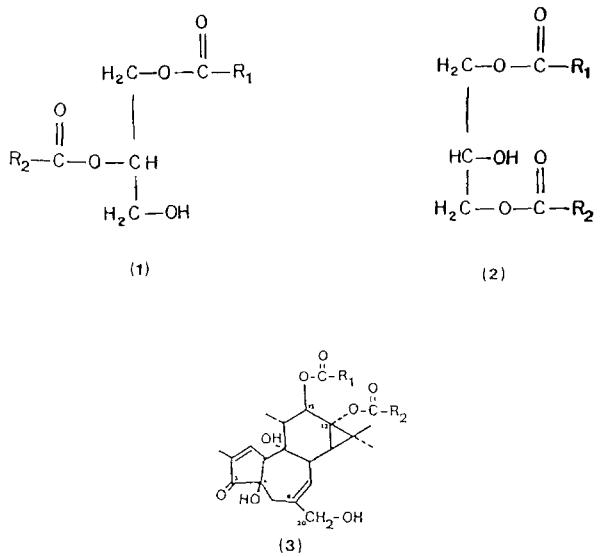

Fig. 2. Chemical structures (1) 1,2-diacylglycerol (2) 1,3-diacylglycerol and phorbol ester (12-0-tetradecanoylphorbol-13-acetate).

ent forms of brain PK-C respond differently to free unsaturated FFA. Thus type I PK-C is less sensitive to arachidonic acid activation than are type II and III protein kinases C. It is not known at present whether unsaturated FFA interact with PK-C in a way similar to diacylglycerols or not. However, it may be possible that FFA bind to the hydrophobic domain of PK-C without Ca^{2+}. It must be emphasized here that the concentrations of unsaturated FFA used by the above investigators (53–56) are very high and unphysiological. Such levels of FFA do not occur in vivo even under pathological situations (ischemia and spinal cord trauma) in which FFA and their metabolites have been reported to have perturbing effects on biological membranes (16–18).

d. Stimulation by Gangliosides. The requirement of phospholipids for PK-C activity, and the activation of PK-C by DAG raise the question as to whether other lipids may modulate PK-C activity. Gangliosides are complex glycosphingolipids which form complexes with Ca^{2+} through their sialic acid moieties, so it seems reasonable that they might modulate the activities of some enzymes. Indeed, it has been found that gangliosides added exogenously to assays of Na^+,K^+-ATPase (57), cyclic nucleotide phosphodiesterase (58), and adenylate cyclase (59) alter the activities of these enzymes. The latter two are both Ca^{2+}-calmodulin dependent systems. Goldenring et al. (60) studied the effects of gangliosides on protein kinase activities in rat brain membranes. In the presence of Ca^{2+}, exogenous gangliosides stimulated the phosphorylation of several proteins with Mr of 45 kDa and higher, which was not seen with Ca^{2+} in the absence of gangliosides.

The pattern of protein phosphorylation stimulated by gangliosides was similar to that stimulated by calmodulin, but the kinase activities stimulated by the latter were much more sensitive to trifluoperazine that the former. From these results it was suggested that there might be a separate kinase system stimulated by Ca^{2+}-ganglioside complex. However, it was also possible that the stimulatory effect was due to gangliosides acting through the calmodulin-dependent kinase system.

Exogenous gangliosides also inhibited the phosphorylation of both the large and small MBP and a 78 kD protein whether the added ganglioside was present as the Na^+ or Ca^{2+} complex. MBP is phosphorylated by PK-C (61). It was postulated that ganglioside was inhibiting PK-C and that the 78 kD protein was the autophosphorylated product of PK-C. Inhibition of rat brain MBP phosphorylation could not be produced with asialoGM1, ceramide, or NeuNAc, and there was a quantitatively different inhibiting effect among the gangliosides (GT1b > GD1b > GD1a > GM1) (62). This inhibitory effect could not be overcome by elevating Ca^{2+} concentration, nor did high concentrations of Ca^{2+} alter the inhibition constants for specific gangliosides, thus indicating that ganglioside was not causing this effect simply by removing available Ca^{2+} by chelation.

Further evidence for a direct inhibitory effect of gangliosides on PK-C was obtained using partially purified preparations of PK-C isolated from HL-60 promyelocytic leukemia cells and bovine brain (63). A mixture of gangliosides inhibited the stimulation of PK-C activity by diacylglycerols. This inhibition was almost complete at 100 μM and half-maximal at 30 μM but was not seen at unphysiologically high (over 10^{-5} M) concentrations of Ca^{2+}. There was a differential inhibitory effect among the gangliosides tested (GQ1b = GT1b > GD1a = GD1b > GM1 = GM3). However, none was as potent individually as a mixture in the ratio equivalent to that isolated from brain, suggesting a synergism among the different types. Similar results were obtained when PK-C was assayed using type III-S histone, porcine MBP, or a chemically syn-

thesized myelin basic peptide fragment. Gangliosides have a negative charge so it is possible that decreased phosphorylation of MBP or histone could be due to gangliosides binding to these substrates and thereby interfering with the phosphorylation reaction. Nevertheless, there was no difference in kinase activity towards histone over a six-fold range of substrate concentration, making it likely that there was a direct inhibitory effect of ganglioside on PK-C. Both the Na^+ and Ca^{2+} salts of gangliosides were effective inhibitors, indicating that the mechanism of this inhibitory effect is probably different from that of the stimulatory effect of Ca^{2+}-gangliosides on phosphorylation of higher molecular weight proteins.

The inhibitory effect of gangliosides on endogenous phosphorylation of MBP in purified myelin was confirmed by Chan (64, 65) who also found that ganglioside inhibits dephosphorylation of MBP. Nevertheless, this same author found that in the presence of Ca^{2+} and PtdSer, gangliosides stimulated the initial rate of MBP phosphorylation by purified PK-C, although gangliosides alone had little effect on PK-C activity. Gangliosides only slightly increased the total amount of MBP phosphorylation by PK-C from 4.4 to 4.8 mol phosphate/mol MBP. From studies on tryptic digests of MBP it was concluded that gangliosides do affect the phosphorylation of myelin basic protein in myelin not through modulation of PK-C but via the cAMP-dept-PK. Resolution of these apparently contradictory findings must await further experimentation.

The Ca^{2+} complex of GQ1b stimulated a kinase system in plasma membrane of GOTO human neuroblastoma cells to phosphorylate both endogenous proteins as well as exogenous histone H1 and tubulin (66). The latter author believes that this kinase system is different from PK-C, calmodulin activated kinase, and cyclic nucleotide dependent kinase systems because GQ1b had no effect on their activities. In contrast, Kreutter et al. (63) found GQ1b to have an inhibitory effect on partially purified PK-C equivalent to that of GT1b.

Maximum activity of rat brain PK-C can be obtained when GM3 substitutes for PS and the enzyme is stimulated by TPA (67). However, when PK-C is stimulated by DAG, substituting GM3 for PS does not activate the kinase system over the level obtained with Ca^{2+} alone. Polysialogangliosides (GT1b, GD1a, GD3) are less effective than monosialogangliosides (GM4, GM3, GM1) in this capacity. GM3 had a similar effect when this system was stimulated with several other tumor promotors which activate PK-C.

There is evidence that in mouse Swiss 3T3 cells exogenous GM1 and GM3 increase the affinity of PDGF for its receptor and inhibit PDGF stimulated tyrosine phosphorylation of the PDGF receptor (68). In epidermoid carcinoma cells GM1 and GM3 inhibit EGF stimulated tyrosine phosphorylation of the EGF receptor, but do not affect binding of EGF to its receptor (68). GM1 and GM3 both inhibit growth of the cells in which these studies were conducted. Therefore, gangliosides may be involved in regulating phenomena such as cellular division and differentiation through PK-C and other protein kinase systems. Indeed, one kinase which is stimulated by ganglioside and apparently different from other known kinase systems has been identified in both myelin (64) and synaptosomal plasma membranes (65).

Neither asialogangliosides nor sialic acids separately have been found to alter the activity of PK-C, suggesting that the modulating effects of gangliosides are properties of the entire molecule. Sphingosine alone can inhibit rat brain PK-C, raising the possibility that catabolic products of gangliosides may also play a role in regulating PK-C activity (37, 38). Such inhibition by sphingosine does not involve the active site of PK-C. Rather, it seems that through competitive interactions with PtdSer, Ca^{2+} and PK-C, sphingosine displaces activators such as DAG or phorbol esters, thus preventing formation of the active lipid-enzyme complex. Although the concentration of sphingosine in biological membranes is quite low, it is possible that transient elevations in membrane sphingosine levels could occur as a consequence of sphingolipid catabolism with consequent inhibition of PK-C activity. Evidence for a possible physiological role of sphingosine in such a capacity was found for the differentiation of HL-60 cells and oxidative bursts in human neutrophils (66).

e. Stimulation by Sulfatides. With TPA, sulfatides, the 3-sulfate ester of cerebrosides, stimulate PK-C to the same extent as phosphatidylserine (70). Other sphingolipids such as cerebrosides, sphingomyelins, or ceramides did not cause any stimulation of PK-C. Thus the sulfate group of the sulfatides may be necessary for the activation of PK-C. Fujiki et al. (70) also reported although both sulfatide and phosphatidylserine activated PK-C to the same extent in the presence of TPA, their activa-

tions were different in the presence of 1,2-diolein. With DAG (1,2-diolein) PK-C activity in the presence of sulfatide was 4-times lower than in the presence of phosphatidylserine.

The mechanism of activation of PK-C by sulfatides is not understood at present. The binding to PK-C may be similar for the sulfatide-Ca^{2+} complex and the phosphatidylserine-Ca^{2+} complex. It must be noted here that the ratio of specific to non-specific [^{3}H]TPA binding to PK-C was lower in the presence of sulfatide (53.9) than of phosphatidylserine (93.6), indicating that sulfatide caused more non-specific binding than phosphatidylserine. Further, the sulfatide-Ca^{2+} complex may have a different affinity for phospholipid bilayers than the phosphatidylserine-Ca^{2+} complex. It would be interesting to know if other sulfolipids, such as ceramide dihexoside sulfate and sulfogalactosyldiacylglycerol, also activate PK-C and if the extent of activation depends on the fatty acid composition (saturated fatty acid vs unsaturated by hydroxy fatty acid vs non-hydroxy). At present nothing is known either about the affinity of Ca^{2+} for different sulfolipids or the stability of the sulfolipid-Ca^{2+} complexes.

f. Stimulation by Lipoxin A and Other Eicosanoids. Oxygenated products of the "arachidonic acid cascade" are known to have profound effects on cellular metabolism (71). Hansson et al. (72) have found that lipoxin A (5,6,15L-trihydroxy-7,9,11,13-eicosatetraenoic acid), a recently discovered metabolite of arachidonic acid, activates human placental PK-C in the presence of calcium at a 30 fold lower concentration than does arachidonic acid or 1,2-dioleoylglycerol (Table III). The methyl esters of lipoxin A were much less active than lipoxin A, indicating the importance of a free carboxylic group in the lipoxin A induced activation of PK-C. The free acids of leukotriene B_4 as well as two lipoxin B isomers had no effect on PK-C activity. In contrast, linolenic acid, leukotriene C_4, certain mono- and dihydroxylated eicosanoids, and one lipoxin B isomer produced a stimulatory effect on PK-C activity.

Activation of PK-C in vitro results in autophosphorylation of this enzyme (73, 74). Thus the addition of Ca^{2+} and phosphatidylserine to the purified enzyme preparation resulted in increased phosphorylation of native PK-C (Mr 80,000). A slight stimulation of the autophosphorylation was also observed in the presence of calcium and TPA, whereas lipoxin A and B did not stimulate this type of phosphorylation. In contrast, lipoxin A stimulates the phosphorylation of at least four proteins. Three phosphoprotein bands; one at Mr 30,000 and a doublet at Mr 60,000 were similarly affected by the addition of TPA or dioleoylglycerol, whereas the increased phosphorylation of phosphoprotein (Mr 97,000) was most pronounced after lipoxin A stimulation. The mechanism of lipoxin induced activation of PK-C is not known. However, lipoxin A probably binds to the hydrophobic domain of PK-C in a manner similar to unsaturated FFA, but with higher affinity.

g. Stimulation of PK-C by Lipopolysaccharide. The active lipid moiety of lipopolysaccharides of Gram-negative bacteria, diacylglucosamine 1-phosphate, markedly stimulates the activity of PK-C (75). It has been suggested that the stimulation may be due to the structural similarity of diacylglucosamine 1-phosphate to phosphatidylserine and some biological effects of lipopolysaccharide may be mediated by its interaction with PK-C.

h. Stimulation of PK-C by Organic Solvents. Organic solvents such as chloroform, benzene, and toluene are tumor promoters (76, 77). They also markedly stimulate PK-C but without competing with TPA for its binding site, indicating that the mechanism of action of tumor promotor solvents is different from the phorbol esters.

III. ROLE OF PK-C

PK-C is an ubiquitous enzyme found in a variety of mammalian tissues (21). This enzyme has attracted particular attention because it plays a pivotal role in controlling many cellular functions. Nishizuka's group was the first to suggest that PK-C plays an important role in stimulus-response coupling for the release of serotonin from platelets (76). Subsequent studies by many investigators have confirmed the role of PK-C not only in vesicular release from platelets but also in exocytosis of cellular constituents from a variety of endocrine and exocrine tissues (12, 13). Two signal pathways, PK-C activation and Ca^{2+} mobilization, can be selectively and independently induced in vitro by the addition of permeable DAG (or phorbol ester) for the former, and the calcium ionophore A23187 for the latter. On the basis of these studies it has been sug-

gested that the two signal pathways (protein phosphorylation and Ca^{2+} mobilization) often act synergistically to elicit the full cellular response (12, 18). The paramount role of PK-C in signal transduction at the cell surface has been extended also to neuronal functions, such as release of neurotransmitters in the peripheral and central nervous systems and the modulation of membrane conductance (16, 79, 80). According to Nishizuka (12) this enzyme may also play an important role in gene expression and cell proliferation.

As shown in Table II PK-C phosphorylates a number of enzymes and stimulates or inhibits their activities. The dephosphorylation of phosphorylated enzymes is catalyzed by a Ca^{2+}-dependent protein phosphatase. These phosphorylation/dephosphorylation cycles may have important effects on cellular metabolism and function (2, 3). PK-C also phosphorylates a number of receptors related to control of cell growth and differentiation as well as modulation of the immune system (18, 81, 82). There are a number of cytoskeletal proteins which appear to be selectively phosphorylated by PK-C (12), suggesting that this kinase may have a role in control of cell shape, movement, and intracellular transport. PK-C is also responsible for the phosphorylation and activation of the Na^+-H^+ exchanger (83). Changes in Na^+-H^+ exchanger activity may be reflected in alterations in cell volume. The activation of PK-C is essential for triggering the stimulation of amino acid transport in mouse embryo fibroblast (84). Activation of PK-C has also been implicated in the development of mouse mammary gland (85, 86). This enzyme may also be involved in the regulation of Ca^{2+} gating (80, 87). On the basis of his studies on the regulation of synaptic plasticity, Miller (88) has recently concluded that PK-C is a key regulator of neuronal excitability.

As discussed earlier, tumor-promoting phorbol esters have a structure similar to DAG and they activate PK-C both in vitro and in vivo (48, 50). Thus PK-C is a receptor for that tumor promotor. It must be mentioned that cellular responses to phorbol ester and DAG are quite different. For example, phorbol ester does not always increase intracellular levels of free calcium while this is consistently observed with DAG. Another possibility is that interactions with phorbol ester result in an active form of PK-C which is catalytically distinct from PK-C activated by DAG. Finally, the half life of DAG in cells is very short (48), while phorbol ester is only slightly degraded. This suggests that phorbol esters probably effect some limited phase of cellular response which is similar but not identical to the sequence of events during DAG stimulation.

The treatment of the Daudi human β-lymphoblastoid cell line with human β-interferon results in a 4-fold increase in the concentration of diacylglycerols and a marked increase in PK-C activity (89, 90) but the action of interferon in Daudi cells does not involve changes in the concentration of cytosolic Ca^{2+}. This suggests that PK-C may play an important role in the action of interferon in the cells, but the precise biochemical pathway remains unknown.

PK-C may also be involved in the regulation of arachidonic acid metabolism because the treatment of several types of cells with phorbol esters results in increased levels of prostaglandins. The rate limiting step in prostaglandin biosynthesis is the release of arachidonic acid from phospholipids catalyzed by phospholipase A_2, or from DAG by diacylglycerol and monoacylglycerol lipases. The activities of the lipolytic enzymes are regulated by inhibitory proteins called lipocortins (91). Khanna et al. (92) have reported that PK-C phosphorylates lipocortin in vitro. Phosphorylation of a lipocortin has been shown to decrease its inhibition of phospholipase A_2 (93, 94).

Another mechanism by which a phorbol ester might activate phospholipase A_2 is by activation of PK-C resulting in activation of a tyrosine kinase leading to phosphorylation of lipocortin (95). Increasing intracellular calcium would be another way PK-C may stimulate phospholipase A_2 indirectly.

V. CONCLUSION

It is obvious that PK-C activity in vitro is regulated by several lipids including DAG, gangliosides, sulfatides, unsaturated FFA, and lipoxin A. The lipid activators of PK-C can be classified into groups. The first group of activators including DAG, phosphatidylserine, gangliosides, and sulfatides, acts through Ca^{2+} binding. Lipid-Ca^{2+} complexes can interact with the lipophilic domain of PK-C and produce marked stimulation of enzymic activity. This suggestion is supported by the work of Walsh et al. (96) who have shown that PK-C becomes more hydrophobic in the presence of Ca^{2+}. The increase in the hydrophobicity of this enzyme may increase its affinity to the lipid activator. The second group of activators, including unsaturated FFA and li-

poxin A, does not require calcium and probably interacts with PK-C through a different mechanism. Multiple forms of PK-C are found in mammalian tissues (27). Some forms of PK-C may require a lipid-Ca^{2+} complex for activation whereas others are directly stimulated by *cis* unsaturated free fatty acids and lipoxin A. Mammalian cells also contain several types of receptors on their surface, and stimulation of various types of receptors may require different activators which may bring about phosphorylation of different proteins. Thus, the lipid activators may also be responsible for the specificity of phosphorylation by PK-C.

Association and dissociation of PK-C with membrane lipids is an important event for the regulation of its activity. These interactions control not only the amount of active enzyme but also its subcellular localization. Redistribution of PK-C to cell membranes or specific regions of cell membrane could occur if the lipid activator accumulates in localized regions of the cell membrane. Better understanding of the modes of action and mechanisms of activation of PK-C by various lipids is essential for elucidating the role of PK-C in the regulation of multiple cellular functions.

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REFERENCES

1. Lefkowitz, R. J., Caron, M. G., and Stiles, G. L., 1984. Mechanisms of membrane receptor regulation. New Eng. J. Med. 310:1570–1579.
2. Nestler, E. J., Walaas, S. I., and Greengard, P. 1984. Neuronal phosphoproteins: Physiological and clinical implications. Science 225:1357–1364.
3. Cohen, P. 1982. The role of protein phosphorylation in neural and hormonal control of cellular activity. Nature (London) 296:613–620.
4. Rasmussen, H., and Barrett, P. Q. 1984. Calcium messenger system: an integrated view. Physiol. Rev. 64:938–984.
5. Ebashi, S. 1985. Ca^{2+} in biological systems. Experientia 41:978–981.
6. Murer, E. H. 1985. The role of platelet calcium. Seminars Hemat. 22:313–323.
7. Levine, B. A., and Dalgarno, D. C. 1983. The dynamics and function of calcium-binding proteins. Biochim. Biophys. Acta 726:187–204.
8. Cox, J. A. 1984. Sequential events in calmodulin on binding with calcium and interaction with target enzymes. Fed. Proc. 43:3000–3004.
9. Berridge, M. J. 1986. Growth factors, oncogenes and inositol lipids. Cancer Surveys 5:413–430.
10. Berridge, M. J. 1985. Intracellular signalling through inositol trisphosphate and diacylglycerol. Biol. Chem. Hoppe-Seyler 367:447–456.
11. Berridge, M. J. 1984. Inositol trisphosphate and diacylglycerol as second messengers. Biochem. J. 220:345–360.
12. Nishizuka, Y. 1986. Studies and perspectives of protein kinase C. Science 233:305–312.
13. Nishizuka, Y. 1984. The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature (London) 308:693–698.
14. Sekar, M. C., and Hokin, L. E. 1986. The role of phosphoinositides in signal transduction. J. Membrane Biol. 89:193–210.
15. Abdel-Latif, A. A. 1986. Calcium-mobilizing receptors, polyphosphoinositides and the generation of second messengers. Pharm. Rev. 38:227–272.
16. Williamson, J. R. 1986. Role of inositol lipid breakdown in the generation of intracellular signals—state of the art lecture. Hypertension 8:II 140–156.
17. Bell, R. M. 1986. Protein kinase C activation by diacylglycerol second messenger. Cell 45:631–632.
18. Cuatrecasas, P. 1986. Hormone receptors, membrane phospholipids and protein kinases. The Harvey Lectures Series 80:89–128.
19. Kikkawa, U., Takai, Y., Minakuchi, R., Inohara, S., and Nishizuka, Y. 1982. Calcium-activated, phospholipid dependent protein kinase from rat brain—subcellular distribution, purification and properties. J. Biol. Chem. 257:13341–13348.
20. Takai, Y., Kishimoto, A., Kikkawa, U., Mori, T., and Nishizuka, Y. 1979. Unsaturated diacylglycerol as a possible messenger for the activation of calcium activated phospholipid-dependent protein kinase system. Biochem. Biophys. Res. Commun. 91:1218–1224.
21. Kuo, J. F., Anderson, R. G. G., Wise, B. C., Mackerlova, L., Salomonsson, I., Brackett, N. L., Katoh, N., Shoji, M., and Wrenn, R. W. 1980. Calcium dependent protein kinase: Widespread occurrence in various tissues and phyla of the animal kingdom and comparison of effect of phospholipid, calmodulin and trifluoperazine. Proc. Natl. Acad. Sci. (USA) 77:7039–7043.
22. Katoh, N., and Kuo, J. F. 1982. Subcellular distribution of phospholipid-sensitive, calcium dependent protein kinase in guinea pig heart, spleen and cerebral cortex and inhibition of the enzyme by Triton X-100. Biochem. Biophys. Res. Commun. 106:590–595.
23. Helfman, D. M., Appelbaun, B. D., Vogler, W. R., and Kuo, J. F. 1983. Phospholipid-sensitive Ca^{2+} dependent protein kinase and its substrate in human neutrophils. Biochem. Biophys. Res. Commun. 111:847–853.
24. Wise, B. C., Raynor, R. L., and Kuo, J. F. 1982. Phospholipid sensitive Ca^{2+}-dependent protein kinase from heart: Purification and general properties. J. Biol. Chem. 257:8481–8488.
25. Parker, P. J., Goris, J., and Merlevede, W. 1986. Specificity of protein phosphatases in the dephosphorylation of protein kinase C. Biochem. J. 246:63–67.
26. Bignon, Y. J. and Plagne, R. 1986. Oncogenes. Biomed. 40:138–142.
27. Coussens, L., Parker, P. J., Rhee, L., Yang-Feng, T. L., Chen, E., Waterfield, M. D., Francke, U., and Ullrich, A. 1986. Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. Science 233:859–866.
28. Huang, K. P., Nakabayashi, H., and Huang, F. L. 1986a. Isozymic forms of rat brain Ca^{2+}-activated and phospho-

lipid-dependent protein kinase. Proc. Natl. Acad. Sci. 83:8535–8539.
29. May, W. S., Sahyoun, N., Wolf, M., and Cuatrecasas, P. 1985. Role of intracellular calcium mobilization in the regulation of protein kinase C mediated membrane processes. Nature (London) 317:549–557.
30. Kishimoto A., Takai, Y., Mori, T., Kikkawa, U., and Nishizuka, Y. 1980. Activation of calcium and phospholipid dependent protein kinase by diacylglycerol. Its possible relation to phosphatidylinositol turnover. J. Biol. Chem. 255:2273–2276.
31. Cabot, M. C., and Jakens, S. 1984. Structural and chemical specificity of diacylglycerols for protein kinase C activators. Biochem. Biophys. Res. Commun. 125:163–169.
32. Lapetina, E. G., Reep, B., Ganong, B. R., and Bell, R. M. 1985. Exogenous sn-1,2-diacylglycerols containing saturated fatty acids function as bioregulators of protein kinase C in human platelets. J. Biol. Chem. 260:1358–1361.
33. Boni, L. T., and Rando, R. R. 1985. The nature of protein kinase C activation by physically defined phospholipid vesicles and diacylglycerols. J. Biol. Chem. 260:10819–10825.
34. Nomura, H., Ase, K., Sekiguchi, K., Kikkawa, U., Nishizuka, Y., Nakano, Y., and Satoh, T. 1986. Stereospecificity of diacylglycerol for stimulus-response coupling in platelets. Biochem. Biophys. Res. Commun. 140:1143–1151.
35. Brockerhoff, H. 1986. Membrane protein-lipid hydrogen bonding: evidence from protein kinase C, diglyceride and tumor promotors. FEBS Lett. 201:1–4.
36. Hannun, Y. A., Loomis, C. R., and Bell, R. M. 1985. Activation of protein kinase C by Triton X-100 mixed micelles containing diacylglycerol and phosphatidylcholine. J. Biol. Chem. 260:10039–10043.
37. Hannun, Y. A., Loomis, C. R., and Bell, R. L. 1986. Protein kinase C activation in mixed micelles. Mechanistic implications of phospholipids, diacylglycerol and calcium interdependencies. J. Biol. Chem. 261:7184–7190.
38. Hannun, Y. A., Loomis, C. R., Merrill Jr., A. H., and Bell, R. M. 1986. Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets. J. Biol. Chem. 261:12604–12609.
39. Dawson, R. M. C., Hemington, N., and Irvine, R. F. 1985. The inhibition of diacylglycerol-stimulated intracellular phospholipases by phospholipids with a phosphocholine-containing polar group. Biochem. J. 230:61–68.
40. Michell, R. H., Allan, D., and Finean, J. B. 1976. Significance of minor glycerolipids in membrane structure and function. Adv. Exp. Biol. Med. 72:3–13.
41. Bouscarel, B., and Exton, J. H. 1986. Regulation of hepatic glycogen phosphorylase and glycogen synthase by calcium and diacylglycerol. Biochim. Biophys. Acta 888:126–134.
42. Goldberg, W. J., Dorman, R. V., Dabrowiecki, Z., and Horrocks, L. A. 1985. The effects of ischemia and CDPamines on Na^+, K^+-ATPase and acetylcholinesterase activities in rat brain. Neurochem. Path. 3:237–248.
43. Kido, H., Fukusen, N., Ishidoh, K., and Katunuma, N. 1986. Diacylglycerol amplified the induction in vivo of tyrosine aminotransferase and ornithine decarboxylase by glucocorticoid. Biochem. Biophys. Res. Commun. 138: 275–282.
44. Verma, A. K., Pong, R. C., and Erickson, D. 1986. Involvement of protein kinase C activation in ornithine decarboxylase gene expression in primary culture of newborn mouse epidermal cells and in skin tumor promotion by 12-0-tetradecanoylphorbol-13-acetate. Cancer Res. 46:6149–6155.
45. Lin, C. H., Bishop, H., and Strickland, K. P. 1986. Properties of diacylglycerol kinase purified from bovine brain. Lipids 21:206–211.
46. Farooqui, A. A., Taylor, W. A., and Horrocks, L. A. 1986. Membrane bound diacylglycerol lipases in bovine brain. Pages 181–190, *in* Horrocks, L. A. Toffano, G., and Freysz, L. (eds.), Phospholipids in the Nervous System, Fidia Research Series, Liviana Press, Italy.
47. Farooqui, A. A., Taylor, W. A., and Horrocks, L. A. 1987. Membrane bound diacylglycerol lipases of bovine brain. Pages 729–746, *in* Goheen, S. C. (ed), Proc. Membrane Protein Symposium, Bio-Rad Laboratories.
48. Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., and Nishizuka, Y. 1982. Direct activation of Ca^{2+}-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J. Biol. Chem. 257:7847–7857.
49. Vandenbark, G. R., Kuhn, L. J., and Niedel, J. E. 1984. Possible mechanism of phorbol diester induced maturation of human promyelocytic leukemia cells. J. Clin. Invest. 73:448–457.
50. Brasseur, R., Cabiaux, V., Huart, P., Castagna, M., Bazter, S., and Ruysschaert, J. M. 1985. Structural analogies between protein kinase C activators. Biochem. Biophys. Res. Commun. 127:969–976.
51. Weinstein, I. B., Gattoni-Celli, B., Kirschmeier, P., Lambert, M., Hsiao, W., Backer, J., and Jeffrey, A. 1984. Stereochemical model of phorbol esters. Pages 229–237, *in* Levine, A., van Woode, G., Toppe, W., and Waston, J. (eds.), Cancer cells, Cold Spring Harbor Laboratory Symposium 1.
52. Sharkey, N. A., Leach, K. L., and Blumberg, P. M. 1984. Competitive inhibition by diacylglycerol of specific phorbol ester binding. Proc. Natl. Acad. Sci. USA 81:607–610.
53. McPhail, L. C., Clayton, C. C., and Snyderman, R. 1984. A potential second messenger role for unsaturated fatty acids: activation of Ca^{2+}-dependent protein kinase. Science 224:622–625.
54. Murakami, K., and Routtenberg, A. 1985. Direct activation of purified protein kinase C by unsaturated fatty acids (oleate and arachidonate) in the absence of phospholipids and Ca^{2+}. FEBS Lett. 192:189–193.
55. Murakami, K., Chan, S. Y., and Routtenberg, A. 1986. Protein kinase C activation by cis fatty acid in the absence of Ca^{2+} and phospholipids. J. Biol. Chem. 261: 15424–15429.
56. Sekiguchi, K., Tsukuda, M., Ogita, K., Kikkawa, U., and Nishizuka, Y. 1987. Three forms of rat brain protein kinase C: Different response to unsaturated fatty acids. Biochem. Biophys. Res. Commun. 145:797–802.
57. Leon, A., Facci, L., Toffano, G., Sonnino, S., and Tettamanti, G. 1981. Activation of (Na^+, K^+)-ATPase by nanomolar concentrations of GM1 ganglioside. J. Neurochem. 37:350–357.
58. Davis, C. W., and Daly, J. W. 1980. Activation of rat cerebral cortical 3′,5′-cyclic nucleotide phosphodiesterase activity by gangliosides. Molec. Pharm. 17:206–211.
59. Partington, C. R., and Daly, J. W. 1981. Effect of gangliosides on adenylate cyclase activity in rat cerebral cortical membranes. Molec. Pharm. 15:484–491.
60. Goldenring, J. R., Otis, L. C., Yu, R. K., and DeLorenzo, R. J. 1985. Calcium/ganglioside-dependent protein kinase activity in rat brain membrane. J. Neurochem. 44:1229–1234.
61. Turner, R. S., Chou, C-H, J., Kibler, R. F., and Kuo, J. F. 1982. Basic protein in brain myelin is phosphorylated by endogenous phospholipid-sensitive Ca^{2+} dependent protein kinase. J. Neurochem. 39:1397–1404.
62. Kim, J. Y. H., Goldenring, J. R., DeLorenzo, R. J., and Yu, R. K. 1986. Gangliosides inhibit phospholipid-sensitive Ca^{2+} dependent kinase phosphorylation of rat myelin basic proteins. J. Neurosci. Res. 15:159–166.

63. Kreutter, D., Kim, J. Y. H., Goldenring, J. R., Rasmussen, H., Ukomadu, C., DeLorenzo, R. J., and Yu, R. K. 1986. Regulation of protein kinase C activity by gangliosides. J. Biol. Chem. 262:1633–1637.
64. Chan, K. F. J. 1987. Ganglioside-modulated protein phosphorylation in myelin. J. Biol. Chem. 262:2415–2422.
65. Chan, K. F. J. 1987. Ganglioside-modulated protein phosphorylation. Partial purification and characterization of a ganglioside-stimulated protein. J. Biol. Chem. 262:5248–5255.
66. Tsuji, S., Nakajima, J., Sasaki, T., and Nagai, Y. 1985. Bioactive gangliosides. IV. Ganglioside GQ1b/Ca^{2+} dependent protein kinase activity exists in the plasma membrane fraction of neuroblastoma cell line, GOTO. J. Biochem. 97:969–972.
67. Momoi, T. 1986. Activation of protein kinase C by ganglioside GM3 in the presence of calcium and 12-0-tetradecanoylphorbol 13-acetate. Biochem. Biophys. Res. Commun. 138:865–871.
68. Bremer, E. G., Schlessinger, J., and Hakomori, S-I. 1986. Ganglioside-mediated modulation of cell growth. J. Biol. Chem. 261:2434–2440.
69. Merrill Jr., A. H., Sereni, A. M., Stevens, V. L., Hannun, Y. A., Bell, R. M., and Kinkade Jr., J. M. 1986. Inhibition of phorbol ester-dependent differentiation of human promyelocytic leukemic (HL-60) cell by sphingosine and other long-chain bases. J. Biol. Chem. 261:12610–12615.
70. Fujiki, H., Yamashita, K., Suganuma, M., Horiuchi, T., Taniguchi, N., and Makita, A. 1986. Involvement of sulfatide in activation of protein kinase C by Tumor promoters. Biochem. Biophys. Res. Commun. 138:153–158.
71. Irvine, R. F. 1982. How is the level of free arachidonic acid controlled in mammalian cells? Biochem. J. 204:3–16.
72. Hansson, A., Serhan, C. N., Haeggstrom, J., Ingelman-Sundberg, M., Samuelsson, B., and Morris, J. 1986. Activation of protein kinase C by lipoxin A and other eicosanoids: Intracellular action of oxygenated products of arachidonic acid. Biochem. Biophys. Res. Commun. 134:1215–1222.
73. Wolf, M., Sahyoun, N., LeVine III, H., and Cuatrecasas, P. 1984. Protein kinase C: Rapid enzyme purification and substrate-dependence of the diacylglycerol effect. Biochem. Biophys. Res. Commun. 122:1268–1275.
74. Huang, K. P., Chan, K. F. J., Singh, T. J., Nakabayashi, H., and Huang, F. L. 1986b. Autophosphorylation of rat brain Ca^{+}-activated and phospholipid dependent protein kinase. J. Biol. Chem. 261:12134–12140.
75. Wightman, P. D., and Raetz, C. R. H. 1984. The activation of protein by kinase C biologically active lipid moieties of lipopolysaccharide. J. Biol. Chem. 259:10048–10052.
76. Roghani, M., Da Silva, C., and Castagna, M. 1987a. Tumor promotor chloroform is a potent protein kinase C activator. Biochem. Biophys. Res. Commun. 142:738–744.
77. Roghani, M., Da Silva, C., Guvelli, D., and Castagna, M. 1987b. Benzene and toluene activate protein kinase C. Carcinogen 8:1105–1107.
78. Kaibuchi, K., Takai, Y., and Nishizuka, Y. 1981. Cooperative roles of various membrane phospholipids in the activation of calcium activated phospholipid dependent protein kinase. J. Biol. Chem. 256:7146–7149.
79. Gerrard, J. M., Israels, S. J., and Friesen, L. L. 1985. Protein phosphorylation and platelet secretion. Nouv. Rev. Fr. Hematol. 27:267–273.
80. Levitan, I. B. 1985. Phosphorylation of ion channels. J. Memb. Biol. 87:177–190.
81. O'Flaherty, J. T., Redman, J. F., and Jacobson, D. P. 1986. Protein kinase C regulates leukotriene B_4 receptors in human neutrophils. FEBS Lett. 206:279–282.
82. Ginsberg, J., and Murray, P. G. 1986. Protein kinase C activators modulate differentiated thyroid function in vitro. FEBS Lett. 206:309–312.
83. Grinstein, S., Cohen, S., Goetz, J. D., Rothstein, A., Mellors, A., and Gelfand, E. W. 1986. Activation of the Na^{+}-H^{+} antiport by changes in cell volume and by phorbol esters; possible role of protein kinase. Current Topics Memb. Trans. 26:115–134.
84. Kitagawa, K., Nishino, H., and Iwashima, A. 1986. Possible involvement of protein kinase C in stimulation of amino acid transport by phorbol ester platelet-derived growth factor and A23187 in Swiss 3T3 cells. Experientia 42:1038–1040.
85. Caulfield, J. J., and Bolander Jr., F. F. 1986. Involvement of protein kinase C in mouse mammary gland development. J. Endocr. 109:29–34.
86. Holladay, C. S., and Bolander Jr., F. F. 1986. Hormonal regulation of protein kinase C in the mouse mammary gland. Proc. Soc. Exp. Biol. Med. 183:343–347.
87. Sagi-Eisenberg, R. 1985. Possible role for a calcium-activated, phospholipid dependent protein kinase in mode of action of DSCG. Trends Pharm. Sci. 6:198–200.
88. Miller, R. J. 1986. Protein kinase C: a key regulator of neuronal excitability. Trends Neurosci. 9:538–541.
89. Yap, W. H., Teo, T. S., McCoy, E., and Tan, Y. H. 1986. Rapid and transient raise in diacylglycerol concentration in Daudi cells exposed to interferon. Proc. Natl. Acad. Sci. USA 83:7765–7769.
90. Hamilton, T. A., Becton, D. L., Somers, S. D., Gray, P. W., and Adams, D. O. 1985. Interferon-γ modulates protein kinase C activity in murine peritoneal macrophages. J. Biol. Chem. 260:1378–1381.
91. Flower, R. J. 1985. Background and discovery of lipocortins. Agents and Actions 17:255–262.
92. Khanna, N. C., Tokuda, M., and Waisman, D. M. 1986. Phosphorylation of lipocortins in vitro by protein kinase C. Biochem. Biophys. Res. Commun. 141:547–554.
93. Hirata, F. 1981. The regulation of lipomodulin, a phospholipid inhibitory protein, in rabbit neutrophils by phosphorylation. Biol. Chem. 256:7730–7733.
94. Hirata, F. 1985. Receptor mediated cascade of phospholipid metabolism. Pages 99–105., *in* Horrocks, L. A., Kanfer, J. N., and Porcellati, G. (eds.), Phospholipids in the Nervous System Vol. 2, Raven Press, New York.
95. Hirata, F., Matsuda, K., Notsu, Y., Mattori, T., del Carmine, R. 1984. Phosphorylation at a tyrosine residue of lipomodulin in mitogen stimulated murine thymocytes. Proc. Natl. Acad. Sci. 81:4717–4721.
96. Walsh, M. P., Valentine, K. A., Ngai, P. K., Carruthers, C. A., and Hollenberg, M. D. 1984. Ca^{2+}-dependent hydrophobic-interaction chromatography—Isolation of a novel binding protein and protein kinase C from bovine brain. Biochem. J. 224:117–127.
97. Kuo, J. F., Schatzman, R. C., Turner, R. S., and Mazzei, G. J. 1984. Phospholipid-sensitive, Ca^{2+}-dependent protein kinase: A major protein phosphorylation system. Mol. Cellu. Endocrin. 35:65–73.
98. Kishimoto, A., Takai, Y., and Nishizuka, Y. 1977. Activation of glycogen phosphorylase kinase by a Ca^{2+} activated, cyclic nucleotide-independent protein kinase system. J. Biol. Chem. 252:7449–7452.
99. Hofer, H. W., Schlatler, S., and Graefe, M. 1985. Phosphorylation of phosphofructokinase by protein kinase C changes the allosteric properties of the enzyme. Biochem. Biophys. Res. Commun. 129:892–897.
100. Rider, M., and Hue, L. 1986. Phosphorylation of purified bovine heart and rat liver 6-phosphofructo-2-kinase by protein kinase C and comparison of the fructose-2,6-bisphos-

phatase activity of the two enzymes. Biochem. J. 240:57–61.
101. Nettelblad, F. A., Forsberg, P. O., Humble, E., and Engstrom, L. 1986. Aspects on the phosphorylation of muscle phosphofructokinase by protein kinase C—Inhibition by phosphofructokinase stabilizers. Biochem. Biophys. Res. Commun. 136:445–453.
102. Naghshineh, S., Noguchi, M., Huang, K. P., and Londos, C. 1986. Activation of adipocyte adenylate cyclase by protein kinase C. J. Biol. Chem. 261:14534–14538.
103. Nishikawa, M., Hidaka, H., and Adelstein, R. S. 1983. Phosphorylation of smooth muscle heavy meromyosin by calcium activated, phospholipid dependent protein kinase. J. Biol. Chem. 258:14069–14072.
104. Nishikawa, M., Seller, J. R., Adelstein, H., and Hidaka, H. 1984. Protein kinase C modulates in vitro phosphorylation of the smooth muscle heavy meromyosin by myosin light chain kinase. J. Biol. Chem. 259:8808–8812.
105. Beg, Z. H., Stonik, J. A., and Brewer Jr., H. B. 1985. Phosphorylation of hepatic 3-hydroxy-3-methylglutaryl coenzyme a reductase and modulation of its enzymic activity by calcium activated and phospholipid dependent protein kinase. J. Biol. Chem. 260:1682–1687.
106. Forsberg, P. O., Humble, E., and Engstrom, L. 1986. Aspects in phosphorylation of muscle phosphofructokinase by protein kinase C. Inhibition by fructokinase stabilizers. Biochem. Biophys. Res. Commun. 136:445–453.
107. Vuilliet, J., Woodgett, R., Ferrari, S., and Hardie, D. G. 1985. Characterization of the sites phosphorylated on tyrosine hydroxylase by Ca^{2+} and phospholipid-dependent protein kinase, calmodulin-dependent multiprotein kinase and cyclic AMP-dependent protein kinase. FEBS Lett. 182:335–339.
108. Papini, E., Grzeskowiak, M., Bellavite, and Rossi, F. 1985. Protein kinase C phosphorylates a component of NADPH oxidase of neutrophils. FEBS Lett. 190:204–208.
109. DeWald, B., Payne, T. G., and Baggiolini, M. 1984. Activation of NADPH oxidase of human neutrophils. Potentiation chemotactic peptide by diacylglycerol. Biochem. Biophys. Res. Commun. 125:367–373.
110. Vilgrain, I., Defaye, G., and Chambaz, E. M. 1984. Adrenocortical cytochrome P-450 responsible for cholesterol side chains cleavage is phosphorylated by the calcium activated phospholipid-sensitive protein kinase (protein kinase C). Biochem. Biophys. Res. Commun. 125:554–561.
111. Zwiller, J., Revel, M., and Malviya, A. N. 1985. Protein kinase C catalyzes phosphorylation of guanylate cyclase in vitro. J. Biol. Chem. 260:1350–1353.
112. DePaoli-Roach, A., Roach, P. J., Zucker, K. E., and Smith, S. S. 1986. Selective phosphorylation of human DNA methyltransferase by protein kinase C. FEBS Lett. 197:149–153.
113. Connolly, T. M., Lawing, W. J., and Majerus, P. W. 1986. Protein kinase C phosphorylates human platelet inositol trisphosphate 5′-phosphomonoesterase, increasing the phosphatase activity. Cell 46:951–958.
114. Hincke, M. T., and Tolnai, S. 1986. Phosphorylation of bovine cardiac calcium-activated neutral protease by protein kinase C. Biochem. Biophys. Res. Commun. 137:559–565.
115. Tung, H. Y. L. 1986. Phosphorylation of the calmodulin-dependent protein phosphatase by protein kinase C. Biochem. Biophys. Res. Commun. 138:783–788.
116. Limas, C. J. 1980. Phosphorylation of cardiac sarcoplasmic reticulum by a calcium activated phospholipid dependent protein kinase. Biochem. Biophys. Res. Commun. 96:1378–1383.
117. Greene, D. A., and Lattimer, S. A. 1986. Protein kinase C agonists acutely normalize decreased ovabain-inhibitable respiration in diabetic rabbit nerve. Implications for (Na^+,K^+)-ATPase regulation and diabetic complications. Diabetes 35:242–245.
118. Villalba, M., Pagares, M. A., Renart, M. F., and Mato, J. M. 1987. Protein kinase C catalyses the phosphorylation and activation of rat liver phospholipid methyltransferase. Biochem. J. 241:911–916.
119. Ryu, S. H., Lee, K. Y., Suh, P. G., Choi, W. C., Rhee, S. G., and Huang, K. P. 1987. Phosphorylation of phospholipase C enzymes by protein kinase C. Fed. Proc. 46:2286 (Abstr.)
120. Bennett, C. F., Angioli, M. P., and Crooke, S. T. 1987. Phosphorylation of PI-specific phospholipase C by protein kinase C in vivo. Fed. Proc. 46:2287 (Abstr.).
121. Leach, K. L., and Blumberg, P. M. 1985. Modulation of protein kinase C activity and [^{3}H]phorbol 12,13-dibutyrate binding by various tumor promotors in mouse brain cytosol. Cancer Res. 45:1958–1963.
122. Ramsdell, J. S., Pettit, G. R., and Tashjian, A. H. 1986. Three activators of protein kinase C, bryostatins, dioleins and phorbol esters, show differing specificities of action on GH_4 pituitary. J. Biol. Chem. 261:17073–17080.
123. Ahmad, Z., Lee, F. T., DePaoli-Roach, A., and Roach, P. J. 1984. Phosphorylation of glycogen synthetase by the Ca^{2+}- and phospholipid activated protein kinase (protein kinase C). J. Biol. Chem. 259:8743–8747.
124. Dawson, R. M. C., Hemington, N., and Irvine, R. F. 1983. Diacylglycerol potentiates phospholipase attack upon phospholipid bilayers: possible connection with cell stimulation. Biochem. Biophys. Res. Commun. 117:196–201.
125. Dawson, R. M. C., Irvine, R. F., Bray, J., and Quinn, B. J. 1984. Long-chain unsaturated diacylglycerols cause a perturbation in the structure of phospholipid bilayers rendering them susceptible to phospholipase attack. Biochem. Biophys. Res. Commun. 125:836–842.
126. Roach, P. J., and Goldman, M. 1983. Modification of glycogen synthase activity in isolated rat hepatocytes by tumor-promoting phorbol ester: Evidence for differential regulation of glycogen synthease and phosphorylase. Proc. Natl. Acad. Sci. (USA) 80:7170–7172.
127. O'Flaherty, J. T., Schmitt, J. D., McCall, C. E., and Wykle, R. L. 1984. Diacylglycerols enhance human neutrophil degranulation responses: Relevancy to a multiple mediator hypothesis of cell function. Biochem. Biophys. Res. Commun. 123:64–70.
128. Exton, J. H. 1986. Mechanisms involved in calcium-mobilizing agonists responses. Adv. Cyclic Nucleotide Protein Phosphorylation Res. 20:211–262.
129. Walaas, S. I., Horn, R. S., Adler, A., Albert, K. A., and Walaas, O. 1987. Insulin increases membrane protein kinase C activity in rat diaphragm. FEBS Lett. 220:311–318.
130. Klucis, K., and Polya, G. M. 1987. Calcium-independent activation of two plant leaf calcium-regulated protein kinases by unsaturated fatty acids. Biochem. Biophys. Res. Commun. 147:1041–1047.
131. Choy, P. C., Farren, S. B., and Vance, D. E. 1979. Lipid requirements for the aggregation of CTP-phosphocholine cytidylyltransferase in rat liver cytosol. Can. J. Biochem. 57:605–612.