Journal of Cellular Biochemistry 29:143–155 (1985)
Membrane Skeletons and Cytoskeletal–Membrane Associations 47–59

Role of Protein Kinase C in Transmembrane Signaling

Yoshimi Takai, Kozo Kaibuchi, Terutaka Tsuda, and Masahiko Hoshijima

Department of Biochemistry, Kobe University School of Medicine, Kobe 650, Japan

Many extracellular signals elicit Ca^{2+} mobilization and diacylglycerol formation in their target cells. Diacylglycerol is derived from the receptor-linked phosphoinositide turnover and serves as a second messenger for the activation of protein kinase C in the presence of Ca^{2+} and phosphatidylserine. Unique diacylglycerols such as 1-oleoyl-2-acetyl-glycerol, which activate intracellular protein kinase C when added to intact cells, have been synthesized. Tumor-promoting phorbol esters substitute for such diacylglycerols and directly activate protein kinase C in both intact cell and cell-free systems. Under appropriate conditions, the synthetic diacylglycerols and phorbol esters induce protein kinase C activation without Ca^{2+} mobilization, whereas Ca^{2+} ionophore A23187 induces Ca^{2+} mobilization without protein kinase C activation. Using these substances, we have obtained evidence that both protein kinase C and Ca^{2+} are involved in and play a synergistic role in exocytosis, cell division, and other cellular functions. In this article, the role of protein kinase C in transmembrane signaling is discussed.

Key words: membrane receptor, protein kinase, phosphoinositide, Ca^{2+}, tumor promoters, exocytosis, cell division

It has been known that many extracellular signals such as hormones, neurotransmitters, growth factors, and other biologically active substances induce both Ca^{2+} mobilization and phosphoinositide turnover in their target cells [1]. Ca^{2+} has been shown to serve as a second messenger for these extracellular signals and its mode of action has been extensively investigated [2]. However, physiological significance of phosphoinositide turnover had been unclear for a long time since the first discovery by Hokin and Hokin in 1953 [3]. In 1979, we found that the receptor-linked phosphoinositide turnover is directly linked to the activation of protein kinase C [4–7]. This protein kinase is distributed in most mammalian tissues [8,9]. The enzyme is usually inactive but is activated by diacylglycerol in the presence of Ca^{2+} and phosphatidylserine. The diacylglycerol is derived from the phosphoinositide turnover

Abbreviations used: TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PHA, phytohemagglutinin; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; PAF, platelet-activating factor.

Received March 19, 1985, revised and accepted June 17, 1985.

in a receptor-linked manner. Evidence has been obtained that protein kinase C and Ca^{2+} are essential and synergistically effective for eliciting the activation of cellular functions [10,11]. Moreover, we found incidentally that the tumor-promoting phorbol esters substitute for diacylglycerol and activate protein kinase C in a manner similar to that of diacylglycerol [12]. It is now generally accepted that protein kinase C as well as Ca^{2+} plays a role of crucial importance in the actions of many extracellular signals [13].

Ca^{2+} is well known as a factor that regulates the dynamic rearrangement of the cytoskeletal systems [14]. For instance, Ca^{2+} regulates the length and cross-linkage of actin filaments through Ca^{2+}-sensitive proteins such as gelsolin, fragmin, villin, brevin, actinogelin, and caldesmon [14]. Ca^{2+} affects the interaction of microtuble-associated proteins with microtubles through calmodulin [15]. Ca^{2+} stimulates the phosphorylation of myosin light chain through the activation of Ca^{2+}-activated, calmodulin-dependent myosin light chain kinase [16], and the phosphorylation of this protein is considered to be essential for the interaction between myosin and actin filaments [17]. On the other hand, protein kinase C has been shown to phosphorylate several cytosketal proteins such as myosin light chain [18], troponin [19], vinculin [20,21], α-actinin [21], filamin [21], spectrin [13], and microtuble-associated proteins [13]. Although the function of phosphorylation of these proteins by protein kinase C has not yet been clear, it is likely that protein kinase C as well as Ca^{2+} may be directly or indirectly related to the regulation of cytoskeletal systems. Therefore, it may be important to understand the role of protein kinase C and Ca^{2+} in the actions of various extracellular signals for the investigation of the cytoskeletal system. In this article, the mode of activation and properties of protein kinase C will be briefly reviewed, and then the role of protein kinase C in transmembrane signaling will be discussed.

MATERIALS AND METHODS

Materials and Chemicals

Protein kinase C is purified to near homogeneity from the soluble fraction of rat brain as described [22]. Human washed platelets and peripheral lymphocytes are prepared as described earlier [23,24]. H1 histone and [γ-^{32}P]ATP used for the substrates of protein kinase C are prepared as described earlier [4]. 1-Oleoyl-2-acetyl-glycerol and 1-acetyl-2-oleoyl-glycerol are synthesized as described [25]. Diolein, TPA, Ca^{2+} ionophore A23187, and PHA are obtained from Nakarai Chemicals, P-L Biochemicals, Calbiochem, and Difco Laboratories, respectively. $H_3{}^{32}PO_4$, [^{3}H]phorbol-12,13-dibutyrate, and [^{3}H]methyl-thymidine are obtained from Japan Radioisotope Association, Amersham, and New England Nuclear, respectively. Other chemicals and materials are obtained as described [4–8,10–12,22–27].

Assays

Protein kinase C is assayed by measuring the incorporation of γ-^{32}P of ATP into H1 histone as a model substrate in the presence of diolein, Ca^{2+} and phospholipid under the conditions described previously [22]. Protein phosphorylation in intact cells are carried out as follows: Intact cells are prelabeled with ^{32}Pi and then stimulated by each agonist. The radioactive proteins are directly subjected to sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis, stained, dried on a filter paper, and

exposed to an x-ray film to prepare an autoradiograph as described earlier [24,26,27]. The relative intensity of each band is quantitated by densitometric tracing. Phosphoinositide turnover is assayed by measuring the formation of radioactive diacylglycerol or the incorporation of ^{32}P into phosphatidic acid or phosphatidylinositol. Diacylglycerol and each phospholipid are separated by thin-layer chromatography on silica gel plate as described [27]. Cytoplasmic free Ca^{2+} concentration is measured by means of the fluorescent Ca^{2+} indicator quin 2 [28]. Serotonin release from platelets is assayed by measuring the radioactivity of [^{14}C] serotonin released into the medium after the stimulation by each agonist as described previously [26,27]. DNA synthesis of lymphocytes is assayed by measuring the incorporation of [^{3}H]thymidine into DNA as described [24].

RESULTS AND DISCUSSION

Mode of Activation and Properties of Protein Kinase C

Activation of protein kinase C by diacylglycerol. The principal pathway of signal transduction is schematically shown in Figure 1. In resting cells, diacylglycerol is almost absent. When cells are stimulated by an agonist, diacylglycerol is rapidly produced. For instance, in platelets, it has been proposed that this diacylglycerol is initially derived from the hydrolysis of phosphatidylinositol-4,5-bisphosphate, and subsequently from the hydrolysis of phosphatidylinositol-4-phosphate and phosphatidylinositol by the action of phospholipase C [29]. Inositol-1,4,5-trisphosphate, another product of the hydrolysis of phosphatidylinositol-4,5-bisphosphate, has been shown to serve as a trigger for the intracellular translocation of Ca^{2+} [30]. Ca^{2+} increased

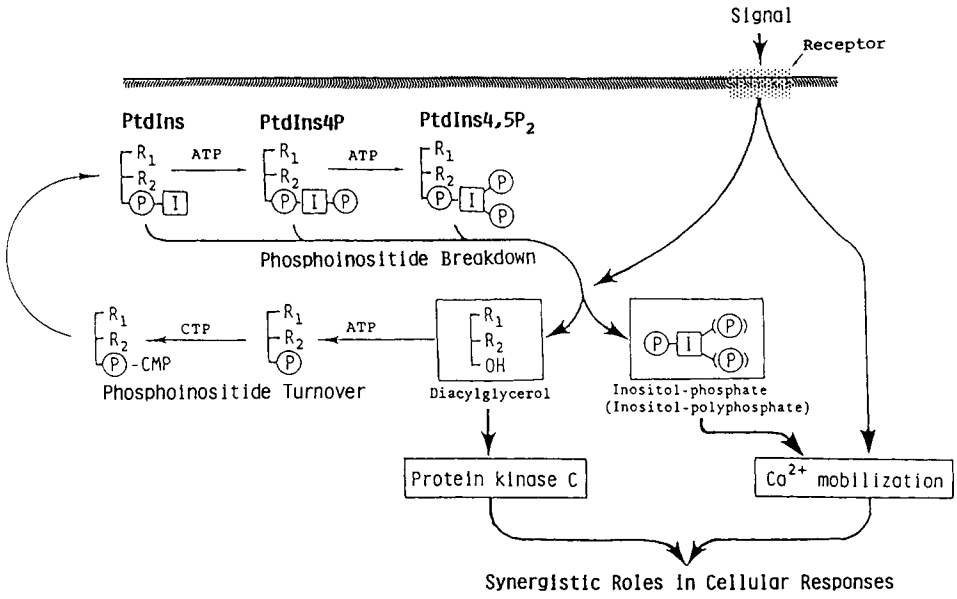

Fig. 1. Phosphoinositide turnover and signal transduction. PtdIns, phosphatidylinositol; PtdIns4P, phosphatidylinositol-4-phosphate; PtdIns4,5P_2, phosphatidylinositol-4,5-bisphosphate; R_1 and R_2, fatty acyl group; I, inositol; P, phosphoryl group.

in this way then facilitates further the hydrolysis of other phosphoinositides and production of diacylglycerol. The diacylglycerol produced activates protein kinase C in the presence of Ca^{2+} and phosphatidylserine. Kinetically, diacylglycerol dramatically increases the affinity of protein kinase C for Ca^{2+} and phosphatidylserine and renders the enzyme fully active without a net increase of the concentration of this divalent cation [5–7]. Extracellular signals that induce phosphoinositide turnover are listed in Table I, and it is conceivable that protein kinase C is activated by the action of these substances.

To obtain evidence that the diacylglycerol produced in a receptor-linked manner initiates the activation of protein kinase C, unique diacylglycerols have been synthesized. These are 1-oleoyl-2-acetyl-glycerol and 1-acetyl-2-oleoyl-glycerol. The diacylglycerols derived from the hydrolysis of phosphoinositides in intact cells are mostly 1-palmitoyl- or stearoyl-2-arachidonyl-glycerol. These diacylglycerols with two long acyl moieties are too hydrophobic to intercalate into membranes when added exogenously to intact cells. The synthetic diacylglycerols mentioned above, in contrast, easily intercalate into membranes and are converted to phosphatidic acid *via* the pathway of phosphoinositide turnover [10]. Evidence has been obtained that these synthetic diacylglycerols induce the activation of protein kinase C in many cell types including platelets, neurotrophils, mast cells, lymphocytes, and fibroblasts [10,24,31–33]. In platelets, a 40-kilodalton protein has been identified as an endogenous substrate for protein kinase C [26,27]. When platelets are stimulated by thrombin, a natural agonist for platelet aggregation and release reaction, the 40-kilodalton protein is rapidly and heavily phosphorylated as shown in Figure 2. Similarly, when the cells are stimulated by either 1-oleoyl-2-acetyl-glycerol or 1-acetyl-2-oleoyl-glycerol, this protein is heavily phosphorylated. However, the synthetic diacylglycerol does not induce endogenous diacylglycerol formation and Ca^{2+} mobilization, although natural stimuli induce both reactions. Essentially similar observations have been made in other cell types described above. These results strongly suggest that diacylglycerol derived from the receptor-linked turnover of phosphoinositide may serve as a messenger for the activation of protein kinase C.

TABLE I. Extracellular Signals Inducing Phosphoinositide Turnover [1,30]

Extracellular signals	Target tissues
Acetylcholine (m)	Various
Adrenaline (α1)	Various
Histamine (H1)	Intestinal smooth muscle, brain
Pancreozymin, caerulein	Pancreas, intestinal smooth muscle
Bombesin	Pancreas
Substance P	Parotid gland
Angiotensin, vasopressin	Liver, vascular smooth muscle
Bradykinin	Vascular endothelium
Glucose	Langerhans' islet
Antigen, concanavalin A	Mast cells
fMet-Leu-Phe, zymosan	Polymorphonuclear leukocytes
TSH	Thyroid gland
ADP, thrombin, collagen, PAF	Platelets
PHA, con A	Lymphocytes
Platelet-derived growth factor	Fibroblasts
Epidermal growth factor	A431 epidermal carcinoma cells

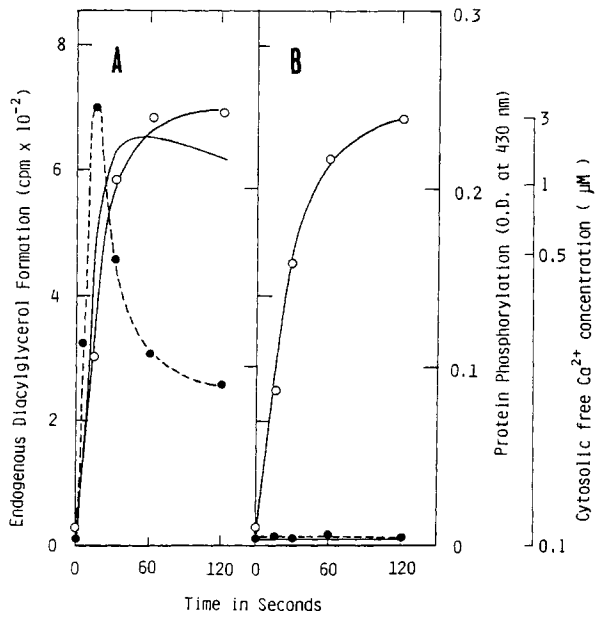

Fig. 2. Time courses of diacylglycerol formation, 40-kilodalton protein phosphorylation and Ca^{2+} mobilization. Radioactive platelets were stimulated by thrombin (0.8 unit/ml) or 1-oleoyl-2-acetyl-glycerol (50 μg/ml) at 37°C for various periods of time as indicated. Other details are described under Materials and Methods. A) stimulated by thrombin; B) stimulated by 1-oleoyl-2-acetyl-glycerol. (●----●), diacylglycerol formation; (○——○), 40-kilodalton protein phosphorylation; (———), Ca^{2+} mobilization.

Properties of protein kinase C. Protein kinase C has been found in all tissues so far tested [8,9]. The molecular weight is about 77,000 as estimated by gel filtration. The isoelectric point is about pH 5.6. The enzyme uses ATP as a phosphate donor, but other nucleoside triphosphates are inactive. The enzyme phosphorylates many proteins as far as tested in a cell-free system, and the proteins that are proposed as substrates for this enzyme are listed in Table II. Protein kinase C phosphorylates either seryl- or threonyl-residues of these proteins but does not phosphorylate tyrosyl-residue. Protein kinase C is a single polypeptide with no subunit structure, but is composed of at least two functionally different domains. One is a hydrophobic domain that binds to the membrane, and the other is a hydrophilic domain that carries a catalytically active center. These two domains can be separated by limited proteolysis with Ca^{2+}-dependent protease, which is designated as calpain, only in a cell-free system. This proteolysis does not occur in intact cells upon stimulation by natural agonists.

Protein Kinase C and Tumor-Promoting Phorbol Esters

Activation of protein kinase C by phorbol esters. TPA, one of the most potent tumor-promoting phorbol esters, directly activates protein kinase C in the presence of Ca^{2+} and phosphatidylserine in a cell-free purified system [12]. Kinetically, TPA substitutes for diacylglycerol and greatly increases the affinity of this enzyme for Ca^{2+} and the phospholipid. TPA is more potent than diacylglycerol for

TABLE II. Proteins Proposed as Substrates for Protein Kinase C

Proposed substrates	Reference no.
Nuclear proteins	
Histone in calf thymus	[4]
Protamine in salmon sperm	[4]
High-mobility-group proteins in lamb thymus	[34]
Cytoskeletal proteins	
Myosin light chain in chicken gizzard	[18]
Troponin in bovine cardiac muscle	[19]
Vinculin in chicken gizzard	[20,21]
Filamin in chicken gizzard	[21]
Spectrin in human erythrocytes	[13]
Microtubule-associated proteins in rat brain	[13]
Receptor proteins	
Insulin and somatomedin C receptor in human B-lymphocytes	[35]
EGF receptor in A431 human epidermal carcinoma cells	[36,37]
Transferrin receptor in HL 60 cells	[38]
Interleukin 2 receptor in human T-cell leukemia	[39]
Retinoid-binding protein in calf liver and uterus	[40]
Enzyme and enzyme-related proteins	
Glycogen phosphorylase kinase in rabbit skeletal muscle	[4]
Glycogen synthetase in rabbit skeletal muscle	[4]
Phospholamban in chicken cardiac sarcolemma	[41,42]
Ribosomal S6 protein	[43]
Initiation factor 2	[44]
Miscellaneous proteins	
Myelin basic protein in rat and rabbit brain	[45]
Human fibrinogen	[46]
Middle T antigen in polyoma-virus-infected 3T6 cells	[47]
Class I HLA antigens in human platelets, lymphocytes, and HL-60 leukemic cells	[48]
Bovine chromobindins	[49]
Synthetic peptide Arg-Arg-Ala-Ser-Gly-Pro-Pro-Val	[50]
40-K protein in human platelets	[26,27]
87-K protein in rat brain synaptosome	[51]
50-K protein (B-50 protein) in rat brain synaptic membrane	[52]
45-K and 56-K proteins in human peripheral lymphocytes	[24]
22-K, 31-K, 34-K, and 60-K proteins in rat mast cells	[32]
80-K protein in Swiss 3T3 mouse fibroblasts	[33]
35-K, 56-K, and 70-K proteins in rat hepatocytes	[53]
36-K protein in rat basophilic leukemia cells	[54]
38-K protein in human myelocytic leukemia cells	[55]
59-K, 65-K, 80-K, and 82-K proteins in rat GH_3 pituitary cells	[56]

the activation of protein kinase C, and this tumor promoter, at an amount of roughly 1/1,000 to 1/200 of that of diacylglycerol, is able to cause the full activation of protein kinase C as shown in Figure 3. Various phorbol derivatives showing tumor-promoting activity, eg, phorbol-12,13-didecanoate, phorbol-12,13-dibutyrate, and phorbol-12, 13-dibenzoate, are able to activate protein kinase C. On the other hand, phorbol-12-tetradecanoate, phorbol-13-acetate, 4α-phorbol-12,13-didecanoate, and phorbol itself, which are all inactive for tumor promotion, are ineffective for the activation of this enzyme. Teleocidin and mezerein, which are also known as other groups of tumor promoters, have been shown to activate protein kinase C [57,58].

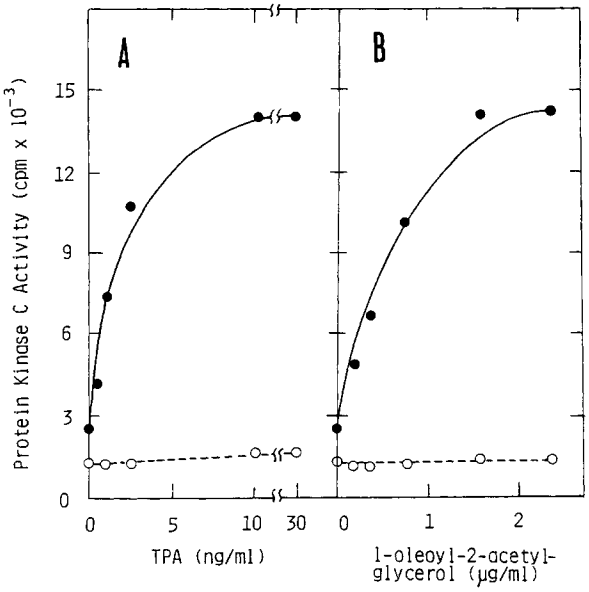

Fig. 3. Activation of protein kinase C by TPA and diacylglycerol in a cell-free system. Protein kinase C was assayed under the standard conditions in the presence of 20 μg/ml of phospholipid, 1×10^{-5} M $CaCl_2$ and various amounts of either TPA or 1-oleoyl-2-acetyl-glycerol as indicated. Other details were described elsewhere [12]. A) with TPA; B) with 1-oleoyl-2-acetyl-glycerol. (●——●), in the presence of phospholipid and $CaCl_2$; (○----○), in the presence of $CaCl_2$ and without phospholipid.

Tumor-promoting phorbol esters activate protein kinase C not only in a cell-free system but also in an intact cell level. This evidence has been obtained in several cell types including platelets, lymphocytes, mast cells, leukocytes, and fibroblasts [11,12,24,31–33]. For example, in platelets, TPA induces the phosphorylation of the 40-kilodalton protein, which is an endogenous substrate for protein kinase C as described above. Moreover, when resting platelets are disrupted by sonication in the presence of EGTA to remove free Ca^{2+}, protein kinase C is recovered mostly in the soluble fraction. However, in platelets treated with TPA, protein kinase C is recovered mostly in the particulate fraction. Presumably, TPA increases dramatically the affinity of the enzyme for Ca^{2+} and thereby renders the enzyme more tightly bound to the membrane even in the presence of EGTA. These observations strongly suggest that TPA activates protein kinase C in intact cells. It can be emphasized that TPA does not induce endogenous diacylglycerol formation or Ca^{2+} mobilization, at least in an early period when protein kinase C is activated.

Protein kinase C as a phorbol ester receptor. Experiments using [^{3}H]phorbol-12,13-dibutyrate indicate that this radioactive tumor promoter binds to the enzyme in the presence of Ca^{2+} and phospholipid, resulting in the activation of this enzyme [59,60]. This phorbol ester does not bind to protein kinase C or phospholipid alone irrespective of the presence or absence of Ca^{2+}. Since protein kinase C binds to phosphatidylserine in the presence of Ca^{2+} to make a ternary complex, the phorbol ester may bind to this ternary complex to make an active quaternary complex. Among various phorbol derivatives, only tumor-promoting

derivatives inhibit [^{3}H]phorbol-12,13-dibutyrate-binding to protein kinase C in a competitive manner. The active diacylglycerol also inhibits [^{3}H]phorbol-12,13-dibutyrate-binding to protein kinase C.

The receptor of tumor-promoting phorbol esters has been previously shown to be distributed widely in many tissues [60–63]. Protein kinase C is also distributed widely in various tissues as described above. The distribution pattern of both entities is apparently very similar, and both the phorbol ester receptor, as measured by [^{3}H]phorbol-12,13-dibutyrate-binding activity, and protein kinase C are copurified by several column chromatographies [60]. The evidence, together with that described above, suggests that protein kinase C complexed with Ca^{2+} and phospholipid may be a receptor of the phorbol esters and play pleiotropic actions, if not all, of the phorbol esters as well as other tumor promoters.

Role of Protein Kinase C in Transmembrane Signaling

Role in exocytosis. Many secretagouges induce both Ca^{2+} mobilization and phosphoinositide turnover [1]. Previously, only Ca^{2+} has been considered to be a messenger for eliciting release reactions; however, evidence is now available that the diacylglycerol derived from the receptor-linked turnover of phosphoinositide may also be involved in secretory processes. Among various cell types, the role of protein kinase C in secretion has been studied most extensively in platelets.

Platelets normally respond to many secretagouges—such as thrombin, collagen, and PAF—and secrete ADP, serotonin, lysosomal enzymes, growth factors, and many other constituents [64]. These secretagouges are known to induce both Ca^{2+} mobilization and phosphoinositide turnover [64]. Ca^{2+} mobilization is linked to the activation of calmodulin and stimulates the phosphorylation of myosin light chain through the activation of its specific protein kinase [65]. The phosphoinositide turnover is directly linked to the activation of protein kinase C, which phosphorylates the 40-kilodalton protein [26,27]. The phosphorylation of myosin light chain and 40-kilodalton protein is associated with the release reaction from platelets [10,11], although the precise functions of these proteins remain to be clarified. As described above, it is possible to activate protein kinase C without Ca^{2+} mobilization by a membrane-permeable synthetic diacylglycerol or phorbol ester. On the other hand, under appropriate conditions, Ca^{2+} ionophore A23187 induces Ca^{2+} mobilization without protein kinase C activation. When intact platelets are stimulated by either the synthetic diacylglycerol or the phorbol ester in the presence of A23187, the release reaction is induced as shown in Figure 4. The magnitude of serotonin release in response to these agents is nearly the same as that induced by thrombin. Low concentrations of the synthetic diacylglycerol or the phorbol ester alone are almost inactive; however, high concentrations of these agents by themselves induce the release reaction to some extent. The exact reason for this phenomenon is not known, but it is possible that the diacylglycerol and phorbol ester may act as a membrane fusigen in the secretory processes. Likewise, A23187 at the concentration employed (0.4 μM) does not induce protein kinase C activation or serotonin release. However, at the concentrations higher than 0.5 μM, A23187 alone causes both protein kinase C activation and Ca^{2+} mobilization, resulting in the release reaction. The activation of protein kinase C by the action of high concentrations of A23187 may be due to a large increase in the Ca^{2+} concentrations. These results indicate that either the activation of protein kinase C or Ca^{2+} mobilization alone is not sufficient and both are necessary for eliciting the

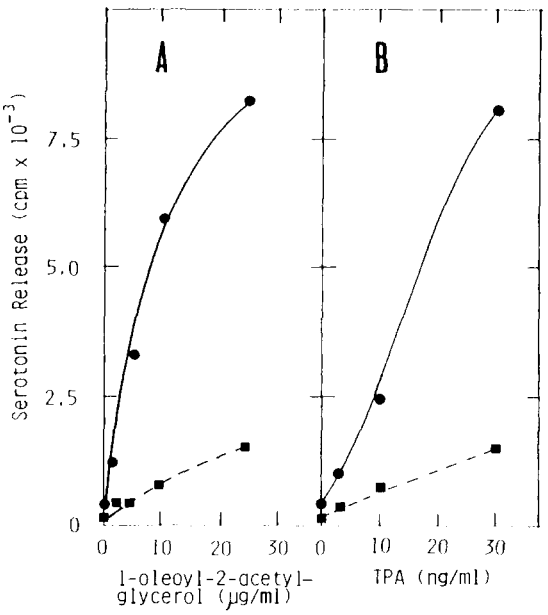

Fig. 4. Synergistic effects of Ca^{2+} ionophore and 1-oleoyl-2-acetyl-glycerol or TPA on serotonin release. Platelets were labeled with [^{14}C]serotonin, and then stimulated by various concentrations of 1-oleoyl-2-acetyl-glycerol or TPA in the presence or absence of A23187 (0.4 μM) as indicated. Other details are described under Materials and Methods. A) stimulated by 1-oleoyl-2-acetyl-glycerol; B) stimulated by TPA. (●——●), in the presence of A23187; (■----■), in the absence of A23187.

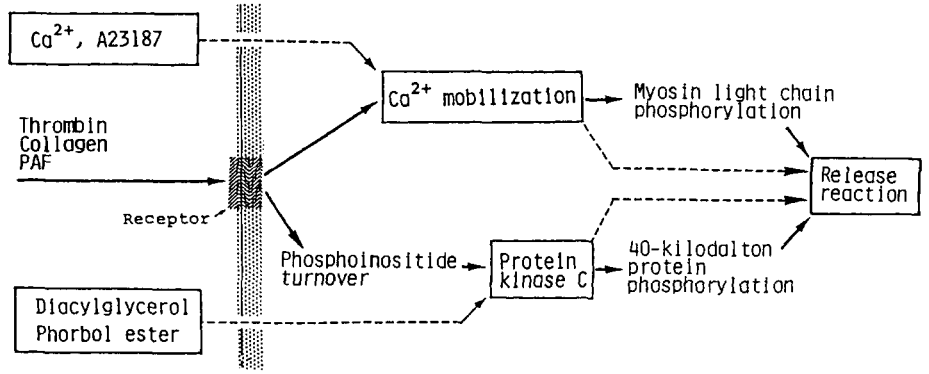

Fig. 5. Synergistic action of protein kinase C activation and Ca^{2+} mobilization in release reaction from platelets.

release reaction. The role of protein kinase C and Ca^{2+} in the release reaction from platelets is schematically shown in Figure 5. Essentially similar observations have been made in other types of cells, which are listed in Table III. Therefore, it is most likely that protein kinase C and Ca^{2+} may play a synergistic role in the release reactions elicited by many secretagouges.

TABLE III. Release Reactions Controlled by Protein Kinase C and Ca^{2+}

Types of cells	Released materials	Reference no.
Platelets	Serotonin and lysosomal enzyme	[10,11,31]
Neutrophils	Lysosomal enzyme	[31]
Mast cells	Histamine	[32]
Adrenal medullary cells	Catecholamine	[66]
Adrenal glomerulosa cells	Aldosterone	[67]
Pancreatic islets	Insulin	[68]
Pancreatic acini	Amylase	[69]
Ileal cholinergic nerve	Acetylcholine	[70]

Role in cell division. Some growth factors such as platelet-derived growth factor and epidermal growth factor have been shown to increase the intracellular Ca^{2+} concentration, which is essential for cell division [71,72]. These growth factors have been recently shown to stimulate phosphoinositide turnover, suggesting that protein kinase C as well as Ca^{2+} may also be involved in the regulation of cell division. To obtain the evidence that protein kinase C may play a role in the regulation of cell division, the following experiments have been conducted in our laboratory using human peripheral lymphocytes as a model system.

In macrophage-depleted human peripheral T-lymphocytes, DNA synthesis is markedly stimulated by TPA in the presence of a small concentration of Ca^{2+} ionophore A23187 and PHA as shown in Figure 6. TPA (0.1 nM) alone causes only a two- to threefold increase in DNA synthesis over that found in unstimulated lymphocytes. This tumor promoter at higher concentrations (1–10 nM) induces DNA synthesis at most twice as much as that caused at 0.1 nM, and the DNA synthesis induced by TPA alone is far less than that observed in the presence of three factors—TPA, PHA, and Ca^{2+} ionophore. Over this wide range of TPA concentrations (0.1–10 nM) Ca^{2+} ionophore (0.25–0.5 μM) shows practically no effect on DNA synthesis, unless a small quantity of PHA is added. In the presence of PHA, in contrast, TPA at higher concentrations (1–10 nM) significantly enhances DNA synthesis without addition of Ca^{2+} ionophore. The exact reason for this enhancement is not clear, but the tumor-promoting phorbol ester at higher concentrations shows various nonspecific effects on membranes presumably by acting as a perturber or a weak Ca^{2+} ionophore. Ca^{2+} ionophore A23187 alone shows practically no effect as mentioned above; but again, at higher concentrations (more than 0.5 μM) it slightly enhances DNA synthesis, probably owing to the activation of protein kinase C by a large increase in Ca^{2+} concentration, as described for its action on platelets. Essentially similar results are obtained with OAG instead of TPA as shown in the same figure. These results suggest that protein kinase C and Ca^{2+} are essential but not sufficient for the initiation of DNA synthesis in lymphocytes. The action of PHA is required in addition to protein kinase C activation and Ca^{2+} mobilization, but the mode of action of PHA is unclear. Further investigation is needed for understanding the definitive role of protein kinase C and Ca^{2+} in the regulation of lymphocyte proliferation.

Recently, other several lines of evidence have also been described that protein kinase C may be involved in the regulation of cell division. The receptors for insulin [35], epidermal growth factor [36,37], and transferrin [38] are phosphorylated by protein kinase C. Both the phorbol ester and synthetic diacylglycerol, which activate intracellular protein kinase C, stimulate DNA synthesis of Swiss mouse 3T3 fibro-

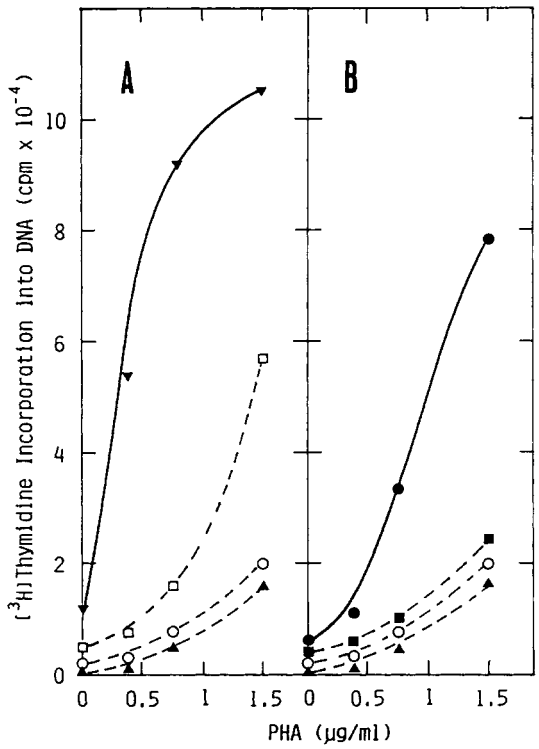

Fig. 6. Synergistic effects of A23187 and TPA or OAG on PHA-induced DNA synthesis in macrophage-depleted peripheral lymphocytes. Macrophage-depleted peripheral lymphocytes were incubated for 72 hr at 37°C with various concentrations of PHA in the presence or absence of A23187 (0.25 μM), TPA (0.1 nM), and OAG (3 μg/ml) as indicated. Other details are described under Materials and Methods. A) with TPA; B) with OAG. (○——○), stimulated by PHA alone; (▲---▲), stimulated by PHA and A23187; (□---□), stimulated by PHA and TPA; (■---■), stimulated by PHA and OAG; (▼——▼), stimulated by PHA, A23187, and TPA; (●——●), stimulated by PHA, A23187, and OAG.

blasts synergistically in the presence of a low concentration of insulin [73]. These results, together with our observations described above, strongly suggest that protein kinase C and Ca^{2+} may play a role of crucial importance in the regulation of cell division of various types of cells.

Role in other cellular functions. In addition to exocytosis and cell division, protein kinase C has been suggested to regulate other cellular functions such as glycogenolysis in hepatocytes [74], superoxide generation in neutrophils [75,76], and contraction of smooth muscle [77]. Presumably, protein kinase C may be related to the regulation of a wide variety of cellular functions that are elicited by extracellular signals listed in Table I, and the role of protein kinase C in transmembrane signaling will be more definitely clarified in the near future.

ACKNOWLEDGMENTS

This investigation has been supported in part by research grants from the Scientific Research Fund of the Ministry of Education, Science, and Culture (Japan).

The authors are grateful to Dr. Y. Nishizuka for his critical reading of this manuscript and to Miss Ikuko Ueda and Junko Yumaguchi for their secretarial assistance.

REFERENCES

1. Michell RH: Bichim Biophys Acta 415:81, 1975.
2. Rasmussen H, Goodman DBP: Physiol Rev 57:421, 1977.
3. Hokin MR, Hokin LE: J Biol Chem 203:967, 1953.
4. Takai Y, Kishimoto A, Iwasa Y, Kawahara Y, Mori T, Nishizuka Y: J Biol Chem 254:3692, 1979.
5. Takai Y, Kishimoto A, Kikkawa U, Mori T, Nishizuka, Y: Biochem Biophys Res Commun 91:1218, 1979.
6. Kishimoto A, Takai Y, Mori T, Kikkawa U, Nishizuka Y: J Biol Chem 255:2273, 1980.
7. Kaibuchi K, Takai Y, Nishizuka Y: J Biol Chem 256:7146, 1981.
8. Minakuchi R, Takai Y, Yu B, Nishizuka Y: J Biochem 89:1651, 1981.
9. Kuo JF, Anderson RGG, Wise BC, Mackerlova L, Salomonsson I, Brackett NL, Katoh N, Shoji M, Wrenn RW: Proc Natl Acad Sci USA 77:7039, 1980.
10. Kaibuchi K, Takai Y, Sawamura M, Hoshijima M, Fujikura T, Nishizuka Y: J Biol Chem 258:6701, 1983.
11. Yamanishi J, Takai Y, Kaibuchi K, Sano K, Castagna M, Nishizuka Y: Biochem Biophys Res Commun 112:778, 1983.
12. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U, Nishizuka Y: J Biol Chem 257:7847, 1982.
13. Takai Y, Kikkawa U, Kaibuchi K, Nishizuka Y: Adv Cyclic Nucleotide Protein Phosphorylation Res 18:119, 1984.
14. Craig SW, Pollard TD: Trend Biochem Sci 7:88, 1982.
15. Kakiuchi S, Sobue K: Trend Biochem Sci 8:59, 1983.
16. Daniel JL, Adelstein RS: Biochemistry 15:2370, 1976.
17. Fox JEB, Phillips DR: J Biol Chem 257:4120, 1982.
18. Endo T, Naka M, Hidaka H: Biochem Biophys Res Commun 105:942, 1982.
19. Katoh N, Wise BC, Kuo JF: Biochem J 209:189, 1982.
20. Werth DK, Niedel JE, Pastan I: J Biol Chem 258:11423, 1983.
21. Kawamoto S, Hidaka H: Biochem Biophys Res Commun 118:736, 1984.
22. Kikkawa U, Takai Y, Minakuchi R, Inohara S, Nishizuka Y: J Biol Chem 257:13341, 1982.
23. Baenziger NL, Majerus PW: Methods Enzymol 31:149, 1974.
24. Kaibuchi K, Takai Y, Nishizuka Y: J Biol Chem 260:1366, 1985.
25. Mori T, Takai Y, Yu B, Takahashi J, Nishizuka Y, Fujikura T: J Biochem 91:427, 1982.
26. Kawahara Y, Takai Y, Minakuchi R, Sano K, Nishizuka Y: Biochem Biophys Res Commun 97:309, 1980.
27. Sano K, Takai Y, Yamanishi J, Nishizuka Y: J Biol Chem 258:2010, 1983.
28. Rink TJ, Sanchez A, Hallam TJ: Nature 305:317, 1983.
29. Wilson DB, Bross TE, Hofmann SL, Majerus PW: J Biol Chem 259:11718, 1984.
30. Berridge MJ, Irvine RF: Nature 312:315, 1984.
31. Kajikawa N, Kaibuchi K, Matsubara T, Kikkawa U, Takai Y, Nishizuka Y: Biochem Biophys Res Commun 116:743, 1983.
32. Katakami Y, Kaibuchi K, Sawamura M, Takai Y, Nishizuka Y: Biochem Biophys Res Commun 121:573, 1984.
33. Rozengurt E, Rodriguez-Pena A, Smith KA: Proc Natl Acad Sci USA 80:7244, 1983.
34. Ramachandran C, Yau P, Bradburg EM, Shyamala G, Yasuda H, Walsh DA: J Biol Chem 259:13495, 1984.
35. Jacobs S, Sahyoun NE, Saltiel AR, Cuatrecasas P: Proc Natl Acad Sci USA 80:6211, 1983.
36. Cochet C, Gill GN, Meisenhelder J, Cooper JA, Hunter T: J Biol Chem 259:2553, 1984.
37. Iwashita S, Fox CF: J Biol Chem 259:2559, 1984.
38. May WS, Jacob S, Cuatrecasas P: Proc Natl Acad Sci USA 81:2016, 1984.
39. Schackelford DA, Trombridge IS: J Biol Chem 259:11706, 1984.
40. Cope FO, Staller JM, Mahsen RA, Boutwell RK: Biochem Biophys Res Commun 120:593, 1984.
41. Iwasa Y, Hosey MM: J Biol Chem 259:534, 1984.
42. Movesian MA, Nishikawa M, Adelstein RS: J Biol Chem 259:8029, 1984.
43. Le Peuch CJ, Ballester R, Rosen OM: Proc Natl Acad Sci USA 80:6858, 1983.

44. De Haro C, De Herreros AG, Ochoa S: Proc Natl Acad Sci USA 80:6843, 1983.
45. Turner RS, Chou CHJ, Kibler RF, Kuo JF: J Neurochem 39:1397, 1982.
46. Papanikolau P, Humble E, Engström L: FEBS Lett 143:199, 1982.
47. Hirata F, Matsuda K, Notsu Y, Hattori T, Carmine RD: Proc Natl Acad Sci USA 81:4717, 1984.
48. Feuerstein N, Monos DS, Cooper HL: Biochem Biophys Res Commun 126:206, 1985.
49. Summers TA, Creutz CE: J Biol Chem 260:2437, 1985.
50. O'Brian CA, Lawrence PS, Kaiser ET, Weinstein IB: Biochem Biophys Res Commun 124:197, 1984.
51. Wu WCS, Walaas SI, Nairn AC, Greengard P: Proc Natl Acad Sci USA 79:5249, 1982.
52. Aloyo VJ, Swiers H, Gispen WH: Prog Brain Res 56:303, 1982.
53. Garrison JC, Johnson DF, Campanile CP: J Biol Chem 259:3283, 1984.
54. Teshima R, Ikebuchi H, Terao T: Biochem Biophys Res Commun 125:867, 1984.
55. Drust DS, Martin, TFJ: J Biol Chem 259:14520, 1984.
56. Sakurada K, Miyazaki T, Kimura K, Yamabe M, Katabami F, Koyama M, Uehara Y, Katoh N: Cancer Res 45:903, 1985.
57. Fujiki H, Tanaka Y, Miyake R, Kikkawa U, Nishizuka Y, Sugimura T: Biochem Biophys Res Commun 120:339, 1984.
58. Miyake R, Tanaka Y, Tsuda T, Kaibuchi K, Kikkawa U, Nishizuka Y: Biochem Biophys Res Commun 121:649, 1984.
59. Kikkawa U, Takai Y, Tanaka Y, Miyake R, Nishizuka Y: J Biol Chem 258:11442, 1983.
60. Niedel JE, Kuhn LJ, Vanderbark GR: Proc Natl Acad Sci USA 80:36, 1983.
61. Ashendel CL, Staller JM, Boutwell RK: Biochem Biophys Res Commun 111:340, 1983.
62. Sando JJ, Young MC: Proc Natl Acad Sci USA 80:2642, 1983.
63. Leach KL, James ML, Blumberg PM: Proc Natl Acad Sci USA 80:4208, 1983.
64. Gordon JL: "Platelets in Biology and Pathology, 2." Amsterdam: Elsevier/North Holland Biomedical Press, 1981.
65. Daniel JL, Holmsen H, Adelstein RS: Thrombos Haemost 38:984, 1977.
66. Knight DF, Scrutton MC: FEBS Lett 160:98, 1984.
67. Kojima I, Lippes H, Kojima K, Rasmussen H: Biochem Biophys Res Commun 116:555, 1983.
68. Zawalich W, Brown C, Rasmussen H: Biochem Biophys Res Commun 117:448, 1983.
69. de Pont JJHHM, Flueren-Jakobs AMM: FEBS Lett 170:64, 1984.
70. Tanaka C, Taniyama K, Kusunoki M: FEBS Lett 175:165, 1984.
71. Habenicht AJR, Glomset JA, King WC, Nist C, Mitchell CD, Ross R: J Biol Chem 256:12329, 1981.
72. Sawyer ST, Cohen S: Biochemistry 20:6280, 1981.
73. Rozengurt E, Rodriguez-Pena A, Coombs M, Sinnet-Smith JA: Proc Natl Acad Sci USA 81:5748, 1984.
74. Fain JN, Li SY, Litosch I, Wallace M: Biochem Biophys Res Commun 119:88, 1984.
75. Robinson JM, Badwey JA, Karnovsky ML, Karnovsky MJ: Biochem Biophys Res Commun 122:734, 1984.
76. Dale MM, Penfield A: FEBS Lett 175:170, 1984.
77. Rasmussen H, Forder J, Kojima I, Scriabine A: Biochem Biophys Res Commun 122:776, 1984.