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## Calcium, Phospholipid Turnover and Transmembrane Signalling

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*Phil. Trans. R. Soc. Lond. B* 1983 **302**, 101-112

doi: 10.1098/rstb.1983.0043

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## Calcium, phospholipid turnover and transmembrane signalling

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Turnover of phosphatidylinositol, which is provoked by various neurotransmitters, peptide hormones and many other biologically active substances, appears to serve as a signal for the transmembrane control of protein phosphorylation through activation of a novel protein kinase (C-kinase). The activation of this enzyme absolutely requires  $\text{Ca}^{2+}$  and phosphatidylserine. Diacylglycerol derived from the receptor-linked breakdown of phosphatidylinositol dramatically increases the affinity of C-kinase for  $\text{Ca}^{2+}$ , and thereby renders this enzyme fully active without a net increase in the concentration of  $\text{Ca}^{2+}$ . Under appropriate conditions synthetic diacylglycerol directly added to intact cell systems activates C-kinase fully without interaction with surface receptors. By using such synthetic diacylglycerol and the  $\text{Ca}^{2+}$  ionophore A23187, it is shown that either receptor-linked protein phosphorylation or  $\text{Ca}^{2+}$  mobilization alone is merely a prerequisite but not a sufficient requirement, and both are synergistically effective for causing a full physiological cellular response. In some tissues cyclic nucleotides, both cyclic AMP and cyclic GMP, may inhibit the receptor-linked breakdown of phosphatidylinositol, and appear to provide negative control that prevents over-response.

## 1. PROPOSED FUNCTIONS OF PHOSPHATIDYLINOSITOL TURNOVER

Cellular functions and proliferation are frequently activated by the interaction of extracellular messengers with their specific cell-surface receptors, and the molecular basis of such signal transmission across the membrane has attracted great attention. A wide variety of neurotransmitters including muscarinic cholinergic and  $\alpha$ -adrenergic stimulators, peptide hormones, secretagogues, chemoattractants, growth factors, and many other biologically active substances have been shown repeatedly to provoke phosphatidylinositol turnover in their target tissues (for reviews see Michell (1975, 1979) and Hawthorne & White (1975)). In general, the stimulation of most of these receptors mobilizes  $\text{Ca}^{2+}$  immediately, and frequently increases cyclic GMP but not cyclic AMP. Although phosphatidylinositol turnover has been sometimes postulated to be involved in  $\text{Ca}^{2+}$ -gate opening (Michell 1975), the causal relation between phospholipid turnover and  $\text{Ca}^{2+}$ -gate opening is still a matter for discussion (Hawthorne 1982; Michell 1982). The receptor-linked breakdown of phosphatidylinositol is initiated by the reaction of phospholipase C, and the primary products have been identified as diacylglycerol and inositol phosphate (partly recovered as inositol 1,2-cyclic phosphate) (Kemp *et al.* 1961; Dawson *et al.* 1971). However, all attempts to explore roles of inositol phosphates have been unsuccessful. Instead, diacylglycerol is proposed to serve as a membrane fusigen or perturber in some exocytotic processes (Allan & Michell 1975; Allan *et al.* 1976). It also seems possible that phosphatidate derived from diacylglycerol may act a  $\text{Ca}^{2+}$  ionophore (Tyson *et al.* 1976; Michell *et al.* 1977; Serhan *et al.* 1981). Diacylglycerol (Bell *et al.* 1979) and phosphatidate (Billah *et al.* 1981) are known to supply arachidonate for prostaglandin synthesis, since most phosphatidylinositol molecules in mammalian tissues contain arachidonate at

position 2 (Marcus *et al.* 1969; Holub *et al.* 1970). Recent studies in this laboratory have suggested that diacylglycerol derived from phosphatidylinositol plays roles in the transmembrane control of protein phosphorylation through activation of a novel protein kinase, which is tentatively referred to as C-kinase (Takai *et al.* 1979; Kishimoto *et al.* 1980). The protein kinase absolutely requires  $\text{Ca}^{2+}$  and phospholipid, particularly phosphatidylserine for enzymatic activity, but at physiologically low concentrations of  $\text{Ca}^{2+}$  the enzyme seems to be dependent solely upon diacylglycerol for its activation. In other words, kinetic analysis indicates that a small quantity of diacylglycerol dramatically increases the apparent affinity of C-kinase for  $\text{Ca}^{2+}$  and thus fully activates this enzyme at less than micromolar concentrations of  $\text{Ca}^{2+}$  (Kishimoto *et al.* 1980; Kaibuchi *et al.* 1981). C-kinase is found in various tissues and organs so far examined (Nishizuka 1980; Kuo *et al.* 1980; Minakuchi *et al.* 1981), and has been recently purified to homogeneity from soluble fractions of rat brain (Kikkawa *et al.* 1982) and bovine heart (Wise *et al.* 1982). Some of the physical and kinetic properties of this protein kinase have been described (for reviews see Nishizuka (1980, 1983), Nishizuka & Takai (1981) and Takai *et al.* (1982*b, c*)). The following sections are devoted to describing evidence that this protein kinase is directly coupled to phosphatidylinositol turnover, and plays roles of crucial importance in some physiological processes such as release reactions. It will be also shown that the transmembrane control of protein phosphorylation and mobilization of  $\text{Ca}^{2+}$  are equally essential and synergistically effective in causing a full cellular response. Cyclic AMP and cyclic GMP do not appear to antagonize each other, but both prevent C-kinase activation by inhibiting phosphatidylinositol breakdown, presumably through actions of the respective cyclic nucleotide-dependent protein kinases. Cyclic AMP-dependent and cyclic GMP-dependent protein kinases will be referred to as A-kinase and G-kinase, respectively. Human platelets were used for the subsequent studies unless otherwise specified.

## 2. EVIDENCE FOR A LINK WITH PROTEIN PHOSPHORYLATION

Platelets are often employed as a model system for exploring the molecular basis of hormone action, since various extracellular messengers such as thrombin, collagen and platelet-activating factor cause aggregation and release reactions, and this activation of platelet function is antagonized by cyclic-AMP-elevating agents such as prostaglandins and prostacyclin. When platelets are stimulated, two endogenous proteins with approximate molecular masses of 40 kDa (40 k protein) and 20 kDa (20 k protein) are selectively and heavily phosphorylated, and these reactions are known to be associated with the release of serotonin (Lyons *et al.* 1975; Haslam & Lynham 1977). The 20 k protein is myosin light chain, and a specific calmodulin dependent protein kinase has been proposed to catalyse this phosphorylation reaction (Hathaway & Adelstein 1979). It will be shown below that  $\text{Ca}^{2+}$  mobilization appears to be essential for the phosphorylation of 20 k protein. On the other hand, the function of 40 k protein has not yet been defined, but C-kinase may be responsible for the phosphorylation of this platelet protein without a net increase in the  $\text{Ca}^{2+}$  concentration. This reaction is dependent on diacylglycerol, as shown schematically in figure 1 (Kawahara *et al.* 1980; Sano *et al.* 1983).

Under normal conditions diacylglycerol is almost absent from membranes, but is produced transiently in a signal-dependent manner. When platelets are stimulated by thrombin or collagen, diacylglycerol is produced rapidly, and this reaction is immediately followed by phosphorylation of 40 k protein and release of serotonin, as shown in figure 2. Quantitative

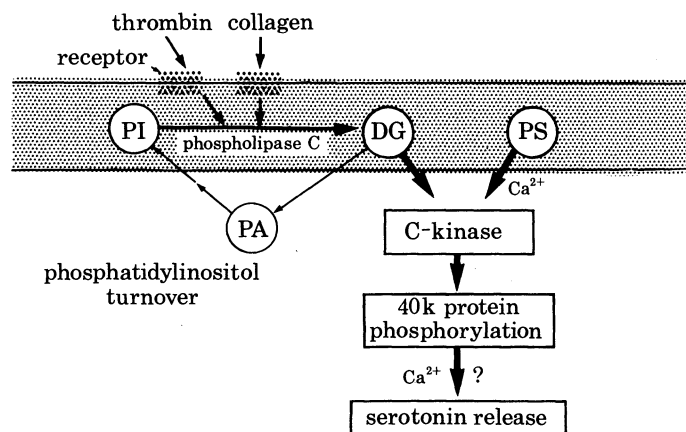


FIGURE 1. A possible mechanism of transmembrane control of protein phosphorylation in platelets. PI, phosphatidylinositol; DG, diacylglycerol; PS, phosphatidylserine; PA, phosphatidate.

analysis of various phospholipids has indicated that this diacylglycerol is most probably derived from the receptor-linked breakdown of phosphatidylinositol, as described by Rittenhouse-Simmons (1979), Bell & Majerus (1980) and Kawahara *et al.* (1980). Essentially similar results have been obtained with platelet-activating factor instead of thrombin and collagen (Ieyasu *et al.* 1982). Another set of experiments has shown that, upon stimulation of platelets with these extracellular messengers, the incorporation of radioactive phosphate into phosphatidylinositol as well as into phosphatidate is greatly increased as a result of this enhanced phospholipid turnover. The accumulation of diacylglycerol is only transient. Usually the amount of diacylglycerol accumulated is less than that of phosphatidylinositol expended. This poor stoichiometry as well as the rapid disappearance of diacylglycerol is probably due to its conversion to

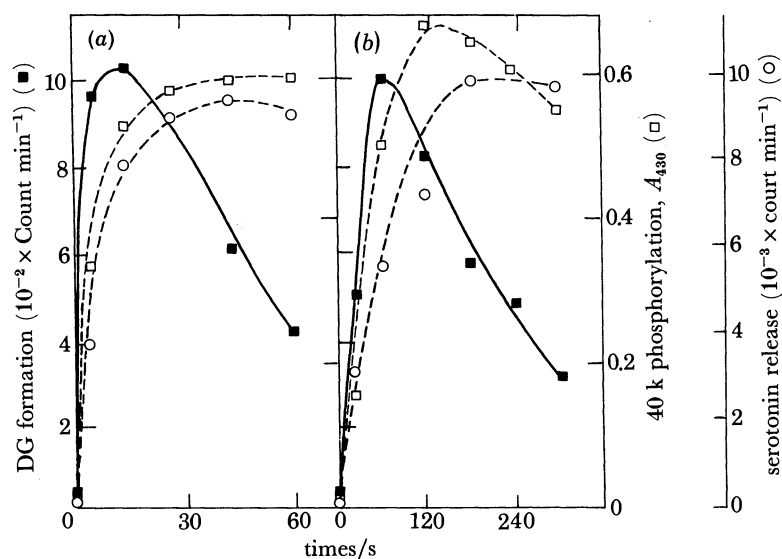


FIGURE 2. Activation of platelets by thrombin (a) and collagen (b). Human platelets were labelled with [ $^3\text{H}$ ]arachidonate,  $^{32}\text{P}_i$  or [ $^{14}\text{C}$ ]serotonin, and then stimulated by thrombin or collagen at 37 °C for various periods of time as indicated. Diacylglycerol formation (■), phosphorylation of 40 k protein (□) and release of serotonin (○) were determined. Detailed experimental conditions were as described elsewhere (Sano *et al.* 1983).

phosphatidylinositol by way of phosphatidate, and also due to its degradation to arachidonate for thromboxane synthesis. Under all conditions so far tested, the receptor-linked formation of diacylglycerol is always associated with phosphorylation of 40 k protein.

Human platelets contain a large quantity of C-kinase showing the highest specific activity among various tissues and organs so far examined. With calf thymus H1 histone as a model substrate, C-kinase in platelets is 20 times more active than A-kinase. C-kinase appears to lack tissue and species specificity, at least in its physical and kinetic properties. The enzyme preferentially phosphorylates 40 k protein partly purified from human platelets, and this reaction is absolutely dependent on the simultaneous presence of diacylglycerol and phospholipid at less than micromolar concentrations of  $\text{Ca}^{2+}$  (Sano *et al.* 1983). The fingerprint mapping patterns of tryptic phosphopeptides prepared from the radioactive 40 k protein samples, which are phosphorylated in purified cell-free systems and in intact platelets activated by thrombin, collagen and platelet-activating factor, are all identical, indicating that C-kinase may be responsible for the phosphorylation of 40 k protein observed during platelet activation (Ieyasu *et al.* 1982; Sano *et al.* 1983).

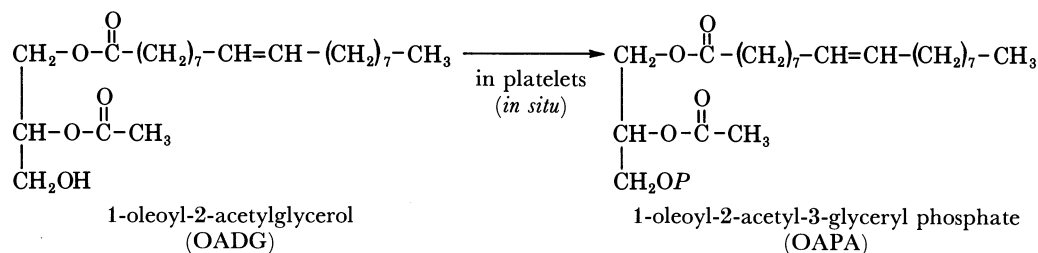


FIGURE 3. Structure of an active synthetic diacylglycerol and its conversion to phosphatidate in intact platelets.

In another set of experiments it has been shown that under appropriate conditions C-kinase may be activated by the direct addition of diacylglycerol to intact platelets (Kaibuchi *et al.* 1982a). A previous report (Mori *et al.* 1982) has described that the unique role of diacylglycerol in the activation of C-kinase appears not to be specific for the arachidonyl moiety in the glycerol backbone, and that various synthetic diacylglycerols possessing one short-chain fatty acid such as acetic acid at either position 1 or 2 are also sufficiently effective to activate C-kinase in the presence of  $\text{Ca}^{2+}$  and phospholipid. For instance, 1-oleoyl-2-acetyl-glycerol (OADG), shown in figure 3, is active *in vitro* in supporting enzymic activity. It is found that this synthetic diacylglycerol is capable of activating C-kinase directly in intact platelets as judged by phosphorylation of 40 k protein. The fingerprint mapping pattern of tryptic phosphopeptides, which are prepared from the 40 k protein phosphorylated in intact platelets, is identical with that prepared with the use of a homogeneous preparation of C-kinase. In the experiments with intact platelets, the synthetic diacylglycerol is suspended by sonication in an aqueous 1% dimethyl sulphoxide solution, and added to the incubation mixture. Under these conditions neither the breakdown of phosphatidylinositol nor the formation of endogenous diacylglycerol has been demonstrated. Dimethyl sulphoxide alone is rather inhibitory, and there is no indication that this effect of synthetic diacylglycerol is simply due to damage of the platelet membranes. It is likely that this synthetic diacylglycerol may be intercalated into the phospholipid bilayer, where it activates C-kinase directly. During the activation of C-kinase this exogenously added synthetic diacylglycerol is rapidly converted *in situ* to the corresponding phosphatidate,



1-oleoyl-2-acetyl-3-glycerol phosphate (OAPA), presumably by the action of diacylglycerol kinase, which is present at the cytoplasmic side of the membrane, as shown in figure 4. This newly produced unique phosphatidate has been isolated from platelets, and identified as such by comparison with an authentic sample of the synthetic product. In intact platelets, diacylglycerols possessing two long fatty acyl moieties such as diolein are far less effective in inducing C-kinase activation, presumably because of their inability to be intercalated into the membrane, although these diacylglycerols are highly active in cell-free systems. For enzyme assay, synthetic diacylglycerol is mixed first with phospholipid in a small volume of chloroform. After chloroform has been removed under nitrogen, the lipid micelles are prepared by sonication in buffer medium before being added to the reaction mixture.

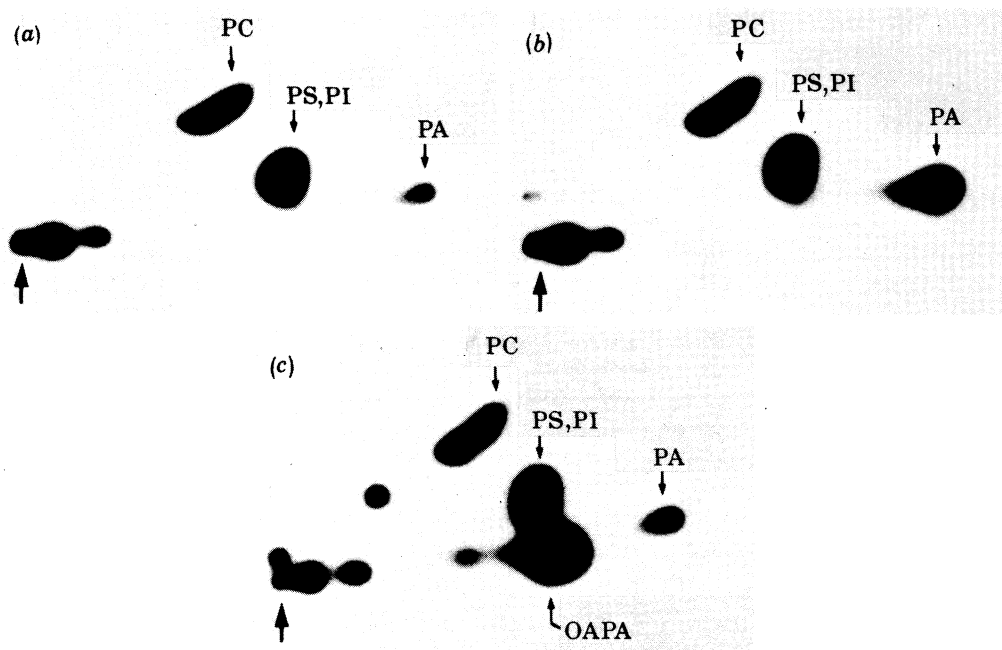


FIGURE 4. Radioautographs of various phospholipids isolated from human platelets stimulated by thrombin or synthetic diacylglycerol. Human platelets were labelled with  $^{32}\text{P}$ , and then stimulated by either thrombin (b) or 1-oleoyl-2-acetyl-3-glycerol (OADG) (c). (a), Control. Phospholipids were extracted direct from platelets, and separated by two-dimensional thin-layer chromatography, after which radioautographs were prepared. Detailed experimental conditions will be described elsewhere. PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidate; OAPA, 1-oleoyl-2-acetyl-3-glycerol phosphate. Arrows indicate the origin.

It is also noted that several phospholipid-interacting drugs such as trifluoperazine, chlorpromazine, dibucaine and tetracaine inhibit C-kinase by competing with phospholipid (Mori *et al.* 1980). In intact platelets these drugs do not interfere with receptor-linked diacylglycerol formation, but block the phosphorylation of 40 k protein and release of serotonin (Nishizuka & Takai 1981).

The experimental results briefly outlined above do not provide conclusive evidence, but strongly suggest that in platelets C-kinase is activated by various extracellular messengers, and that the receptor-linked breakdown of phosphatidylinositol serves as a signal for the transmembrane control of protein phosphorylation. Although cell-surface receptors of various extracellular messengers are presumably different, C-kinase may lie on a common pathway

that eventually leads to the release of serotonin. However, the detailed causal relation between the phosphorylation of 40 k protein and the release reaction remains to be clarified.

### 3. SYNERGISTIC ROLES OF CALCIUM AND PROTEIN PHOSPHORYLATION

It has been generally accepted that stimulation of receptors, related to phosphatidylinositol turnover, simultaneously mobilizes  $\text{Ca}^{2+}$  as shown schematically in figure 5. Although the activation of C-kinase absolutely requires  $\text{Ca}^{2+}$  in addition to phospholipid, synthetic diacylglycerol added directly to intact platelets causes full activation of this enzyme without addition of  $\text{Ca}^{2+}$  in the medium (Kaibuchi *et al.* 1982a). This is presumably due to the fact that diacylglycerol dramatically increases the affinity of C-kinase for  $\text{Ca}^{2+}$  to the  $10^{-7}$  M range, and renders this enzyme fully active without a net increase in the  $\text{Ca}^{2+}$  concentration (Kaibuchi *et al.* 1981).

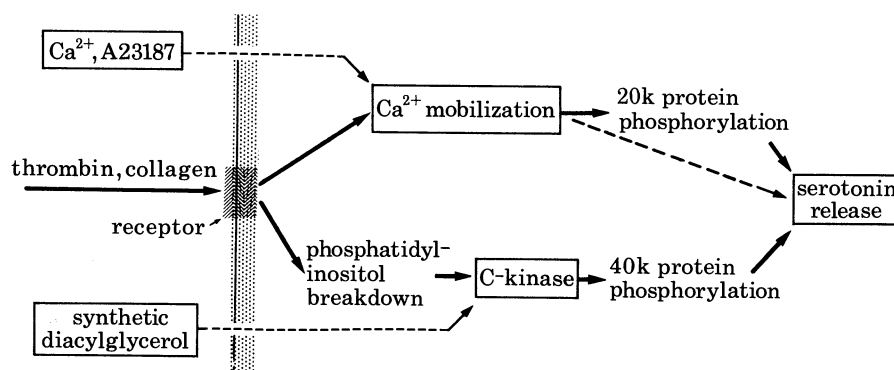


FIGURE 5. Signal pathways for platelet activation.

Thus it seems possible to induce C-kinase activation and  $\text{Ca}^{2+}$  mobilization independently by the exogenous addition of synthetic diacylglycerol and the  $\text{Ca}^{2+}$  ionophore A23187 respectively. In the experiments shown in figure 6, human platelets labelled with radioactive phosphate are stimulated by various concentrations of thrombin, synthetic diacylglycerol or A23187. Then the phosphorylation of 40 k and 20 k proteins is measured by sodium dodecyl sulphate polyacrylamide gel electrophoresis, followed by radioautography and densitometric tracing. When platelets are stimulated by thrombin, both 40 k and 20 k proteins are phosphorylated concomitantly. On the other hand, with synthetic diacylglycerol only 40 k protein is phosphorylated to an extent that is very similar to that induced by the natural extracellular messenger. In contrast, at lower concentrations of A23187 only 20 k protein is phosphorylated, presumably owing to the increase in the  $\text{Ca}^{2+}$  concentration, because a calmodulin-dependent myosin light-chain kinase is responsible for phosphorylation of 20 k protein (Hathaway & Adelstein 1979).

In the next set of experiments, shown in figure 7, radioactive human platelets labelled with  $^{32}\text{P}_i$  or  $[^{14}\text{C}]$ serotonin are stimulated by synthetic diacylglycerol in the presence and absence of a low concentration of the  $\text{Ca}^{2+}$  ionophore. It is shown that 40 k protein is phosphorylated almost equally in the presence and absence of A23187. Nevertheless, the release of serotonin is markedly enhanced by the addition of this ionophore, and the full physiological response may be observed in the simultaneous presence of synthetic diacylglycerol and A23187. This  $\text{Ca}^{2+}$  ionophore alone does not induce the endogenous formation of diacylglycerol and phosphoryla-

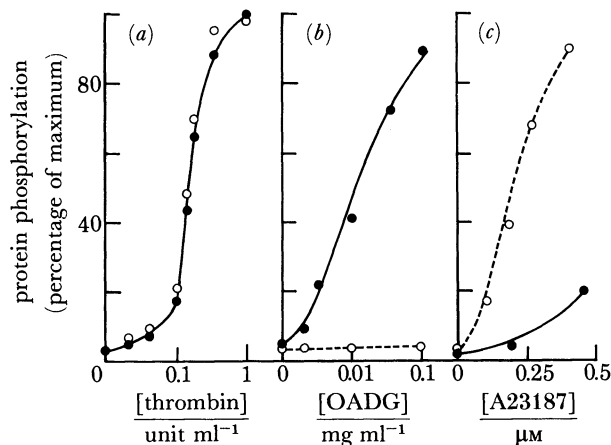


FIGURE 6. Independent induction of phosphorylation of 40 k (●) and 20 k (○) protein in platelets. Human platelets were labelled with  $^{32}\text{P}_i$ , and then stimulated by thrombin (a), synthetic diacylglycerol (b) or A23187 (c). The radioactive phosphoproteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis, and measured by densitometric tracing of the radioautographs prepared. Detailed experimental conditions will be described elsewhere.

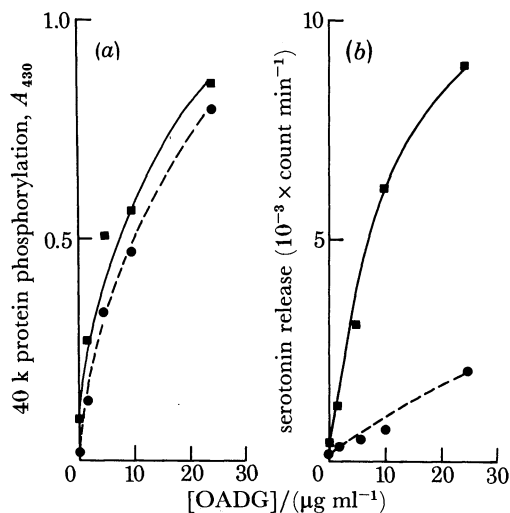


FIGURE 7. Synergistic effects of  $\text{Ca}^{2+}$  ionophore and synthetic diacylglycerol on phosphorylation of 40 k protein (a) and release of serotonin (b). Human platelets were labelled with either  $^{32}\text{P}_i$  or  $^{14}\text{C}$ serotonin, and then stimulated by various concentrations of 1-oleoyl-2-acetyl glycerol in the presence (■) or absence (●) of  $0.4 \mu\text{M}$  A23187 as indicated. Phosphorylation of 40 k protein and release of serotonin were determined under the conditions specified earlier (Kawahara *et al.* 1980). Other detailed experimental conditions will be described elsewhere. (Adapted from Kaibuchi *et al.* (1982a).)

tion of 40 k protein, nor does it cause release of serotonin at the concentration employed in the present experiment. However, the  $\text{Ca}^{2+}$  ionophore at higher than micromolar concentrations itself causes the phosphorylation of 40 k protein as well as serotonin release, probably because of an enhancement of non-specific degradation of phospholipid, and also because of activation of C-kinase by a large increase in  $\text{Ca}^{2+}$  concentrations (Takai *et al.* 1979; Kishimoto *et al.* 1980; Kaibuchi *et al.* 1981). Likewise, this synthetic diacylglycerol alone at more than  $0.1 \text{ mg ml}^{-1}$  causes release of a significant amount of serotonin. The exact reason for this enhanced release reaction is not known, but it is possible that the diacylglycerol or phosphatidate therefrom may



act as a membrane fusigen or  $\text{Ca}^{2+}$  ionophore as mentioned above. Although  $\text{Ca}^{2+}$  may play diverse roles in the release reaction, the results presented above suggest that under limited conditions protein phosphorylation and  $\text{Ca}^{2+}$  mobilization are equally indispensable and synergistically effective for causing a full physiological cellular response.

The synergistic effects of synthetic diacylglycerol and A23187 are also observed in other cell types. For example, when rat polymorphonuclear leucocytes and mast cells are stimulated by synthetic diacylglycerol, lysosomal enzymes and histamine, respectively, are released to some extent. If, however, these cells are incubated with a small quantity of diacylglycerol in the presence of a low concentration (0.3–0.6  $\mu\text{M}$ ) of A23187, the release reactions are dramatically enhanced. The  $\text{Ca}^{2+}$  ionophore alone at this concentration shows only a slight effect. Again, the results are consistent with the supposition that  $\text{Ca}^{2+}$  mobilization and protein phosphorylation may be synergistically involved, even if causally related in some way, in the transmembrane control of cellular functions and proliferation by various extracellular messengers.

#### 4. CYCLIC NUCLEOTIDES AS NEGATIVE MESSAGERS

Stimulation of receptors related to phosphatidylinositol turnover frequently increases cyclic GMP levels, although the precise signal pathway for elevating this cyclic nucleotide has not yet been identified unequivocally. In addition, the role of this cyclic nucleotide in biological regulation is not fully substantiated. It remains a puzzle that G-kinase shows very similar, if not identical, catalytic properties to those of A-kinase (Hashimoto *et al.* 1976). It has been suggested that in human platelets cyclic GMP may act as a negative rather than a positive messenger, and provides an immediate feedback control that prevents over-response (Haslam *et al.* 1980; Takai *et al.* 1981). This assumption is based on the observation that sodium nitroprusside, known as a potent platelet inhibitor, markedly increases cyclic GMP concentration. A series of experiments given in figure 8 has revealed that both sodium nitroprusside and 8-bromo cyclic GMP inhibit thrombin-induced phosphatidylinositol breakdown and diacylglycerol formation as well as the phosphorylation of 40 k protein and release of serotonin

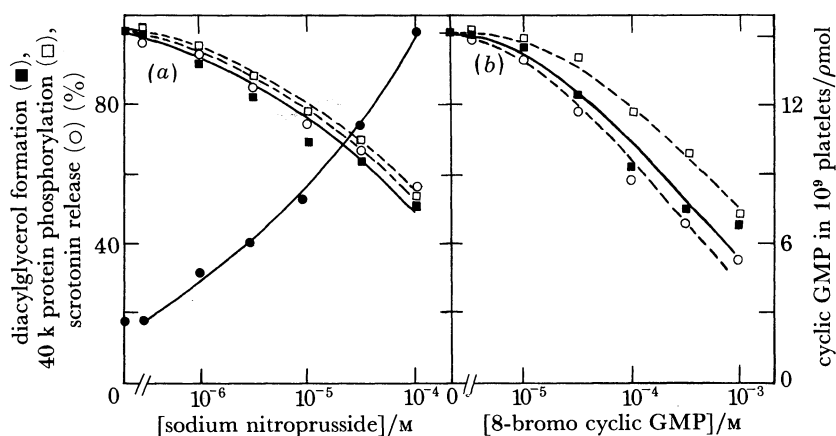


FIGURE 8. Inhibitory effect of cyclic GMP on platelet activation. Human platelets were labelled with [ $^3\text{H}$ ]arachidonate,  $^{32}\text{P}_i$  or [ $^{14}\text{C}$ ]serotonin, and then stimulated by thrombin in the presence of increasing amounts of sodium nitroprusside (a) or 8-bromo cyclic GMP (b). Formation of diacylglycerol (■), phosphorylation of 40 k protein (□) and release of serotonin (○) were determined as described (Kawahara *et al.* 1980). Cyclic GMP (●) was measured by radioimmunoassay. Detailed experimental conditions were as described elsewhere (Takai *et al.* 1981).

(Takai *et al.* 1981, 1982*a-c*). Conversely, these compounds enhance the phosphorylation of a distinct protein with an approximate molecular mass of 50 kDa (50 k protein). Although sodium nitroprusside increases cyclic AMP levels slightly, at most twofold, the observed inhibitory action of this compound may not be mediated by this slight increase in cyclic AMP. A quantitative analysis indicates that phosphorylation of 50 k protein is inversely proportional to phosphorylation of 40 k protein. A similar inhibitory action of cyclic GMP on phosphatidylinositol turnover is also observed for rat aortic smooth muscle, which is stimulated by noradrenalin. The concentrations of sodium nitroprusside and 8-bromo cyclic GMP needed for the inhibition of noradrenalin-induced phosphatidylinositol breakdown and muscle contraction are nearly the same. Although the inhibitory action of cyclic GMP has only been studied with the two selected tissues mentioned above, it seems possible that this cyclic nucleotide may act as a negative rather than a positive messenger, and serves as an intracellular feedback inhibitor that prevents over-response to extracellular messengers. Obviously this proposed function does not necessarily exclude other possible roles of this cyclic nucleotide in biological systems.

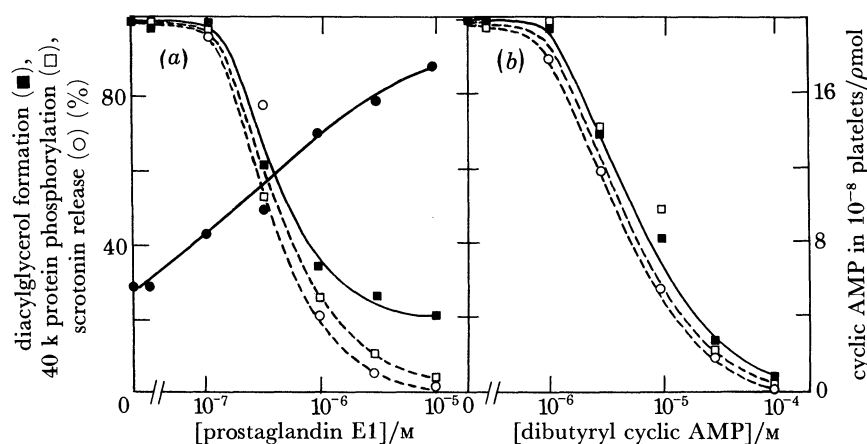


FIGURE 9. Inhibitory effect of cyclic AMP on platelet activation. Detailed conditions are similar to those given in figure 8, except that prostaglandin E1 (*a*) and dibutyryl cyclic AMP (*b*) were employed instead of sodium nitroprusside and 8-bromo cyclic GMP. Cyclic AMP (●) was assayed by radioimmunoassay. ■, Formation of diacylglycerol; □, phosphorylation of 40 k protein; ○, release of serotonin. Other detailed conditions were as described elsewhere (Kawahara *et al.* 1980).

*Bidirectional control* systems occur in most tissues, such as platelets, lymphocytes and polymorphonuclear leukocytes. In these tissues receptors that induce phosphatidylinositol turnover generally promote activation of cellular functions, whereas the receptors that produce cyclic AMP usually antagonize such activation. In contrast, in *monodirectional control* systems in some tissues such as hepatocytes and adipocytes the two receptors mentioned above do not appear to interact with each other but function independently. In hepatocytes, for instance,  $\alpha$ - and  $\beta$ -adrenergic stimulators, which induce turnover of phosphatidylinositol and accumulation of cyclic AMP, respectively, enhance glycogenolysis equally, and cyclic AMP does not inhibit phosphatidylinositol breakdown (Kaibuchi *et al.* 1982*b*). However, in platelets, receptor-linked formation of diacylglycerol, phosphorylation of 40 k protein and release of serotonin are all inhibited progressively in a parallel manner by increasing amounts of prostaglandin E1 as well as by dibutyryl cyclic AMP, as shown in figure 9 (Takai *et al.* 1982*a-c*). A densitometric analysis of a series of radioautographs prepared under different conditions suggests that cyclic

AMP profoundly inhibits phospholipid breakdown, presumably through the phosphorylation of 50 k protein described above, and thereby counteracts C-kinase activation. Thus, 40 k protein and 50 k protein in platelets may be regulated in an opposing manner by the two distinctly different receptor mechanisms elicited by thrombin and prostaglandin E1. Nevertheless, the precise roles of these proteins in platelet function remain to be clarified.

The interaction of the two receptor mechanisms briefly described above may also be observed in other cell types that are activated by various extracellular messengers, but may be inhibited by another group of extracellular messengers that increase cyclic AMP. Again, with human peripheral lymphocytes and polymorphonuclear leucocytes activated by phytohaemagglutinin and formyl-Met-Leu-Phe, respectively, it is shown that phosphatidylinositol turnover is profoundly inhibited by dibutyryl cyclic AMP as well as by prostaglandin E1, which markedly increases cyclic AMP concentration (Takai *et al.* 1982*c*). It is also shown with mast cells that prostaglandin E1 inhibits phosphatidylinositol turnover and histamine release, which are induced by concanavalin A plus phosphatidylserine (Kennerly *et al.* 1979). Although the inhibition of phosphatidylinositol turnover by cyclic nucleotides described above may be mediated through the actions of the respective protein kinases, the molecular basis of this inhibition is unknown.

#### CODA AND PROSPECTS

Although the experimental support described above has resulted from studies with a limited number of tissues, it is suggested that the C-kinase system is potentially important for the activation of cellular functions and proliferation by various extracellular messengers. A series of analyses has indicated that C-kinase shows catalytic properties distinctly different from those of cyclic nucleotide-dependent enzymes (Iwasa *et al.* 1980). However, the target proteins of C-kinase in most tissues remain to be identified. It is possible that some cytoskeletal proteins may serve as substrates as postulated for a group of tyrosine-specific protein kinases. Perhaps an elucidation of the topographical arrangement and compartmentation in the protein kinase – phosphate acceptor protein interaction is essential for a full understanding of the mechanism of transmembrane control of protein phosphorylation.

Figure 10 illustrates a hypothetical scheme, outlined for platelets as a model system. The present studies have also attempted to explore a possible relation between receptor-linked protein phosphorylation and  $\text{Ca}^{2+}$  mobilization, and the results obtained seem to suggest that under certain conditions these two processes are equally indispensable and synergistically effective in causing a full physiological response. It is also possible that, in platelets, cyclic AMP and cyclic GMP do not antagonize each other but have integrated roles in the extracellular and intracellular circuits, eventually leading to feedback inhibition of the receptor-linked phospholipid degradation in activated cell membranes. The mechanism of accumulation of cyclic GMP is not clear, but it is presumed that arachidonate peroxide and prostaglandin endoperoxides serve as activators for guanylate cyclase (Hidaka & Asano 1977; Graff *et al.* 1978). In most tissues, arachidonate is also released from other major phospholipids. It seems possible that, when this class of receptors is stimulated, both phospholipase C and A2 type reactions proceed in a concerted manner. Phospholipases C in mammalian tissues so far described are specific for phosphatidylinositol, and do not appear to be rate-limiting in many tissues. The events occurring immediately after stimulation of the receptors remain largely unexplored. One of the serious barriers to approaching the problems lies in the difficulties of

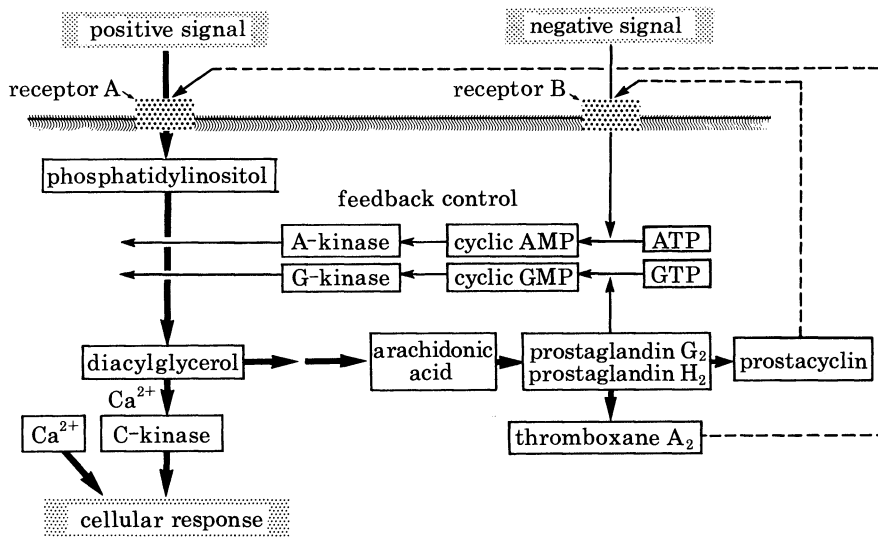


FIGURE 10. A possible role of phosphatidylinositol degradation in transmembrane control of protein phosphorylation. Platelets were used as a model system. (Adapted from Takai *et al.* (1982*a*)).

understanding lipid-protein interactions. Nevertheless, the principles and mechanisms proposed in this article may obviously be modified by future knowledge that will rapidly expand in the next few years.

The author appreciates the invitation to this meeting, and expresses his deep gratitude to the Royal Society and the Naito Science Foundation for the support that made this lecture possible. The author is grateful to his colleagues in the last few years, particularly to Dr Y. Takai, Dr A. Kishimoto, Dr U. Kikkawa, Dr K. Sano, Dr K. Kaibuchi, Dr K. Nishiyama, Dr N. Kajikawa, Dr J. Yamanishi, Dr M. Castagna, Dr Y. Kawahara and Dr R. Minakuchi, who have so effectively carried out the experiments described in this article. Skilful secretarial assistance on the part of Mrs S. Nishiyama, Miss K. Yamasaki and Mrs M. Furuta is also gratefully acknowledged.

This investigation has been supported in part by research grants from the Scientific Research Fund of the Ministry of Education, Science and Culture (1982), the Intractable Diseases Division, Public Health Bureau, the Ministry of Health and Welfare (1981-2), a Grant-in-Aid of New Drug Development from the Ministry of Health and Welfare (1979-82), the Science and Technology Agency (1982), and the Yamanouchi Foundation for Research on Metabolic Disorders (1982).

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