Phosphatidate Accumulation in Hormone-treated Hepatocytes via a Phospholipase D Mechanism*

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Isolated rat hepatocytes responded to a variety of Ca²⁺-mobilizing agents (vasopressin, angiotensin II, epinephrine, epidermal growth factor, ATP, and ADP) with a rapid increase in phosphatidate mass, as measured by a sensitive new method. When hepatocytes were incubated with vasopressin (10⁻⁶ M), phosphatidate levels increased 2–3-fold in 2 min, but there was no significant increase in diacylglycerol at this time. Changes in the fatty acid composition of phosphatidate also preceded those in diacylglycerol. De novo synthesis of phosphatidate from [⁴H]glycerol was unaffected by vasopressin in short-term incubation.

Incubation of washed rat liver plasma membranes with GTPγS caused a time-dependent increase in phosphatidate. When membranes were incubated with GTPγS and [γ-³²P]ATP, no incorporation of ³²P into phosphatidate was observed. This excludes the phospholipase C-diacylglycerol kinase pathway and suggests that a phospholipase D activity produced the phosphatidate. At submaximal concentrations of GTPγS, ATP and ADP stimulated membrane phosphatidate formation, presumably by acting through P₂-purinergic receptors. Only phosphatidylincholine, among the major phospholipids, decreased in the membranes in response to GTPγS. The fatty acid composition of the phosphatidate produced in response to vasopressin in hepatocytes also suggests that phosphatidylcholine may be the source of hormonally elicited phosphatidate. We conclude that Ca²⁺-mobilizing hormones mainly increase phosphatidate levels in hepatocytes by a mechanism that does not involve phosphorylation of diacylglycerol but de novo synthesis but involves a guanine nucleotide-binding protein coupled to phospholipase D.

Phosphatidate has long been recognized as a central metabolite in both phospholipid and triglyceride metabolism and measurements of phosphatidate mass, which is only about 1% of total phospholipid, have been infrequent. Most studies of phosphatidate metabolism have employed radioisotopic labeling and two-dimensional thin layer chromatography of phospholipids. Ca²⁺-mobilizing hormones have been shown to increase [³²P]PO₄ incorporation into phosphatidic acid in a number of cell types (for example, Ref. 1). This increase in labeling is thought to be a consequence of the phosphorylation by diacylglycerol kinase of diacylglycerol formed by the phosphodiesteratic cleavage of inositol phospholipids. In rat hepatocytes, Takenawa et al. (2) have reported that vasopressin (2 nM) elicits a transient increase in [³²P]PO₄-labeled phosphatidate. Thomas et al. (3) have reported a similar increase in phosphatidate labeling following stimulation by vasopressin (10 nM). These workers found that the increase in [³²P] phosphatidate coincided with a small early increase of [³H] arachidonoyl-diacylglycerol labeling, but preceded the bulk of diacylglycerol labeling. This finding suggests that phosphatidate may be formed by a mechanism different from that involving phospholipase C and diacylglycerol kinase.

Hokin-Neaverson and her co-workers (4) have suggested that a phospholipase D might be responsible for inositol production from phosphatidylinositol in response to acetylcholine in mouse pancreas. Cockcroft (5) has shown that, in neutrophils, f-met-leu-phe elicits a 6-fold increase in phosphatidate formation as measured by phosphate analysis. When neutrophils were labeled with [³²P]PO₄, the phosphatidate formed was found to have a much lower specific activity than that of the γPO₄ of ATP. Cockcroft has taken this as evidence of either a direct formation of phosphatidate via phospholipase D or of a partial reversal of the CDP-diacylglycerol synthetase reaction.

Phospholipase D has been widely studied in plants but was once believed not to occur in animals. Kanfer and his co-workers (6, 7) have characterized a phospholipase D from rat brain. This integral membrane protein will not hydrolyze exogenous substrates under most conditions, which probably explains why earlier workers did not observe it. Phospholipase D activity has also been observed in preparations from a variety of rat tissues including liver (7). With the exception of the work of Cockcroft and of Hokin-Neaverson, no studies have linked phospholipase D activity to hormonal action. In this report, we show that in hepatocytes, Ca²⁺-mobilizing hormones elicit an increase of phosphatidate mass as measured by a sensitive new procedure. This increase in phosphatidate precedes the hormone-elicited increase in diacylglycerol. Experiments with rat liver plasma membranes indicate that this increase in phosphatidate does not require ATP and proceeds via a phospholipase D mechanism that is stimulated by a guanine nucleotide-binding protein.

**EXPERIMENTAL PROCEDURES**

**Materials**—HEPES,¹ angiotensin II, (-)-epinephrine, butylated hydroxytoluene, 2-nitrophenylhydrazine, fatty acids, EGTA, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, 1,2-diolein, dipalmityl

¹ The abbreviations used are: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GTPγS, guanosine 5′-(3-O-thio)triphosphate; GDPβS, guanosine 5′-(2-O-thio)di phosphate; EGTA, ethylenediaminetetraacetic acid; HPLC, high performance liquid chromatography.
phosphatidic acid, [8-arginine]vasopressin, phorbol myristate acetate, choline, phosphocholine, choline kinase, alkaline phosphatase, ATP, and ADP were from Sigma. Collagenase Type 1 was from Cooper Biomedical. Lyso phosphatidic acid was from Serdary. Methanol and acetoneitrile were from Burdick and Jackson. Toluene, chloroform, butanol, acetic acid, methylamine, and NH₂OH were from Fisher. Coomassie Blue R250 was from Bethesda Research Laboratories. A23187 was a gift from Dr. D. Chauffoy, Janssen Pharmaceutica, Beerse, Belgium.

**Lipid Extraction from Hepatocytes**—Hepatocytes were prepared from fed male rats (250-300 g) as previously described (8). Hepatocyte suspensions (75-90 mg/ml wet weight) were shaken and gassed with 95% O₂: 5% CO₂ for 15 min at 37 °C before addition of hormones. One ml samples were removed and extracted with 3.75 ml of CHCl₃/MelOH/H₂O, 2:2:0.2. The phosphatidate was deacylated with butanol/acetic acid/H₂O, 6:1:1. Standards of dipalmitoyl phosphatidate and phosphatidylethanolamine were extracted with 2 ml of CHCl₃/MelOH/H₂O: 5:5:1 plus 5 ml of NH₄OH. The plates were air-dried overnight under N₂ and then developed with CHCl₃/MelOH/H₂O, 2:2:0.2. The phosphatidate was deacylated as described by Clarke and Dawson (19). Alkyl and alkenyl lipids were resolved by this chromatographic procedure. The sensitivity of this method is such that 1.4 pg of phosphatidate could be detected.

**Thin Layer Chromatography of Lipids**—Phosphatidate was separated from other lipids by multidevelopment chromatography on 20 x 8 cm Silica Gel 60 F-254 plates (Merck). Samples (lipids from 3-4 mg of hepatocytes) and dipalmitoyl phosphatidate standard were applied in 20 µl of CHCl₃. The plates were developed with butanol/acetic acid/H₂O (6:1:1) (10) to the top of the plate (8 cm). The plates were then dried for 40 min in a lyophilizer, and the top 1 cm of the plates containing the neutral lipids was removed. Through drying at all stages was necessary for optimal resolution and staining. The plates were then developed with CHCl₃/MelOH/NH₄OH (10:30:6) to the top of the plate. The plates were air-dried for 30 min, stained with Coomassie Brilliant Blue R250 (0.03% in 50% CHCl₃), and destained for 30 min in 30% MetOH 100 mM NaCl (11). The plates were air-dried and scanned with an LKB 2202 Ultrasonic laser densitometer. The output was channelled to a Hewlett-Packard analog/digital converter and the data were stored in a Hewlett-Packard 3357 computer (Laboratory Automation System). Integration of thin layer chromatography bands was performed using the Hewlett-Packard CLOT system. Standard curves of dipalmitoyl phosphatidate (0.25, 0.5, and 1 µg) were constructed for each plate. Recovery of added phosphatidate was 60% from hepatocyte extracts.

Neutral lipids were separated on Silica Gel 60 F-254 plates using the solvent system toluene/diethyl ether/EtOH/NaOH/NH₄OH (50:40:2:0.2). 1.2-Diaclyglycerol was quantitated by Coomassie Blue staining and densitometry using 1,2-diolein as a standard.

**Fatty Acid Analysis**—Phosphatidate and diacylglycerol (from 70-90 mg of hepatocytes) were purified by thin layer chromatography as above in N₂-purged chambers. Plates were dried under N₂ (~15 min between developments and before staining). The plates were lightly stained with Coomassie Blue, and the lipid-containing areas were scraped off and extracted with 2 ml of CHCl₃/MelOH/MeOH (1:1) for diacylglycerol and with 2 ml of MeOH/H₂O 1:1 N HCl (20:1) for phosphatidate. The recoveries were about 70% for phosphatidate and 100% for diacylglycerol as assessed by fatty acid analysis of standards. The samples were evaporated under N₂, saponified, and the fatty acids were converted to 2-nitrophenylhydrazides by the method of Miwa et al. (12). The derivatized fatty acids were separated by HPLC using a Beckman Ultrasphere octyl column (5-pM particle size, 250 X 4.6 mm inner diameter). A Waters Associates M6000-A pump, a model U6K injector, and model 450 variable wavelength detector. The column was eluted isocratically with acetoneitrile/H₂O (85:15), and the nitrophenylhydrazides were detected by absorbance at 230 nm (12). Peaks were integrated using the Hewlett-Packard Laboratory Automation System as above. The samples were converted to 2-nitrophenylhydrazides by the method of Miwa et al. (12) and provided a clear resolution of phosphatidate from other lipids in one dimension. Other phospholipids are not well resolved by this system. Lipids were detected by the sensitive method of Nakamura and Handa (11) using Coomassie Blue staining. Separation of hepatocyte lipid extracts using this method followed by chromatography in a second dimension showed that the phosphatidate band consisted of 1 spot comigrating with phosphatidate standard (not shown). When 102 µg of phosphatidic acid purified by this chromatographic procedure was acetylated as under "Experimental Procedures," 1.4 µg of phosphatidate and less than 0.5 µg of lysophosphatidate remained. This set an upper limit of approximately 2% for alkyl and alkenyl phosphatidate in agreement with the low content of alkyl and alkenyl lipids found in liver (21). Fig. 1, panel B shows standard curves of dipalmitoyl phosphatidate, dillinoeoyl phosphatidate, and phosphatidate from egg lecithin measured by this method. The sensitivity of this method is such that we can routinely assay the phosphatidate content of extracts from 3-5 mg wet weight of hepatocytes.

The accumulation of phosphatidate was much more rapid than that of diacylglycerol in hepatocytes treated with vasopressin (10⁻⁸ M) as is shown in Fig. 2. Typically, both lipids

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**Hepatocyte Phospholipid D**
increased 2- to 3-fold after incubation for 5 min with this vasopressin concentration. The control value for phosphatidate was 71 ± 11 ng/mg wet weight (mean ± S.E.; nine separate experiments). The time course and levels of diacylglycerol accumulation were very similar to those found in our earlier study using an HPLC method (22).

As shown in Table I, hepatocyte phosphatidate levels were increased by a variety of agents previously shown to mobilize Ca\(^{2+}\) (23). Vasopressin and ATP elicited a 2-fold increase in phosphatidate levels; angiotensin II, epinephrine, epidermal growth factor, and ADP were slightly less efficacious. The calcium ionophore A23187 (10\(^{-6}\) M) also raised phosphatidate levels. Phosphatidate accumulation was stimulated by vasopressin in a concentration-dependent manner with an EC\(_{50}\) of 1.4 nM similar to that previously found for diacylglycerol accumulation (1.2 nM) (22). These concentrations are higher than that needed for Ca\(^{2+}\) mobilization and phosphorylase activation (EC\(_{50}\) = 90 pM) (23). The depletion of hepatocyte calcium by a 15-min incubation with 5 mM EGTA reduced vasopressin-elicited phosphatidate accumulation by 50%, but had no effect on phosphatidate levels in control cells. Phorbol myristate acetate caused a slow increase in phosphatidate levels with a 1.6-fold stimulation at 10 min.

Since various hormone receptors have been shown to couple to phospholipases through guanine nucleotide-binding proteins, we incubated hepatocytes with toxins that modify cer-
increasing rapidly and palmitate and linoleate declining (Fig. 4). The change in phosphatidate fatty acid composition preceded that in diacylglycerol composition suggesting that the phosphatidate was not derived from diacylglycerol. Diacylglycerol mass at 5 min was 1.9 × control; phosphatidate mass at 5 min was 2.3 × control as estimated by total fatty acid recovery. Using these values, the fatty acid composition of the lipid produced in response to vasopressin can be calculated. Vasopressin-elicited diacylglycerol (at 5 min) had the following fatty acid composition (mol %): 22:6, 4.1%; 20:4, 29.5%; 18:2, 15.7%; 16:0, 18.6%; 18:1, 3.7%; 18:0, 28.6%. Phosphatidate elicited by vasopressin (at 5 min) had the fatty acid composition (mol %): 22:6, 4.7%; 20:4, 34.3%; 18:2, 14.8%; 16:0, 10.9%; 18:1, 2.4%; 18:0, 32.9%.

Studies Using Isolated Plasma Membranes—To assess the mechanism of phosphatidate accumulation, we incubated washed isolated liver plasma membranes with varying amounts of GTP\(_\gamma\)S. This nucleotide has been used widely to activate guanine nucleotide-binding proteins and alter membrane processes in the absence of hormone. GTP\(_\gamma\)S stimulated phosphatidate formation by the plasma membranes (Fig. 5). High concentrations of GTP\(_\gamma\)S (10 \(\mu\)M) caused a 5.7-fold increase in membrane phosphatidate; half-maximal stimulation was seen at 1.5 \(\mu\)M GTP\(_\gamma\)S. GDP\(_\beta\)S (250 \(\mu\)M) was without effect. It should be noted that this experiment was performed without added ATP. At high concentrations of GTP\(_\gamma\)S (20 \(\mu\)M), ATP had no effect on the accumulation of phosphatidate (data not shown) which argues against a role for diacylglycerol kinase in phosphatidate formation in this system. When plasma membranes were incubated with \([\gamma^{32}\text{P}]\text{ATP} \) there was no incorporation of \(^{32}\text{P}\) into membrane phosphatidate under conditions where 4.9 nmol of phosphatidate was formed in response to GTP\(_\gamma\)S at 10 \(\mu\)M (Table II). The time course of membrane phosphatidate formation is shown in Fig. 6. After an initial lag, phosphatidate formation proceeded for at least 10 min. It is very unlikely that sufficient carryover of ATP exists in the washed membrane preparation to phosphorylate 9 nmol of diacylglycerol to phosphatidate. In addition, in intact hepatocytes oleoyl-acyetyl glycerol and dioctanoyl glycerol were not phosphorylated to a detectable extent (by mass) and the diacylglycerol kinase inhibitor (27) ethylene glycol dioctanate (at concentrations from 0.2–1 mm) was without effect on phosphatidate levels in the presence or absence of vasopressin (data not shown). Similarly, another diacylglycerol kinase inhibitor (28), R59 022, was without effect on vasopressin-elicited diacylglycerol accumulation in

min did not affect phosphatidate levels after vasopressin challenge (data not shown). These data suggest that vasopressin does not increase the de novo synthesis of phosphatidate or diacylglycerol in the period of time (5 min) examined in these experiments.

Although phosphatidate accumulation preceded that of diacylglycerol (Fig. 2), phosphatidate could be derived from a rapidly phosphorylated pool of diacylglycerol. In this case we might expect to see a rapid change in the fatty acid composition of diacylglycerol as it is formed from a phospholipid substrate by phospholipase C. This change in diacylglycerol fatty acid composition should precede that of phosphatidate, much as in a radioactive labeling experiment. The fatty acid composition of diacylglycerol did change in response to vasopressin (Fig. 4 and Ref. 22), with an increase in arachidonate and stearate and a parallel decline in palmitate and linoleate. Phosphatidic acid had a different fatty acid composition under basal conditions but underwent a similar change in fatty acid composition after vasopressin with arachidonate and stearate

Fig. 3. The effect of vasopressin on the incorporation of \([^{3}H]\text{glycerol into phosphatidate and diacylglycerol.} \) After hepatocytes were incubated for 15 min, 40 \(\mu\)Ci of \([^{3}H]\text{glycerol (New England Nuclear) was added to each flask. Thirty s later (0 min), one flask was adjusted to vasopressin (10 \(^{-8}\) M) and the other was adjusted with albumin-saline (control). Aliquots were removed at the indicated times and processed as under "Experimental Procedures." The areas containing phosphatidate and diacylglycerol were removed from plates and eluted with CHCl\(_3\)/MeOH, 1:1 (diacylglycerol). The extracts were mixed with 10 ml of Ready Solv NA (Beckman) and counted for \(^{3}H\). Filled symbols represent incubations with vasopressin, open symbols without vasopressin.

![Graph showing the effect of vasopressin on the incorporation of \([^{3}H]\text{glycerol into phosphatidate and diacylglycerol.} \)
branes possess a phospholipase D activity. Taken together, these findings support the hypothesis that hepatic plasmic membranes possess a phospholipase D activity.

The effects of various Ca\(^{2+}\)-mobilizing agents on membrane phosphatidate accumulation at a low concentration of GTP\(_\gamma\)S (0.3 \muM) is shown in Table III. Preliminary experiments with Ca\(^{2+}\)-EGTA buffers showed that EGTA inhibited membrane phosphatidate accumulation by about 33%; this inhibition was reversed by Ca\(^{2+}\) at a calculated free concentration of 72 nM (data not shown). Most experiments were conducted with EGTA because the stimulation by GTP\(_\gamma\)S of the hydrolysis of inositol phospholipids by phospholipase C (data not shown). These agents mobilize Ca\(^{2+}\) and cause phosphatidate accumulation in isolated hepatocytes (Ref. 29 and Table I). They are presumably acting through P\(_2\)-purinergic receptors, but ATP may also prevent the breakdown of GTP\(_\gamma\)S.

Potential phospholipid sources of phosphatidate were investigated by incubating plasma membranes with GTP\(_\gamma\)S (20 \muM) in the absence of Ca\(^{2+}\) (Table IV). The major phospholipids were separated as described under "Experimental Procedures," ashed, and assayed for PO\(_4\) content. Phosphatidate rose from 3.8 nmol/mg protein to 11.6 nmol/mg protein (Table IV). The only lipid that declined was phosphatidylinositol, which was reduced from 146.3 to 130.2 nmol/mg by the inclusion of GTP\(_\gamma\)S. This experiment does not rule out other sources for phosphatidate, but it should be observed that, in plasma membranes, phosphatidylserine and phosphatidylinositol are present in amounts only 4- and 2-fold greater, respectively, than phosphatidate and probably do not contribute to its formation.

To explore the effect of GTP\(_\gamma\)S on phosphatidylcholine breakdown, we measured the production of choline, phosphocholine, phosphatidate, and diacylglycerol by plasma membranes (Fig. 7). Both choline and phosphocholine were produced in response to GTP\(_\gamma\)S (Fig. 7A) in agreement with the findings of Irving and Exton (31), but choline was always increased to a larger extent than phosphocholine. Phospho-

![Fig. 5. The effect of GTP\(_\gamma\)S on phosphatidate accumulation in isolated plasma membranes.](image)

**Table II**

**Effect of incubation of plasma membranes with or without GTP\(_\gamma\)S on formation of 
[\(\beta\gamma\)P]phosphatidic acid**

| GTP\(_\gamma\)S | Incubation | Phosphatidate* | cpm |
|----------------|------------|----------------|-----|
| min | nmol | |
| | 0 | 0.31 | 53 |
| - | 10 | 0.69 | 54 |
| + | 0 | 0.31 | 58 |
| + | 10 | 4.87 | 47 |
| TLC blank | | 41 |

* Using the molecular weight of dioleoyl phosphatidate.

![Fig. 6. Time course of accumulation of phosphatidate (PA) in plasma membranes.](image)

**Table III**

**Effect of various agents on phosphatidate formation by hepatic plasma membranes**

Membranes were incubated for 10 min with the indicated agents and 0.4 mM Ca\(^{2+}\), 0.5 mM EGTA (calculated free Ca\(^{2+}\) = 192 nM) for 10 min as described under "Experimental Procedures" (n = 9, three separate membrane preparations).

| GTP\(_\gamma\)S (0.3 \muM) | Other addition | Phosphatidate |
|----------------|----------------|---------------|
| | \(\mu\)g/mg protein | |
| - | 2.95 ± 0.42 | |
| + | 3.48 ± 0.57 | |
| + | Vasopressin (10^{-8} M) | 4.12 ± 0.64 |
| + | Angiotensin II (10^{-8} M) | 3.31 ± 0.32 |
| + | Epinephrine (10^{-8} M) | 3.21 ± 0.25 |
| + | ATP (10^{-4} M) | 6.82 ± 1.46* |
| + | ADP (10^{-4} M) | 6.34 ± 1.22* |

* Greater than GTP\(_\gamma\)S control, \(p < 0.05\), t test.
Phosphatidate has been thought to function as part of the “phosphoinositide cycle” in which inositol phospholipids are hydrolyzed to diacylglycerol, which is then phosphorylated by diacylglycerol kinase (1). The phosphatidate formed is converted to CDP-diacylglycerol and ultimately back to inositol lipid. The evidence for this scheme rests almost entirely on radioisotopic labeling experiments and in vitro enzyme activities. The results we report here do not support the phospholipase C-diacylglycerol kinase pathway as a major source of phosphatidate in hormone-stimulated rat hepatocytes. We find that increases in phosphatidate mass and changes in phosphatidate fatty acid composition precede similar changes in diacylglycerol (Figs. 2 and 4). Furthermore, the fatty acid composition of phosphatidate and diacylglycerol formed in response to vasopressin more closely resembles that of phosphatidylethanolamine than that of inositol lipids (Fig. 4 and text). Rat liver inositol lipids typically have a stearoyl-arachidonyl glycerol backbone with no other fatty acid comprising more than 5% of total fatty acids (32). Phosphatidylethanolamines, too, have a high proportion of stearate (25%) and arachidonate (25%) but also contain palmitate (22%) and linoleate (12%) (32). The de novo synthesis of phosphatidate and diacylglycerol from glycerol was not stimulated by vasopressin (Fig. 3). These findings raise the intriguing possibility that the Ca2+-mobilizing hormones increased phosphatidate levels through a phospholipase D mechanism.

We have performed experiments with rat liver plasma membranes that strongly suggest that a phospholipase D activity is indeed present and is regulated by a guanine nucleotide-binding protein. GTPγS, a nonhydrolyzable GTP analogue, stimulated the formation of phosphatidate by washed plasma membranes; this formation did not require ATP (Fig. 5). In addition, when membranes were incubated with [32P]ATP and with GTPγS, no incorporation of 32P into phosphatidate was observed (Table II). These experiments effectively excluded phosphorylation of diacylglycerol by diacylglycerol kinase in the formation of phosphatidate in isolated liver membranes.

Recently, Irving and Exton (31) reported an activity in rat liver plasma membranes that hydrolyzed phosphatidylethanolamine. Diacylglycerol, phosphocholine, and choline were formed in response to GTPγS, and the enzyme activity was characterized as a phospholipase C. The formation of phosphatidate under identical reaction conditions (reported here) suggests that phosphatidylethanolamine is also hydrolyzed by phospholipase D to form phosphatidate. Both activities do not require Ca2+ for GTPγS activation (in contrast to the hydrolysis of inositol lipids) (24), are stimulated by ATP and ADP when GTPγS is submaximal (Table III), and are resistant to both cholera and pertussis toxins (31). In addition both activities are stimulated by phorbol myristate acetate. When plasma membranes were incubated with GTPγS, phosphatidate increased
and phosphatidylcholine decreased to a similar extent (Table IV). When intact hepatocytes are labeled with [3H]choline, free choline is released in response to vasopressin.2 Experiments with exogenous substrates are underway to assess the substrate specificity of the phospholipase D activity.

All of the Ca2+-mobilizing hormones tested stimulated phosphatidate accumulation in intact hepatocytes (Table I). In contrast, only ATP and ADP were effective in isolated plasma membranes (Table III). We have no explanation, for the discrepancy at this time, but it should be noted that ATP and ADP are also superior to vasopressin as activators of hydrolysis of phosphatidylinositol 4,5-bisphosphate and phosphatidylcholine hydrolysis in liver plasma membranes (31).

The nature of the guanine nucleotide-binding protein(s) involved in activation of the phospholipase D activity is unknown. It appears that G, and G are not involved as cholera toxin, and pertussis toxin did not alter phosphatidate production. F, which as been used widely to activate guanine nucleotide-binding proteins, inhibited phospholipase D activity. Whether this is a result of an inhibitory coupling protein or direct inhibition of phospholipase D is uncertain. Since F produces Ca2+ mobilization and diacylglycerol accumulation (30) without phosphatidate accumulation, it may be a valuable probe into the function of hormone-elicited phosphatidate.

The relationship of this phospholipase D activity to that which has been used widely to activate guanine nucleotide-binding proteins is obscure. We have found that incubation of plasma membranes (31) without phosphatidate accumulation, it may be a valuable probe into the function of hormone-elicited phosphatidate.

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REFERENCES

1. Putney, J. W. (1982) Cell Calcium 3, 369–383
2. Takenawa, T., Homma, Y., and Nagai, Y. (1982) Biochem. Pharmacol. 31, 2663–2667
3. Thomas, A. P., Marks, J. S., Coll, K. E., and Williamson, J. R. (1983) J. Biol. Chem. 258, 5716–5725
4. Hokin-Neaverson, M., Sadeghian, K., Majumder, A. L., and Eibergen, P. F., unpublished observation.
5. P. F. Blackmore, unpublished observation.
6. P. F. Blackmore, S. B. Bocckino, and J. H. Exton, manuscript in preparation.