The translocation of protein kinase C (PKC) from the cytosolic to the particulate fraction in IIC9 fibroblasts has been studied to define the functions of 1,2-diacylglycerol (DAG) derived from the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) and phosphatidylcholine (PC). α-Thrombin caused a biphasic change in DAG, with two peaks at 15–60 s and 5–15 min, derived from PIP₂ and PC, respectively, while platelet-derived growth factor (PDGF) induced a monophasic DAG increase from PC at 5–15 min. α-Thrombin also induced a rapid, but transient, increase of inositol 1,4,5-trisphosphate and cytosolic Ca²⁺, whereas PDGF did not. Three PKC isozymes, α, ε, and ζ, were identified by Western blotting in IIC9 cells and were mainly localized in the cytosol. A fraction of cytosolic PKC α was rapidly translocated by α-thrombin at 15 s, but its membrane association was lost within 1 min. PKC ε was also rapidly translocated; however, its membrane association was sustained for almost 60 min. PKC ζ was not translocated by α-thrombin or phorbol 12-myristate 13-acetate. PDGF translocated PKC ε at 5 min but had little effect at 15 s and did not translocate PKC α or ζ. Incubation with Bacillus cereus PC- or phosphatidylinositol-specific phospholipase C, which increased DAG but not phosphatidic acid, stimulated translocation of PKC ε, but not PKC α or ζ. Addition of chelators to inhibit the rise in intracellular Ca²⁺ largely blocked PKC α translocation induced by α-thrombin but had no effect on PKC ε translocation. Addition of ionomycin allowed α-thrombin to induce PKC α translocation at 5 min. PKC α translocation was mimicked by 1,2-diacylglycerol plus ionomycin, but not by either alone. On the other hand, PKC ε was translocated by the DAG alone. These results support the conclusion that PIP₂ hydrolysis activates both PKC α and ε at 15 s, whereas PC hydrolysis activates only PKC ε at 5 min. The differential activation at 5 min can be attributed to the failure of PC hydrolysis to increase Ca²⁺ and not to a difference in the molecular species of DAG derived from the phospholipids.

Protein kinase C (PKC) has become the subject of intense investigation because of its role in transmembrane signaling by hormones and growth factors (1). Most isozymes of PKC are activated by 1,2-diacylglycerol (DAG) (1) and undergo translocation from the cytosol to the plasma membrane (1–6). In most agonist-stimulated cells, DAG is produced by the hydrolysis of two different phospholipids, phosphatidylinositol 4,5-bisphosphate (PIP₂) and phosphatidylcholine (PC), frequently resulting in a biphasic DAG increase (1, 7–13).

PKC isozymes are differentially expressed in various cells (2, 4–6, 14–16) and can be divided into groups on the basis of their biochemical and structural properties (1, 14, 17, 18). The classical PKC isozymes α, β₁, βⅡ, and γ are regulated by phosphatidylinerine, DAG, and Ca²⁺. The non-classical PKC isozymes δ, ζ, η, and θ are Ca²⁺-independent because they lack the Ca²⁺-binding C2 domain. Recently, Nakanishi and Exton (19) reported that PKC ζ was not activated by phorbol 12-myristate 13-acetate (PMA) or DAG. This confirmed observations with the expressed enzyme (20, 21).

The function of the second phase of DAG accumulation derived from PC is not known, although there have been some recent reports demonstrating differential functions of PC- and PIP₂-derived DAG in the activation of PKC (2, 22). In GH₃ cells, thyrotropin-releasing hormone (TRH) stimulated a multiphasic elevation in DAG, with a rapid increase at 15 s followed by a sustained increase for 10 min. But Western blotting with a monoclonal antibody raised against 80-kDa PKC showed a rapid but transient membrane association of the cytosolic PKC that was not sustained (22). Kiley et al. (2) also reported that the first phase of DAG caused translocation of PKC, but the second phase of DAG did not.

In IIC9 fibroblasts, α-thrombin stimulates the hydrolysis of PIP₂ and PC, resulting in a biphasic change of DAG (23–25). The first phase is accompanied by a rapid but transient increase of inositol 1,4,5-trisphosphate (IP₃) (23). In contrast to α-thrombin, PDGF induces a monophasic DAG increase derived mainly from PC hydrolysis, with a peak at 5 min (26). A similar monophasic DAG increase is also caused by epidermal growth factor (27). Thus, the IIC9 fibroblast represents a good system to study the functions of DAG from PIP₂ and PC. Recent findings indicate that a possible function of PIP₂-
derived DAG is to activate PKC α in this cell (3), but the function of PC-derived DAG is unknown.

In this report, we show that the second phase of DAG formation is associated with the translocation of PKC ε in IIC9 fibroblasts. Our data also indicate that Ca2+, but not the molecular species of DAG, is the important factor in the differential regulation of PKC α and ε isozymes in these cells.

**EXPERIMENTAL PROCEDURES**

**Materials**—F-12 nutrient (Ham’s) mixture, a-minimum essential medium, Dulbecco’s phosphate-buffered saline, Dulbecco’s modified Eagle’s medium, penicillin, streptomycin, HEPES, glutamine, and polynucleotides raised against synthetic peptides corresponding to specific sequences in PKC isoforms were purchased from Gibco BRL. Fetal bovine serum, human serum-free bovine serum albumin, bovine serum lipase, and rabbit anti-rabbit IgG were from Dyer (30). The bottom layer was dried under N2 gas and applied to a thin layer chromatography Silica Gel 60 A plate. The plate was developed using two sequential solvent systems and the residue was suspended in 50% ethanol. Choline and phosphocholine were measured by the method of Conricode et al. (32). Briefly, the water-soluble upper layer was dried in a Speed Vac concentrator, redissolved in 1 ml of H2O, and then applied to 1 ml of AG 1-X8 resin (200–400 mesh, formate form). Inositol phosphates were eluted from the column according to the procedures of Simpson et al. (31). Choline and phosphocholine were measured by the method of Conricode et al. (32).

**Cell Treatment and Subcellular Fractionation**—Serum-starved cultures were washed twice with serum-free medium, washed twice, and equilibrated as described above. Following stimulation of the cells with a-thrombin or PDGF, cells were quickly rinsed with ice-cold methanol, and then lysed were extracted by the method of Bligh and Dyer (30). The bottom layer was dried under N2 gas and applied to a thin layer chromatography Silica Gel 60 A plate. The plate was developed using two sequential solvent systems.

**Measurement of Water-soluble Inositol Phosphates, Choline, and Phosphocholine**—Cells were labeled with [3H]myo-inositol or [2-14C]choline chloride for 2 days in the inositol-free medium or the serum-free medium and then washed and equilibrated. Cells, activated with a-thrombin or PDGF, were scraped in 3 ml of free medium, washed twice, and equilibrated as described above. The supernatant (cytosolic protein) was applied to a Q-Sepharose column (4 or 6 ml) from each peak were collected and used for Western blotting and in vitro PKC assay. Most of PKC α and γ was recovered from the column, but the recovery of PKC ε was low. A mixture of cells was homogenized in 16 ml of Buffer B containing 20 mM Tris, pH 7.5, 10 mM EGTA, 2 mM EDTA, 250 mM sucrose, 1 mM DTT, 20 μg/ml leupeptin, 20 μg/ml aprotinin, 1 μM P-APMSF, and 0.2 mM magnesium acetate, 120 μM [γ-32P]ATP (approximately 100 cpm/mml), 40 μg/ml phosphatidylserine, 8 μg/ml diolein (or 1 μg/ml PMA), 1 mg/ml histone III-s (or 100 μg/ml myelin basic protein), and enzyme (10 μl). Following incubation at 30°C, the reaction was stopped by spotting 25 μl of the reaction mixture on phosphocellulose plates and incubated immediately in 0.5 M phosphoric acid. The papers were washed three times (each 3–5 min) with the phosphoric acid and then dried. 32P incorporation was quantitated using a scintillation counter.

**Purification of PKC**—PKC isoforms were partially purified from the cytosolic fraction of IIC9 fibroblasts. Two dishes (35 mm) of cells were homogenized in 16 ml of Buffer B containing 20 mM Tris, pH 7.5, 10 mM EGTA, 2 mM EDTA, 250 mM sucrose, 1 mM DTT, 20 μg/ml leupeptin, 20 μg/ml aprotinin, 1 μM P-APMSF, and 0.2 mM magnesium acetate, 120 μM [γ-32P]ATP (approximately 100 cpm/mml), 40 μg/ml phosphatidylserine, 8 μg/ml diolein (or 1 μg/ml PMA), 1 mg/ml histone III-s (or 100 μg/ml myelin basic protein), and enzyme (10 μl). Following incubation at 30°C, the reaction was stopped by spotting 25 μl of the reaction mixture on phosphocellulose plates and incubated immediately in 0.5 M phosphoric acid. The papers were washed three times (each 3–5 min) with the phosphoric acid and then dried. 32P incorporation was quantitated using a scintillation counter.

**Cell Treatment and Subcellular Fractionation**—Serum-starved cultures were washed twice with serum-free medium, equilibrated in the same medium at 37°C for 2 days.

**Differential Translocation of Protein Kinase C**—Cells were homogenized in 16 ml of Buffer B containing 20 mM Tris, pH 7.5, 10 mM EGTA, 2 mM EDTA, 250 mM sucrose, 1 mM DTT, 20 μg/ml leupeptin, 20 μg/ml aprotinin, 1 μM P-APMSF, and 0.2 mM magnesium acetate, 120 μM [γ-32P]ATP (approximately 100 cpm/mml), 40 μg/ml phosphatidylserine, 8 μg/ml diolein (or 1 μg/ml PMA), 1 mg/ml histone III-s (or 100 μg/ml myelin basic protein), and enzyme (10 μl). Following incubation at 30°C, the reaction was stopped by spotting 25 μl of the reaction mixture on phosphocellulose plates and incubated immediately in 0.5 M phosphoric acid. The papers were washed three times (each 3–5 min) with the phosphoric acid and then dried. 32P incorporation was quantitated using a scintillation counter.
mobion-P membranes using a semi-dry transfer apparatus (Bio-Rad) for 18 min at 11 V. The immunoblots were incubated with a blocking solution containing 1 g/100 ml bovine serum albumin and 1 ml/100 ml goat serum in 100 mM Tris, pH 7.5, 0.1 g/100 ml Tween 20, and 0.9 g/100 ml NaCl for 1 h, and then incubated overnight with diluted specific anti-PKC antisera (1 μg/ml). In some cases, PKC isozyme-specific peptides (0.5 μg/ml) were added to the primary antibody solution to block the specific isozyme bands. Following incubation with biotinylated anti-rabbit IgG (5 μg/ml) in the blocking solution without albumin or serum for 30 min, the blots were developed using the Vectastain alkaline phosphatase ABC kit.

RESULTS

α-Thrombin induced a biphasic change in DAG accumulation, with peaks at 15–60 s and 5–15 min, in IIC9 fibroblasts (Fig. 1) in agreement with other findings (23–25). PDGF stimulated a monophasic DAG response, corresponding to the second phase induced by α-thrombin (Fig. 1). As reported already (23–25), the first phase of DAG formation correlated with a sharp increase in IP₃ formation that peaked at 15 s (data not shown), indicating its origin, in part at least, from PIP₂ hydrolysis. The monophasic DAG increase with PDGF and the second phase of DAG increase with α-thrombin correlated with the formation of PA and choline, but not IP₃ (data not shown), indicating their origin from PC via PLD plus PA phosphohydrolase action. Cytosolic Ca²⁺ was also measured to demonstrate that the increase in IP₃ induced by α-thrombin resulted in Ca²⁺ mobilization. As shown in Fig. 2, α-thrombin caused a very rapid increase in cytosolic Ca²⁺, but this returned to control levels within 1–2 min. PDGF did not alter cytosolic Ca²⁺ whether added before or after α-thrombin (Fig. 2).

The distribution of PKC isozymes in IIC9 fibroblasts was determined as the first step to study PKC translocation. Three PKC isozymes (α, ε, and ζ) were identified by Western blotting with polyclonal antibodies raised against isozyme-specific peptides (Fig. 3). Each PKC isozyme band was specifically blocked by the incubation with the specific peptide against which the antibody was raised (Fig. 3). PKC α was also detected with anti-PKC ζ, but PKC ζ was not detected with anti-PKC α (Figs. 3–5). PKC α, ε, and ζ were partially purified from the cytosolic fraction by chromatography on a Q-Sepharose column to confirm the nature of the PKC isozymes identified by Western blotting (Fig. 3). All three PKC isozymes were mainly localized in the cytosolic fraction of unstimulated cells (Fig. 4).

IIC9 cells treated with α-thrombin and PDGF induced differential translocation of PKC isozymes (Figs. 5 and 6). In cells treated with α-thrombin, a fraction of PKC α was translocated to the particulate fraction 15 s after stimulation, but the membrane association of the isozyme was lost within 1 min (Fig. 5). In contrast, membrane association of PKC ε was evident at 15 s, sustained until 5 min, and then gradually lost over 60 min (Fig. 5). PKC ζ was not translocated by α-thrombin at any time (Fig. 5). Although PMA caused a marked translocation of PKC α and ε, it was without effect on PKC ζ (Fig. 5). This latter result reflects previous reports.

Fig. 1. Time course of DAG changes induced by α-thrombin (A) and PDGF (B). Cells were cultured for 2 days, labeled with 10 μCi/dish [3H]myristic acid for 2 days in serum-free medium, and then incubated with 500 ng/ml α-thrombin (approximately 3000 units/mg of protein) or 50 ng/ml PDGF. At the indicated times, the incubations were terminated, lipids were extracted, and the amount of DAG was measured as described under “Experimental Procedures.” Results are means of two separate experiments.
Differential Translocation of Protein Kinase C

FIG. 2. Changes of cytosolic Ca²⁺ with α-thrombin and PDGF. Cells were grown on a microscope coverglass, serum-starved, and incubated with fura-2/AM. Incubations and cytosolic Ca²⁺ measurements were carried out as described under "Experimental Procedures."

FIG. 3. Identification of PKC isozymes α, ε, and ζ. Whole cell protein (4.5 μg) was subjected to 10% SDS-PAGE, and PKC isozymes were identified by Western blotting using polyclonal antibodies raised against PKC isozyme-specific peptides (Ab) as described under "Experimental Procedures." Each PKC isozyme band was specifically blocked by incubation with the isozyme-specific peptide (Ab + Pep). PKC α, ε, and ζ from the cytosolic fraction of IIC9 fibroblasts were partially purified using Q-Sepharose (Q) as described under "Experimental Procedures" and were included to confirm the identity of the isozyme bands.

FIG. 4. Subcellular distribution of PKC isozymes. Untreated cells were fractionated into cytosolic (C) and the particulate (M) fractions as described under "Experimental Procedures." Total protein in the cytosolic fraction was twice that in the particulate fraction. Equal amounts of protein (4.5 μg) were subjected to 10% SDS-PAGE and analyzed by Western blotting using polyclonal antibodies raised against PKC α, ε, and ζ.

FIG. 5. Translocation of PKC isozymes from the cytosolic to the particulate fraction. Cells were cultured for 2 days, incubated in serum-free medium for 2 days, and activated with 500 ng/ml α-thrombin for the indicated times or with 200 nM PMA for 15 min (T). Cytosolic (C) and particulate (M) fractions were prepared, and 4.5 μg of protein was analyzed by Western blotting.

FIG. 6. Translocation of PKC ε induced by PDGF. a, cells, serum-starved for 2 days, were activated with various concentrations of PDGF for 5 min. Cytosolic (C) and particulate (M) fractions were analyzed by Western blotting. b, serum-starved cells were incubated with 50 ng/ml PDGF for the indicated times and fractionated.

FIG. 7. Translocation of PKC isozymes induced by B. cereus PC-specific PLC. Serum-starved cells were incubated with 500 ng/ml α-thrombin for 15 s (α) or with 20 units/ml B. cereus PC-specific PLC for 15 s and 5 min (PLC). Cytosolic (C) and particulate (M) fractions were analyzed by Western blotting.

(19-21) that PKC ζ is not activated by DAG or PMA.

PDGF translocated only PKC ε from the cytosolic to the particulate fraction, but not PKC α or ζ. PDGF caused a maximal translocation of PKC ε at 5 min and was maximally effective at 100 ng/ml (see Fig. 6a for PKC ε, but data not shown for PKC α and ζ). The translocation of PKC ε by PDGF at a half-maximal dose (50 ng/ml) was negligible at 15 s and maximal at 5 min. However, the membrane association slowly declined over 60 min (Fig. 6b). To support the idea that DAG from PC hydrolysis induced by α-thrombin and PDGF activates PKC ε translocation, the effects of B. cereus PC-specific PLC were studied. This PLC at 20 units/ml rapidly translocated PKC ε but did not affect PKC α or ζ (Fig. 7, but data not shown for PKC ζ). The PC-PLC specifically hydrolyzes PC without any detectable hydrolysis of other phospholipids (34). B. cereus PC-PLC also increased DAG to 260% of control at 2 min, and the elevated DAG was

PKC α

PKC ε

PKC ζ

PKC α

PKC ε

PKC ζ
maintained with little decrease until 15 min. However, there was no significant change in PA during this time (data not shown). The translocation of PKC ε by B. cereus PC-PLC was rapid and reached a plateau at 1 min, when DAG increased to 190% of control (data not shown). B. cereus PI-PLC was also applied to IIC9 fibroblasts to compare its effects with those of B. cereus PC-PLC. The PI-PLC also translocated PKC ε but not PKC α. This indicates that DAG derived from PI can also cause translocation of PKCε.

To test the hypothesis that the translocation of PKC α requires Ca++, but that of PKC ε does not, the cells were treated with EGTA and BAPTA/AM to deplete intracellular Ca++. Incubation of the cells with 1 mM EGTA (free concentration in medium) or 25 μM BAPTA/AM for 30 min largely blocked the translocation of PKC α induced by α-thrombin at 15 s but did not affect that of PKC ε (Fig. 8). Cells were also treated with ionomycin and α-thrombin to test the possibility that PC-derived DAG could activate PKC α translocation if Ca++ is supplied. PKC α translocation was induced by incubation with ionomycin plus α-thrombin for 5 min (data not shown), i.e. at a time when α-thrombin alone did not translocate PKC α (Fig. 5). These results confirm that Ca++ is necessary for PKC α translocation and that DAG derived from the two phospholipids can activate PKC α translocation provided Ca++ is elevated.

Because of the preceding results, DOG and ionomycin were applied to the IIC9 cells to confirm the differential translocation of the PKC isozymes. Incubation with DOG for 5 min translocated PKC ε, and the effect was maximal with 100 μg/ml (Fig. 9). This concentration of DOG had a minimal effect on the translocation of PKC α. Ionomycin alone had no effect on PKC α but partly translocated PKC ε as revealed by its continued presence in the cytosolic fraction. The PKC α translocation induced by α-thrombin was fully mimicked by a combination of DOG with ionomycin (Fig. 9).

**DISCUSSION**

Three PKC isozymes (α, ε, and γ) were identified with polyclonal antibodies raised against PKC isozyme-specific peptides in IIC9 fibroblasts. The finding of two more isozymes, PKC ε and γ, extends the previous report of PKC α in these cells (3) and agrees with a very recent study (35). There are several reports on the differential expression of PKC isozymes in various cells (1, 2, 4–6, 14, 16). These observations and the evidence of major differences in the regulation of the different isozymes (1, 14, 17) imply that they have different functions.

Our findings confirm that α-thrombin induces a biphasic change of DAG due to PIP2 and PC breakdown in IIC9 fibroblasts (23, 24), whereas PDGF stimulates a monophasic increase of DAG from PC (26), similar to the second phase of DAG increase induced by α-thrombin. The rapid translocation of PKC α from the cytosolic to the particulate fraction at 15 s, when α-thrombin induces the first phase of DAG increase from PIP2 hydrolysis (Figs. 1 and 5), is also consistent with a previous report (3). However, the present study shows that there is also a rapid translocation of PKC ε, and whereas the membrane association of PKC α is over within 1 min, that of PKC ε is maintained during the second phase of DAG increase (Figs. 1 and 5). Very recently, Leach et al. (35) in a study of the nuclear translocation of PKC isozymes also reported that α-thrombin induced an increase in the PKC ε content of non-nuclear membranes at 1 min in IIC9 cells. Kiley et al. (2) have reported, using digitonin-permeabilized GH3 cells, that TRH rapidly translocated PKC α and ε at 15 s, corresponding to the first phase of DAG increase. They also stated that the second phase DAG did not activate the translocation of any PKC isozymes, whereas the third phase of DAG accumulation down-regulated PKC ε. However, inspection of their data indicates a small translocation and increased autophosphorylation of PKC ε at 10–15 min (see Figs. 3–5 in Ref. 2).

In contrast to α-thrombin, PDGF translocated only PKC ε. Membrane association was minimal at 15 s and maximal at 5 min (Fig. 6), reflecting the slow increase in DAG from PC induced by PDGF. Activation of the translocation of PKC ε, but not PKC α, due to the hydrolysis of PC was also supported by the findings with B. cereus PC-specific PLC (Fig. 7). However, since the time course of PKC ε translocation induced by α-thrombin showed a large effect at 15 s (Fig. 5) and because B. cereus PI-specific PLC caused PKC ε translocation, it seems that the isozyme can also be activated by PIP2 and PI hydrolysis.

We entertain the possibility that the different molecular species of DAG derived from PIP2 and PC may activate different PKC isozymes because of their different fatty acid compositions (24, 26, 36–38). As noted above, it initially seemed that PKC ε could be activated by DAG from both PIP2 and PC, whereas PKC α was only activated by PIP2-derived DAG since it was only transiently translocated by α-thrombin (Fig. 5), and not at all by PDGF. However, when PKC α was partially purified from IIC9 cells and conventional PKC was purified from brain and both preparations were tested with two different species of DAG, one containing stearic and arachidonic acids (corresponding to PIP2) and the other containing palmitic and oleic acids (corresponding to
PC), no significant differences between the effects of the two DAG species were observed.2

The possibility that Ca2+, rather than the molecular species of DAG, was involved in the differential activation of PKC α and ε was therefore tested and was supported by the present findings. The first phase of DAG formation induced by α-thrombin is accompanied by an increase in IP3 and cytosolic Ca2+, whereas the second phase, when PKC α is not translocated, is not (Fig. 2). A role for Ca2+ in the translocation of conventional PKC was indicated by early studies of the binding of the kinase to plasma membranes (39, 40). There have also been reports that Ca2+ influx induced by depolarization or ionophore leads to rapid translocation of conventional protein kinase C (41, 42). In addition, in human neutrophils and rat fibroblasts, the presence of Ca2+ during the disruption of unstimulated cells resulted in an increase of conventional PKC activity and/or immunoreactivity in the particulate fraction (43, 44).

Martin et al. (22) reported that inhibition of the TRH-stimulated Ca2+ increase in GH2 cells resulted in blockade of the hormone-induced PKC translocation of conventional PKC and concluded that elevations in both DAG and Ca2+ were required. Trilivas et al. (44) also found that EGTA inhibited the translocation of PKC α induced by carbachol in 1321N1 cells. However, the chelator did not affect the PKC-mediated phosphorylation of an 80-kDa endogenous protein or an exogenous peptide (45), suggesting that these were phosphorylated by a Ca2+-independent PKC isoform(s).

Our results are consistent with the preceding reports, as follows. Depletion of intracellular free Ca2+ by EGTA, which chelates extracellular Ca2+ and secondarily reduces cytosolic Ca2+, or BAPTA/AM, which enters cells and is then converted to BAPTA, which directly chelates cytosolic Ca2+, largely blocked α-thrombin-induced translocation of PKC α but not of PKC ε (Fig. 8). A role for Ca2+ in PKC α translocation was further shown by the fact that addition of the Ca2+-ionophore ionomycin caused the isoform to be translocated in cells treated with α-thrombin for 5 min. In addition, PKC α translocation was mimicked by the combination of DOG with ionomycin, but not by either agent alone.2 In contrast, PKC ε was translocated by DOG in the absence of ionomycin, as expected, since it lacks the C2 Ca2+-binding domain (1, 17, 18). These findings indicate that Ca2+, but not DAG, is the important factor in the differential activation of PKC isoforms.

The differential translocation of PKC isoforms with respect to different agonists and time of exposure suggests different functions of PKC isoforms in the IIC9 fibroblast. It has been speculated previously that the PKC isoforms may have different functions that can partly explain the diverse responses of cells to various agonists (1, 14). There have been numerous suggestions of possible PKC functions in cells, but there is no information on the cellular functions of specific PKC isoforms. Studies of the differential translocation of PKC isoforms to the plasma membrane, nucleus, and perhaps other intracellular organelles could provide an approach to this intriguing problem.

Acknowledgments—We thank Kim Brewer for expert technical help and Kevin Conricide for helpful discussions. We also thank Stefan Strack for help with Ca2+ measurements and Judy Childs for typing the manuscript.

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3. As seen in Fig. 9, ionomycin alone caused the translocation of PKC α, although the effect was not as large as that seen with α-thrombin. This result was not expected since PKC α lacks a Ca2+-binding domain (1, 14, 17), and its translocation is unaffected by EGTA or BAPTA/AM (Fig. 8). The effect is probably due to the small increase in DAG that is induced by the ionophore, as also observed with 243187 on hepatocytes (46). Based on the findings with DOG (Fig. 9) such an increase would have a much greater effect on PKC ε than PKC α.