Interleukin-1 and Endothelin Stimulate Distinct Species of Diglycerides That Differentially Regulate Protein Kinase C in Mesangial Cells*

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Diglycerides are phospholipid-derived second messengers that serve as cofactors for protein kinase C activation. We have previously shown that, in rat glomerular mesangial cells, the cytokine, interleukin-1α, and the vasoactive peptide, endothelin, generate diglycerides from unique phospholipid precursors. However, neither the molecular species of these diglycerides nor their biological actions were determined. It is now hypothesized that interleukin-1α and endothelin-treated mesangial cells form distinct molecular species of diglycerides which may serve different roles as intracellular signaling molecules. Diglyceride molecular species were resolved and quantified by TLC and high performance liquid chromatography as diglyceride-[14C]acetate derivatives. Endothelin stimulates predominantly ester-linked species (diacylglycerols) in contrast to interleukin-1α which stimulates only ether-linked species (alkyl, acyl- and alkenyl, acylglycerols). In support of these data, interleukin-1α-treated mesangial cells hydrolyze ethanolamine plasmanogens, vinyl ether-linked phospholipids. It has been reported that ether-linked, in contrast to ester-linked, diglyceride molecular species do not activate protein kinase C activity. Thus, we next assessed membrane protein kinase C activity in endothelin- or interleukin-1α-treated mesangial cells. Even though interleukin-1α has no effect upon basal protein kinase C activity, this cytokine, through the formation of ether-linked diglyceride second messengers, inhibits endothelin, platelet-activating factor, or arginine vasopressin-stimulated protein kinase C activity. We further demonstrate that ester-linked diacylglycerols but not alkyl, acyl- or alkenyl, acylglycerols substitute for phorbol esters in a cell-free protein kinase C assay. In addition, alkenylacylglycerols inhibit diacylglycerol-stimulated immunoprecipitated protein kinase C α activity in vitro and total protein kinase C activity in permeabilized mesangial cells ex vivo. Taken together, these data suggest that interleukin-1α-induced formation of ether-linked diglycerides may physiologically serve to down-regulate receptor-mediated protein kinase C activity and that individual molecular species of diglycerides may serve different roles as intracellular signaling molecules.

Classical transmembrane signaling theory suggests that receptors are linked to a phosphatidylinositol 4,5-bisphosphate (PtdIns)(3,4,5)P3-specific phospholipase C generating inositol phosphates and diglycerides (DG). However, distinct phospholipase C and phospholipase D activities that hydrolyze ester- and ether-linked phosphatidylinositol (PtdCho) and phosphatidylethanolamine (PtdEth) may also release unique molecular species of DG and phosphatidic acids. We have previously compared and contrasted the early phospholipid-derived second messengers generated by the vasoconstrictor, endothelin-1 (ET), and the cytokine, interleukin-1α (IL-1) in rat glomerular mesangial cells (MC). ET maintains the myogenic phenotype of these smooth muscle pericytes inducing MC contraction which leads to a decreased glomerular filtration rate through a diminished ultrafiltration coefficient (1). IL-1 induces a phenotypic change in MC, switching these cells from a contractile to a secretory, immune-responsive, state with concomitant changes in matrix formation and adhesion protein expression, events associated with sclerosis (2). ET generates DG initially from PtdIns and secondarily from PtdCho in MC (3). Furthermore, ET-stimulated Ins-1,4,5-P3 responses are transient, while elevated DG responses are sustained (4–6). In contrast, IL-1 generates DG from PtdEth hydrolysis totally independent of any measurable polyphosphoinositide turnover or elevations in intracellular free calcium concentration ([Ca2+]i) in MC (7). Similar results demonstrating IL-1-induced DG derived from PtdCho hydrolysis (8, 9) or from an augmented sequential lysophosphatidic acid acyltransferase/phosphatidic acid phosphohydrolase activity (10) without concomitant Ins-1,4,5-P3 generation have also been reported. This alternative signal transduction pathway which suggests that DG can be generated solely from sources besides the polyphosphoinositides has been demonstrated for diverse growth factors and cytokines including fibroblast growth factor (11, 12), insulin (13), EGF (14), growth hormone (15), interferon α (16), D59Fyr (17), Hara (18), IL-3 (19), and IL-1β (20, 21). Agonists that stimulate hydrolysis of phospholipids besides PtdIns may (interferon, IL-3 (16, 19)) or may not (IL-1, fibroblast growth factor (11, 20, 21, 22)) stimulate PKC. IL-1, in all cell types studied, consistently does not induce PKC translocation or bioactivity. The role of the unique DG species generated by these agonists is still controversial as PtdCho-derived DG has been shown to be

1 The abbreviations used are: PtdIns, phosphatidylinositol; PtdCho, phosphatidylcholine; PtdEth, phosphatidylethanolamine; PtdIns(3,4,5)P3, phosphatidylinositol 4,5-bisphosphate; PtdSer, phosphatidylserine; ET, endothelin-1; IL-1, interleukin-1α; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; R, migration relative to solvent front (variability in the center of the spot); DG, diglyceride(s); diacylglycerol, 1,2-diacyl-sn-glycerol; alkyl, acylglycerol, 1-0-alkyl-2-acyl-sn-glycerol; alkynyl, acylglycerol, 1-0-alkyl-1-enyl-2-acyl-sn-glycerol; [Ca2+]i, cytosolic free calcium concentration; Ins 1,4,5-P3, inositol 1,4,5-trisphosphate; MC, rat glomerular mesangial cell(s); EGF, epidermal growth factor; PKC, protein kinase C; HPLC, high performance liquid chromatography; GTPγS, guanosine 5'-3-O-(thiotriphosphate); ANOVA, analysis of variance.

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an effective (23) or ineffective activator of PKC (24, 25). Agonists, such as IL-1, that generate DG independent of any polyphosphoinositide turnover have never been characterized in terms of their ester- and ether-linked sn-1 configuration. We hypothesize that ET and IL-1 generate discrete molecular species of DG that differentially regulate PKC.

As DG can be generated through distinct biochemical pathways and from different phospholipid precursors, it is very surprising that little information is currently available correlating agonist-induced DG structure with physiological function. Significant ether-linked DG species have been observed in PMA- and fMLP (formylmethionylleucylphenylalanine)-stimulated neutrophils (26) while only diacyl species have been observed with thrombin-stimulated 11 Cg cells (27) and IgG-stimulated mast cells (28). The characterization of molecular species of DG from discrete phospholipid pools is especially important as ether-linked DG (alkyl, acyl- or alkenyl, acyl-DG), in contrast to diacyl species, may inhibit PKC, not important as ether-linked DG (alkyl, acyl- or alkenyl, acyl-DG) stimulated mast cells (28). The characterization of molecular species of DG that differentially regulate PKC. To characterize the molecular species of DG generated by these agonists as either diacyl-, alkyl, acyl-, or alkenyl, acyl-glycerol species. Our second aim was to correlate the formation of these individual molecular species of DG with PKC activation utilizing both cell-free and intact-cell methods.

MATERIALS AND METHODS

ET-1 was obtained from the Peptide Institute (Osaka, Japan) while IL-1 was purchased from U.B.I. (New York). Radiolabeled materials including [3H]glycerol and [14C]acetate were obtained from Amersham. DG and phospholipid standards were obtained from Deva Biologicals (Penn) or Serdy Biochemicals (London, Ontario, Canada). Polyclonal anti-PKC α antibody was obtained from Santa Cruz Biotechnology. All other materials were purchased from either Sigma or Calbiochem.

Gliomerular Mesangial Cell Isolation and Culture—MC were grown from glomeruli obtained from 100-g male Sprague-Dawley rats by a sequential sieving technique (34). MC were grown in RPMI 1640 culture medium supplemented with 8.5% fetal bovine serum, 100 μg/ml streptomycin, 5 μg/ml aprotinin, 10 μg/ml leupeptin, and 20 μg/ml aprotinin for 5 min was accomplished as described (38). Efficiency of permeabilization was determined using [32P]JTP-5 and [3H]glycerol-labeled DG. This procedure did not elevate basal PKC activity or phospholipase C or D activities (38). As should be expected, DG species did not affect membrane protein kinase C activity in non-permeabilized cells.

In Vitro Reconstitution Assay for Immunoprecipitated PKC α—As adapted from previous studies (39, 40), quiescent MC in 100-mm plates were washed twice with ice-cold phosphate-buffered saline supplemented with 1 mM vanadate and lysed on ice with 500 μl of a buffer containing 25 mM HEPEs, pH 7.5, 100 mM NaCl, 20 mM β-glycerophosphate, 5 mM MgCl2, 1 mM vanadate, 0.5 mM EGTA, 0.25 mM EDTA, 0.02% Nonident P-40, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 10 μg/ml aprotinin. 150 μg of cell lysate proteins were precleared with preimmune rabbit sera for 2 h at 4 °C. PKC α was immunoprecipitated from cleared lysates using 0.5 μg of polyclonal anti-PKC α (Santa Cruz Biotechnology). The formed immunocomplexes were subsequently collected with goat anti-rabbit IgG-agarose complex was washed twice and resuspended in a kinase reaction buffer containing 50 mM HEPEs, pH 7.5, 25 mM β-glycerophosphate, 75 mM KCl, 1 mM vanadate, 10 mM MgCl2, and 0.1 mM CaCl2. Phosphorylation assays were performed in 50 μl of kinase buffer in the presence of 1 μCi of [γ-32P]ATP (10 Ci/mmol) per reaction and 20 μM unlabeled ATP, for 20 min at 37 °C. The reaction mixture (50 μl) also contained 10 μg of phosphatase reaction was complete, utilizing 10 μg of histone/reaction and selected alkyl, acyl- or diacylglycerol. The reactions were terminated with the additive...
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RESULTS

Using [14C]acetic anhydride, we have assessed diacyl-, alkyl-, acyl- and alkenylacylglycerol species from vehicle- and ET- or IL-1-treated MC (Fig. 1). Previous experiments using [3H]arachidonate (3, 7, 34) established 5 min as an optimal point to assess DG. These results, expressed as a mass measurement, indicated that ET generates predominately ester-linked species (diacylglycerol) while IL-1 generates only ether-linked (alkyl, acyl- and alkenylacylglycerol) species. ET stimulate a 2.8-fold increase in diester-linked species in contrast to a nonsignificant 1.6-fold increase for ether-linked species. IL-1 treatment significantly increased alkylacylglycerol formation 2.2-fold and alkenylacylglycerol formation 2.8-fold. In data not shown, IL-1 concentrations as high as 100 ng/ml did not stimulate membrane PKC activity. Moreover, in data not shown, in a complementatory manner, ET reduced cytosolic PKC activity. At 5 min, membrane PKC activity was 32, 29, and 71% of total PKC activity for control, IL-1-, and ET-stimulated MC, respectively, suggesting that ET but not IL-1 translocates PKC activity to the membrane. These studies which demonstrate that IL-1 does not stimulate PKC activity or translocation as assessed by phosphorylation of an EGF-receptor binding domain have been confirmed using a PKC pseudosubstrate (Life Technologies, Inc.)

Expressed as ether/ester-DG percentages, control values of 54% were increased to 120% with IL-1 and reduced to 34% with ET. IL-1 restored this diminished ET-induced ether/ester-DG percentage to control levels of 56%. We next analyzed IL-1-stimulated (10 ng/ml) DG species at several time points that correspond to previously published data assessing IL-1-induced [3H]DG formation (7). As depicted in Fig. 2, IL-1 induced alkyl-, acyl- and alkenylacylglycerol species at 1, 5, and 15 min. IL-1-stimulated ether-linked DG returned to baseline values by 30 min, confirming the earlier radioactivity flux measurements (7).

We have utilized a spectrophotometric assay that ascertains the plasmalogen content (a vinyl ether-linked phospholipid) of TLC-separated phospholipid species, to investigate if the elevation in ether-linked DG generation (7) correlates with a decrease in choline-containing phospholipid species. As it has been reported that ether-linked diglyceride species, in contrast to diacylglycerol species, do not activate PKC activity, we treated MC with either IL-1 which generates only ether-linked species or ET which generates predominantly diacyl species and assessed PKC activity (Fig. 4). Using this Triton X-100 mixed micelle EGF-receptor binding domain phosphorylation assay system (Amersham) and a partially purified MC membrane preparation, we have previously demonstrated that PMA activates PKC activity in the presence but not the absence of exogenous PtdSer, calcium, or membrane preparation. ET (100 nm) stimulated membrane PKC activity compared to vehicle- or IL-1 (3 ng/ml)-treated MC. The doses selected elicited maximal DG stimulation in MC. In data not shown, IL-1 concentrations as high as 100 ng/ml did not stimulate membrane PKC activity. Moreover, in data not shown, a complementatory manner, ET reduced cytosolic PKC activity. At 5 min, membrane PKC activity was 32, 29, and 71% of total PKC activity for control, IL-1-, and ET-stimulated MC, respectively, suggesting that ET but not IL-1 translocates PKC activity to the membrane. These studies which demonstrate that IL-1 does not stimulate PKC activity or translocation as assessed by phosphorylation of an EGF-receptor binding domain have been confirmed using a PKC pseudosubstrate (Life Technologies, Inc.).
We have shown previously that IL-1 does not stimulate inositol trisphosphate production or induce elevations in intracellular free calcium concentrations (7). Thus, we used ionomycin (a calcium ionophore) to elevate intracellular calcium concentration to levels observed with 100 nM ET stimulation and then assessed the effects of IL-1 upon membrane PKC activity (Fig. 4). Ionomycin induced a slight increase in PKC activity that was not potentiated with IL-1 suggesting that IL-1 does not activate PKC in the presence or absence of elevation of intracellular calcium. To test if IL-1-generated ether-linked DG have an inhibitory effect upon receptor-activated PKC activity, we pretreated MC with IL-1 or vehicle for 5 min and then assessed ET-stimulated PKC activity for an additional 15 min (Fig. 4). IL-1 had no effect on basal PKC activity but did reduce ET-stimulated PKC activity, suggesting a physiological inhibitory role for IL-1-generated ether-linked DG. In data not shown, this inhibitory effect of IL-1 upon ET-stimulated PKC activity was evident even without preincubation. In addition, IL-1 also inhibited platelet-activating factor- and arginine vasopressin-stimulated PKC activity. ET (100 nM) stimulated membrane PKC activity in a time-dependent manner (Fig. 5), and we chose either a 5-min or 15-min ET stimulation for all subsequent experiments, as these time points correspond to maximal ET-stimulated DG formation and PKC activation. At all time points studied, IL-1 (3 ng/ml) did not stimulate PKC activity above control values.

To test whether ET-induced diacylglycerols but not IL-1-generated ether-linked diglycerols stimulate PKC, we next substituted diacyl-, alkyl, acyl-, and alkenyl, acylglycerol species for PMA in the cell-free PKC assay system (Fig. 6). Substituting diacylglycerol but not alkyl, acyl- and alkenyl, acylglycerols for PMA in the PKC assay elevated basal membrane PKC activity. PtdSer stimulated PKC activity in the absence of PMA and served as baseline control. The mol % of PtdSer and DG have previously been shown to be optimal for this assay (34). To confirm and extend these cell-free studies that assess total PKC activity, we next specifically evaluated immunoprecipitated PKC activity in an in vitro kinase assay (Fig. 7). MC express PKC which is acutely activated by G-protein-linked receptors (43). A 4-min preincubation with 10 nM alkenyl, acylglycerol (AAG) diminished 100 nM diacylglycerol (DAG)-stimulated, immunoprecipitated, PKC activity as assessed by histone phosphorylation. Actual counts/min/lane values for the excised histone bands were, respectively, 3507, 200, and 173 for diacylglycerol-, alkenyl, acylglycerol-, and alkenyl, acylglycerol/ diacylglycerol-treated immunoprecipitates. In addition, alkenyl, acylglycerol inhibited diacylglycerol-induced autophosphorylation of PKC. These data suggest that IL-1- and ET-receptors increase the content of distinct species of DG that differentially activate PKC in MC.

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2 A. Musial, A. Mandal, E. Coroneos, and M. Kester, unpublished data.
To definitively assess the effects of DG molecular species upon PKC activity, we incubated permeabilized MC monolayers for 10 min with 100 nM diacyl-, alkyl, acyl-, or alkenyl, acylglycerol species or vehicle and then assessed membrane PKC activity (Fig. 8). These intact cell experiments are the in vivo equivalents of the cell-free experiments that substitute DG species for PMA in the assay itself. Using the permeabilized cells, diacylglycerol but not ether-linked glyceride species stimulated PKC activity. Ether-linked DG concentrations as high as 1 μM did not stimulate PKC activity (data not shown). Also, in data not shown, maximal concentration of ET (100 nM) and diacylglycerols (100 nM) did not have a synergistic effect upon PKC activity, suggesting activation through a common pathway.

We next tested if alkyl,acylglycerol could inhibit diacylglycerol-induced PKC activation in this intact cell model. Alkyl,acylglycerol species (1 nM through 1 μM) were added 4 min before diacylglycerol species (100 nM) and then membrane PKC was assessed after 10 min. (Fig. 9). Similar results were obtained if the alkyl,acylglycerol species were added simultaneously with the diacylglycerol species (data not shown). Alkyl,acylglycerol inhibited diacylglycerol-induced PKC activity in a dose-dependent manner. Specifically, 1 nM alkyl,acylglycerol reduced 62% of membrane PKC activity induced by 0.1 μM diacylglycerol. These data suggest, but do not prove, that ether-linked DG species compete for the diacylglycerol cofactor site on PKC without activating the enzyme.

**DISCUSSION**

Vasoconstrictors, cytokines, and growth factors stimulate the formation of DG second messengers, yet each agonist leads to separate physiological end points. To further define the biological actions of ET-1 (vasoconstriction and proliferation) and IL-1 (inducibility of genes associated with matrix formation and synthesis of other proinflammatory mediators), we investigated the early signal transduction pathways for these agonists with particular emphasis on the distinct molecular
species of DG generated and their potential regulation of PKC activity. The biochemical information contained in the structure of these unique DG species may determine, in part, the phenotypic changes induced by each of these agonist groups. Solely on the basis of the sn-1 bond linkage of the DG, we have demonstrated that ET and IL-1 generate distinct DG species that differentially regulate PKC. IL-1-treated MC generated only ether-linked DG species in contrast to ET-1-stimulated MC which formed predominantly ester-linked (diacyl) DG species. Furthermore, ET but not IL-1 stimulated basal membrane PKC activity, while IL-1 decreased receptor-stimulated PKC activity. Our results further suggest that it is the elevation of vinyl ether-linked DG species and not a corresponding decrease in diacylglycerols that leads to IL-1 receptor-linked reduction of ET-activated PKC. The action of IL-1-induced ether-linked DG to inhibit ET-stimulated ester-linked DG was shown with both immunoprecipitated PKC α in a cell-free system and with total PKC in a permeabilized cell protocol. Thus, the physiological role of IL-1-induced formation of ether-linked DG may be to down-regulate receptor-mediated PKC activation.

We demonstrate that IL-1 inhibits receptor-stimulated PKC activity by generating ether-linked DG which have been suggested to competitively bind to PKC without activating PKC (44). Alternatively, IL-1 pretreatment of ET-stimulated MC maintains ether/ester-DG percentages at levels that do not favor PKC activation. It has been suggested that the carboxyl groups of the ester bond at the sn-1-position as well as the resulting bond angle are essential to PKC activation (45, 46). It follows that it may be the characteristic kink in the sn-1 ether bond of alkyl- or alkenylphospholipids which allows IL-1 receptors to activate a phospholipase that selectively hydrolyzes these substrates. It is now widely believed that a physical property of DG to lower the binding energy for membrane/PKC interactions favors translocation of the kinase to the membrane (44). Thus, as an alternative mechanism of action, ether-linked DG may not increase the apparent membrane binding constant for PKC. Recent studies have suggested that it is not PKC activation or translocation that is ultimately responsible for an altered phenotype, but, in fact, it is down-regulation and/or proteolysis of the activated PKC isoforms (47). This theory might explain the observation that chronic but not acute phorbol ester treatment is a cell proliferation signal (48). If down-regulation of a specific isotype is critical for activation of a specific cellular phenotype, then a more direct mechanism, competitive inhibition of selected PKC isoforms with ether-linked DG, might also lead to an altered cellular phenotype.

In terms of mass, there is a discrepancy between IL-1-induced hydrolysis of ethanolamine plasmalogens and formation of alkenyl, acylglycerol. Nearly 20 pmol of plasmalogens are hydrolyzed while only 1 pmol of alkyl, acylglycerol is formed. This observation may reflect the fact that IL-1 might activate alkenylphospholipid-selective phospholipases, including A2, and D which would degrade plasmalogens without directly forming alkyl-DG species (49, 50). Vinyl ether-selective phospholipase A2 (plasmalogenase) and lysophospholipase A2 (lysoplasmalogenase) activities have also been described which may be regulated by IL-1-receptors (51, 52). Supporting our data, this phospholipase A2 activity is specific for ethanolamine but not choline plasmalogens (51).

In MC, PKC isoenzymes α, δ, ε, and ζ are expressed, and α, δ, ε but not ζ can be down-regulated by chronic PMA treatment (43). The role of IL-1-induced ether-linked DG to inhibit vasoconstrictor-stimulated PKC α is supported by the following observations: 1) IL-1 inhibits ET-, arginine vasopressin-, and platelet-activating factor-stimulated PKC activity; 2) all of these G protein-linked vasoconstrictors activate PKC α; and 3) ether-linked DG inhibit immunoprecipitate PKC α in an in vitro assay. The putative actions of IL-1-stimulated ether-linked DG to negatively regulate calcium-insensitive PKC isoforms awaits further investigation. Even though IL does not stimulate PKC translocation or activation, it could be that IL-1 induces activation of PKC ζ which is not regulated by DG cofactors. It is for this reason we have used the EGF-receptor binding domain to assess PKC activation as this substrate, and not histones, is phosphorylated in vitro by PKC ζ (53, 54). Moreover, we confirmed our findings by utilizing the alanine to serine mutated pseudosubstrate peptide sequence as a PKC substrate (55).

The DG cofactor substrate specificity for individual PKC isoforms has only recently been investigated as a potential signaling mechanism. In a recent report, supplementation of 1-O-hexadecylglycerol into fibroblasts of Zellweger patients leads to accumulation of ether-linked DG and a concomitant inhibition of bradykinin-induced translocation of PKC α but not ε or ζ (56). Our studies extend these findings by demonstrating that IL-1-induced ether-linked DG inhibit immunoprecipitated PKC α activity in an in vitro assay. In other studies, PtdIns-derived DG preferentially containing arachidonate at the sn-2-position are a better cofactor for PKC α compared with PKC β or γ, in contrast to PtdCho-derived DG species which are equipotent in stimulating PKC isoenzymes (57). Also, the mitogenic effects of insulin have been linked to PtdCho-dependent phospholipase C activity that generates undefined species of DG that specifically activates PKC ζ (58). In a scenario similar to IL-1-receptor signaling in MC, interferon which does not elevate [Ca2+], and forms PtdCho-derived DG, translocates PKC ε but not α in Daudi cells (59). Alternatively, a single agonist may have differential effects on multiple PKC isoforms as retinoic acid-induced differentiation is associated with PKC ε inhibition (60) and PKC α stimulation (61). Thus, signaling mechanisms will be defined in the future by the type of PKC isoforms mobilized as well as the DG cofactor specificity for that PKC isoform.

Our studies are the first report of IL-1 inhibiting receptor-stimulated PKC activity. However, this is not the first instance of IL-1 negatively modulating the actions of vasoconstrictors. IL-1 pretreatment inhibited phenylephrine-induced contraction in rat aortic rings (62) and inhibited cerebral spinal fluid-contracted pial arteries (63). Our data suggest that one component of IL-1α signaling is ether-linked DG inhibition of PKC activity. Other signaling pathways that have been postulated include nuclear targeting of a 16-kDa N-terminal IL-1α cleavage product that may function as a trans-activating factor (64). Alternatively, the carboxyl end of IL-1α bound to the IL-1 receptor also localizes to the nucleus (65). The role of JAK/STAT kinases to amplify early IL-1 receptor signals is an area of active investigation. In addition to inhibiting PKC activity with subsequent effects upon contraction and/or differentiation, ether-linked DG may exert other effects independent of PKC. Ether-linked phospholipids are preferentially esterified at the sn-2-position with arachidonate (66) and, thus, these DG may be a potential substrate for arachidonate release via a DG-lipase. IL-1 has been shown to induce prostanoids in various cell types (67), and the contribution of the DG-lipase mechanism to arachidonate release has not been investigated. Other PKC-independent actions of DG include leukocyte chemotraction (68), adipocyte glucose transport (69), neuronal calcium current regulation (70), K⁺-induced calcium influx regulation (71), and translocation of phosphorylcholine cytidyltransferase activity (72). DG may serve as a cofactor for other kinases besides PKC including c-Raf and PAK-2 (73). Finally, other signaling cascades may augment ether-linked DG-in-
duced inhibition of PKC. For example, IL-1 and PDGF have recently been shown to stimulate sphingomyelin metabolism (74, 75). However, unlike IL-1, in contrast to PDGF, does not stimulate ceramide activity to form the endogenous PKC inhibitor, sphingosine (76).

Our studies suggest that IL-1 and ET stimulate distinct species of DG that differentially regulate PKC in MC. This is the first study to conclusively link agonist-stimulated ether-linked DG formation with a physiologically relevant function; i.e., inhibition of PKC activity. An alternative signal transduction pathway is envisioned by which agonists, such as IL-1, that hydrolyze PtcdCho and PtdEth but not PtdIns predominately form ether- but not ester-linked DG species. Our conclusions lead to the provocative speculation that individual molecular species of DG may serve as key metabolic branching points, coupling discrete receptor populations to specific PKC isotypes.

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