Changing J774A.1 Cells to New Medium Perturbs Multiple Signaling Pathways, Including the Modulation of Protein Kinase C by Endogenous Sphingoid Bases*

(Received for publication, March 25, 1996, and in revised form, October 4, 1996)

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Sphingosine, sphinganine, and other long-chain (sphingoid) bases are highly bioactive intermediates of sphingolipid metabolism that have diverse effects when added to cells, including the inhibition of protein kinase C (PKC) as evaluated by both enzymatic activity and [3H]phorbol dibutyrate ([3H]PDBu) binding. Nonetheless, changes in endogenous sphingoid bases have not been proven to affect PKC or other signal transduction pathways. We have discovered recently that changing J774A.1 cells to new medium results in up to 10-fold increases in sphingoid bases (Smith, E. R., and Merrill, A. H., Jr. (1995) J. Biol. Chem. 270, 18749–18758); therefore, this system was used to elevate sphingosine and sphinganine and determine if PKC was affected. Incubation of J774A.1 cells in new medium for 30 min increased the levels of these endogenous sphingoid bases to approximately 0.5 nmol/mg of protein and decreased [3H]PDBu binding by 40–60%. Addition of NH4Cl, which suppresses the change in sphingosine, restored [3H]PDBu binding. Elevation of endogenous sphinganine by a second method (addition of fumonisin B1, an inhibitor of ceramide synthase) also reduced [3H]PDBu binding; therefore, elevations in sphingosine and sphinganine can both affect PKC. The elevation in sphingoid bases was also associated with an increase in the amount of PKC-δ (the major PKC isoform in J774A.1 cells) in the cytosol, as determined by activity assays and immunoblot analyses. Changing the culture medium affected other PKC isozymes, increased cellular levels of diacylglycerol, dihydroceramide, and ceramide, and altered the expression of two genes (the expression of JE was increased, and the induction of MnSOD by TNF-α was potentiated). Thus, changing the culture medium has numerous effects on J774A.1 cells, including the modulation of PKC by endogenous sphingoid bases.

Sphingosine and other long-chain (sphingoid) bases, the structural backbones of sphingolipids, have been found to affect diverse cellular systems when added to in vitro assays, cells, and even applied to skin (1). These affected systems include, but are not limited to, protein kinase C (PKC) (2), Na⁺,K⁺-ATPase (3), phosphatidic acid phosphatase (4–7), phospholipases (including phospholipase D) (8, 9), retinoblastoma protein phosphorylation (10, 11), and sphingosine-activated protein kinase(s) (12). The inhibition of PKC has been studied most thoroughly in vitro using mixed micellar assays of the purified enzyme (2), as well as by evaluation of cellular functions dependent on this enzyme in platelets (2), neutrophils (13), HL-60 cells (14), and many other systems (1). Sphingosine inhibits PKC by acting as a competitive inhibitor of activation by diacylglycerol (DAG), phorbol dibutyrate (PDBu), and (for some isozymes) calcium (2) and also blocks activation by unsaturated fatty acids and other lipids (15). The exact mechanism by which sphingoid bases inhibit PKC remains unknown; however, since PKC binds to membranes through interactions with DAG and negatively charged phosphatidylserine (PS), sphingosine may be localized in regions of acidic lipids and block enzyme binding or activity (2, 14, 16, 17).

Cells normally contain low levels of free long-chain bases (1–10 nmol/g of tissue (wet weight) or 10–100 pmol/10⁶ cells) (18–21), and the levels have been found to change in response to various treatments (22, 23). Nonetheless, there has been no direct link between changes in endogenous sphingoid bases and inhibition of PKC. We have found that the change from conditioned to new culture medium stimulates relatively rapid increases in free sphingoid bases in J774 macrophages (24, 25). Similar observations have been made in other cultured cell systems, including Swiss 3T3 fibroblasts (26), NIH-3T3 fibroblasts, A431 cells, and NG108-15 cells (27). The levels of free sphingoid bases that are achieved within 30–60 min of incubation of J774 cells in new medium (~0.5 nmol/mg of protein) are comparable with the levels of exogenous long-chain bases that inhibit PKC in human neutrophils (22). Therefore, this system serves as a model to determine if endogenous sphingoid bases can affect PKC, as well as to explore some of the implications of changing culture medium on cellular signal transduction pathways.

Using this model, we have found that changes in endogenous sphingoid bases affect [3H]PDBu binding, PKC activity, and the subcellular localization of one PKC isoform in J774 cells. In contrast, modulation of endogenous ceramide after the medium change did not affect the expression of two genes that are known to be induced by TNF-α: JE (28) and MnSOD (29). The change in culture medium did, however, alter JE expression and potentiate TNF-α induction of MnSOD.

* The abbreviations used are: PKC, protein kinase C; TNF-α, tumor necrosis factor-α; DAG, 1,2-sn-diacylglycerol; PDBu, phorbol 12,13-dibutyrate; PMA, 12-O-tetradecanoylphorbol-13-acetate; FB1, fumonisin B1; MnSOD, Mn²⁺-dependent superoxide dismutase; FBS, fetal bovine serum; DMEM, Dulbecco’s modified Eagle’s medium; PS, phosphatidylserine; NM, new medium; CM, conditioned medium.

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EXPERIMENTAL PROCEDURES

Materials—[a-32P]Phorbol 12,13-dibutyrate (44.8 Ci/mmol), [γ-32P]ATP (>3000 Ci/mmol), [α-32P]dATP (>3000 Ci/mmol), and the ECL kit were purchased from Amersham Corp. Escherichia coli DAG kinase was purchased from Lipidex, Inc. (Westfield, NJ). 1,2-Dioleyl-sn-glycerol and cardiolipin came from Avanti Polar Lipids (Alabaster, AL). Monoclonal antibodies to PKC isozymes (α, βI, βII, γ, and δ) came from Calbiochem. Pumonin B (PB) was purchased either from Sigma or from the Division of Food Sciences and Technology, Council for Scientific and Industrial Research ( Pretoria, South Africa). Fetal bovine serum was purchased either from Life Technologies, Inc. or from Biocell (Rancho Dominguez, CA). QuikHyb and the random-primed DNA labeling kit were purchased from Stratagene (La Jolla, CA) and Boehringer Mannheim, respectively. Zeta-Probe GT membrane (nylon membrane for nucleic acid transfer) was purchased from Bio-Rad. Cetus Corp. generously supplied human recombinant TNF-α. C. Caramidae was prepared according to the method of Gaver and Sweeney (30). All other reagents were from Sigma or were of the highest available research quality.

Cell Culture—J774A.1 cells (American Type Culture Center number TIB 67), a murine macrophage-like transformed cell line, were routinely grown in suspension at 37°C in a spinner flask (Corning Glass) in DMEM supplemented with 10% FBS, penicillin-G (100 units/ml), streptomycin sulfate (100 μg/ml), and sodium bicarbonate (3.7 g/liter). Cells were passaged every 2–3 days by 1:4 dilution with fresh culture medium to yield a cell density of approximately 2 × 10^7 cells/ml. Unless indicated differently, cells were removed from the spinner flask, pelleted by gentle centrifugation, resuspended in new culture medium, and plated at a density of 5 × 10^5 cells/ml in 5 ml of culture dishes to culture dish size on 10 × 7.5–7.5 × 2 cm 2 of membrane for 2 ml of medium (for PKC studies) or plated at 1–2.5 × 10^6 cells/100-mm dish in 6 ml of medium (for RNA studies). Plates were incubated for 3 days at 37°C, 5% CO2 before beginning the experiments.

C3HA cells, a mouse embryo fibroblast line (31), were grown as described previously (32). These cells are an immortalized, contact-inhibited cell line and were grown on 15 cm dishes in DMEM supplemented with 10% fetal calf serum (Intergen, Inc.), 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. For isolation of RNA, conditioned medium was removed from dishes of confluent C3HA cells, the cells were washed quickly with ice-cold sterile PBS, and TNF-α was added in 7 ml of fresh culture medium for 6 h to yield a final concentration of 400 units/ml.

[3H]Phorbol Dibutyrate Binding—For these experiments, J774 cells (Corning) at a density of 10^6 cells/ml were incubated with 0.1% diethylpyrocarbonate-treated water and aspirated from dishes, the cells were washed twice with ice-cold PBS for 2 h, and the amount and purity of RNA loaded was verified by ethidium bromide staining. For determination of activity, PKC was partially purified over DEAE-Sephadex columns (34, 35). Membrane and cytosolic fractions were passed over individual 0.5-ml DEAE-Sephadex columns equilibrated with buffer B (20 mM Tris, pH 7.5, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM EDTA, 0.5 mM EGTA, 0.5 mM dithiothreitol, 50 μg/ml leupeptin, 20 μg/ml aprotinin). The columns were washed with approximately 5 column volumes of buffer B, and the bound enzyme was eluted with 1–1.5 ml of buffer B containing 0.5 mM NaCl and immediately assayed for activity.

PKC activity was determined by measuring the incorporation of [3H]phorbol 12,13-dibutyrate (PB) into autophosphorylation products. Calcium-dependent activity was measured in the presence of DAG (0.04 μg/μl), PS (0.04 μg/μl), and 5 μM EGTA, whereas calcium-dependent activity was measured in the presence of 0.4 mM CaCl2, PS, DAG, but without EGTA. Values have been normalized for protein, which was determined by the Bio-Rad Bradford method (36).

Immunoblot Analyses—Immediately following subcellular fractionation, the isolated cytosolic and membrane extracts were boiled for 10–15 min in SDS sample buffer (37). Approximately 50–100 μg per sample was analyzed by SDS-polyacrylamide gel electrophoresis on 10% gels. Proteins were electrophoretically transferred at 30 V overnight onto nitrocellulose membranes (Schleicher & Schuell), which were blocked for 30 min with 5% powdered milk, incubated with primary rabbit immunoglobulin G antibodies against PKC isozymes (1:1000 dilution in 1% powdered milk in PBS (20 mM Tris, pH 8.0, 150 mM NaCl)) for 2 h at room temperature, washed three times with 0.5% Triton X-100 in PBS, and probed for 1 h with a donkey anti-rabbit IgG conjugated to horseradish peroxidase (1:10,000 dilution). Blots were probed with ECL detection reagents according to the manufacturer’s directions and exposed to film for 1–60 min.

RNA Isolation—RNA was isolated from cells according to the acid guanidinium thiocyanate-phenol-chloroform extraction protocol (38). Following the indicated treatments and incubations, the medium was aspirated from dishes, the cells were washed twice with ice-cold PBS (0.12 mM NaCl, 2.7 mM KCl, 1.2 mM KH2PO4, 1 mM NaH2PO4), and 1 ml of denaturation solution was added to each dish. The final RNA extract was desalted in 100 μl 0.1% diethyl pyrocarbonate-treated water and stored at -75°C.

Northern Hybridizations—Northern blot hybridizations were performed as described by Thomas (39), with the modifications specified in Boss et al. (40). Five micrograms of total RNA were separated by electrophoresis on 1.5% agarose (1.5× formaldehyde) gels (50–60 V for 2 h). The amount and purity of RNA loaded was verified by ethidium bromide staining of the gels. RNA was transferred overnight to nylon membranes (Hybond N, 0.2 μm nitrocellulose, 0.3 μm polyamide, 0.2 μm polyethyleneimine) (41) and cross-linked to the filters using 1200-μl UV light (Stratalinker). Filters were prehybridized for 2–3 h and hybridized overnight (12–18 h) at 42°C in deionized formamide:2× Northern solution (0.1 mM sodium phosphate, pH 7.0, 10× SSC, 2% SDS, 10× Denhardt’s solution, 20× dextran sulfate) (1.1, v/v), which contained 100 μg/ml boiled sonicated calf thymus DNA. Alternatively, some filters were hybridized in 10–15 ml of QuikHyb solution/filter according to the manufacturer’s instructions (Stratagene, Inc., La Jolla, CA).

Labeling of DNA Probes—JE and MnSOD hybridization probes were generated by the random primer labeling method (42, 43) or according to the prescribed protocol in the random primer labeling kit (Boehringer Mannheim). The amount of RNA loaded was normalized to β-actin, which was monitored by hybridization to a mouse β-actin oligonucleotide labeled at the 5’-end with [γ-32P]ATP (41). Autoradiography was carried out for 4–24 h at ~75°C using intensifying screens. Autoradiographs were analyzed by laser densitometry (Personal Densitometer, Molecular Dynamics) using the ImageQuant software system.
RESULTS

To examine whether endogenous long-chain bases can affect PKC, several indices of the state of this enzyme were examined in J774 cells under conditions that increase free sphingosine and sphinganine (24, 25) (e.g. changing the medium) versus conditions where this increase is suppressed (conditioned medium or new medium containing NH₄Cl). Results are expressed as described under "Experimental Procedures." Results were determined as described under "Experimental Procedures." Results are expressed as the mean ± S.E. (n = 6).

[^3H]PDBu Binding—After 30 min in fresh culture medium,[^3H]PDBu binding decreased 40–60%, from 0.189 ± 0.028 to 0.083 ± 0.021 pmol[^3H]PDBu bound per 10⁶ cells compared with cells that were returned to conditioned medium (cf. 0 mM NH₄Cl versus "conditioned medium" in Fig. 1). Since exogenous sphingosine and other long-chain bases cause dose-dependent inhibition of[^3H]PDBu binding by PKC in mixed micellar assays (2) and intact cells (1, 2), this reduction in[^3H]PDBu binding suggests that endogenous long-chain bases can affect PKC, the major intracellular receptor for phorbol esters in most cells (44).

[^3H]PDBu binding was also assessed in cells incubated in new culture medium with increasing concentrations of NH₄Cl, which blocks the generation of total sphingoid bases (Fig. 1), although this is due to a reduction in sphingosine and not sphinganine (25). Ammonium chloride restored[^3H]PDBu binding in a concentration-dependent manner (Fig. 1), and by 4 mM NH₄Cl, the binding had returned to the level obtained in conditioned medium. At this concentration of NH₄Cl, sphingosine levels were reduced by 20% and sphingosine by over 50%. At a higher NH₄Cl concentration (8 mM),[^3H]PDBu binding exceeded that found in conditioned medium.

Similar studies were conducted using chloroquine (25 μM) instead of NH₄Cl. This lysoosmotrophic agent suppressed the elevation in sphingosine to the same extent as 4 mM NH₄Cl and restored[^3H]PDBu binding to the level in conditioned medium (data not shown). Chloroquine was not studied further, however, since it is a hydrophobic amine that, like sphingoid bases, might affect PKC activity.

These results establish that changing the medium to elevate endogenous long-chain bases results in a reduction of[^3H]PDBu binding by PKC and that changing the medium with suppression of the generation of sphingosine restores[^3H]PDBu binding. To elevate free sphingoid bases independently of changing the medium, the cells were treated with fumonisin B₁ (FB₁), a fungal toxin that inhibits ceramide synthase (45). After 3 days, sphingoid base levels were elevated 2–3-fold (Table I), with the sphinganine mass increasing 600% over control at time 0 and 1800% after 60 min in new medium. Phorbol ester binding was 30% lower in the FB₁-treated cells at time 0 (i.e. 0.68 ± 0.07 versus 0.95 ± 0.21 pmol/mg of protein), and after 60 min in new medium, binding was approximately 50% lower than control levels (p < 0.05). These results establish that the elevation of sphingosine is also associated with reduced[^3H]PDBu binding.

PKC Isozymes in J774 Cells and Subcellular Localization—To examine another aspect of PKC behavior, and to take into account the possibility that only some isoforms might be affected (46–48), immunoblot analyses were conducted using cytosolic and membrane fractions prepared from cells after incubation for 30 min in new or conditioned medium, with or without 1.6 μM PMA (Figs 2 and 3). This concentration of PMA was chosen, since it fully activates superoxide production in J774 macrophages (49) and would thus be expected to elicit significant changes in PKC.

Immunoblot analysis of cytosolic and membrane fractions indicated that J774A.1 cells contain PKC-α, PKC-βI, PKC-βII, and PKC-δ. PKC-δ, a member of the nPKC class that does not require calcium for activation (46, 47), was found to be the major isozyme, based on Western blot analysis and activity assays (discussed below). This finding is consistent with its prominence in hematopoietic cells (34, 50). PKC-δ was located primarily in the membrane fraction in cells incubated in either conditioned or new medium (Fig. 2A); however, the change to new culture medium reproducibly increased PKC-δ in the cytosolic fractions. Incubation in conditioned medium, or with PMA in either new or conditioned medium, did not evoke a similar redistribution. PKC-δ appeared to have a slightly reduced mobility on 10% SDS-polyacrylamide gels after PMA

TABLE I

[^3H]PDBu binding in fumonisin B₁-treated cells

J774A.1 cells were incubated with 10 μM FB₁ for 3 days, with fresh DMEM containing 10% FBS and 10 μM FB₁ added every 24 h. On the 3rd day, the cells were incubated in fresh DMEM for 0 or 60 min before measurement of[^3H]PDBu binding, as described under "Experimental Procedures." Long-chain base (LCB) measurements include both sphingosine and sphinganine mass and were made as described under "Experimental Procedures." Results are expressed as the mean ± S.E. of triplicate samples.

| Time | Control | FB₁ | % difference in[^3H]PDBu bound | LCB(−FB₁) pmol/mg protein (mean ± S.E.) | LCB(+FB₁) (% control) |
|------|---------|-----|---------------------------------|--------------------------------------|----------------------|
| min  | pmol[^3H]PDBu bound per mg cell protein (mean ± S.E.) | | | |
| 0    | 0.95 ± 0.21 | 0.68 ± 0.07 | −29 | 289 ± 26 | 537 ± 114* (186) |
| 60   | 1.43 ± 0.08 | 0.76 ± 0.09* | −47 | 256 ± 7.6 | 878 ± 76* (343) |

* Statistically different from control, p < 0.05, determined by Student’s t test.
treatment, which indicates phosphorylation (34, 48, 51), but this was not explored further in this study. Other bands that did not correspond to the molecular weight of PKC were also detected; it is unclear whether these bands represent proteolytic fragments of the enzyme or nonspecific binding of the antibody.

The appearance of PKC-δ in the cytosol indicated a displacement of PKC from the membrane (presumably due to increases in free long-chain bases), as has been seen in studies in which exogenous sphingosine was used (2, 14, 16). PKC-δ localization, therefore, was determined in cells incubated with sphingosine in conditioned medium. The concentrations of sphingosine were similar to the increases in sphingoid bases determined after cells are incubated in new medium, and which have also been found to affect PKC in other cells, including Chinese hamster ovary cells (0.75–4 μM) (52), neutrophils (1–3 μM) (13, 16), and HL-60 cells (~4 μM) (53). As shown in Fig. 2B, 0.5 μM sphingosine caused little or no change in PKC-δ distribution, but 5.0 μM increased the amount of PKC-δ in the cytosolic fractions compared with conditioned medium alone (and to approximately the same level found in new medium). Addition of 10 mM NH₄Cl in new medium prevented PKC-δ redistribution to the cytosolic fraction. Thus, endogenous (and exogenous) sphingoid bases increase the amount of PKC-δ in the cytosol, and the factors that suppress the endogenous sphingoid bases reversed this change.

The classical PKC (cPKC) isozymes, which require calcium in addition to PS and DAG for activation (42), were detected in both cytosol and membrane fractions of cells at time 0, although the amount of cytosolic isozyme exceeded membrane-bound in all cases (Fig. 3). Incubation of cells in new medium eliminated PKC-α and substantially reduced the amount of PKC-βI and βII in the cytosol; all three were eliminated in the membrane fractions. When incubated, instead, in conditioned medium, these losses of PKC-α, βI, and βII did not occur. Addition of PMA with new medium further reduced the amount of cPKCs present in the cytosolic fraction, but PMA did not alter the amount or distribution of cPKCs in conditioned medium (Fig. 3, lanes 4 and 5 under “Cytosol” and “Membrane” fractions). As shown in Fig. 4, NH₄Cl did not prevent the loss of PKC-α in new medium alone. Similar results were obtained for PKC-βI and PKC-βII (not shown).

**PKC Activity in Isolated Cytosolic and Membrane Fractions**—To determine whether the changes in PKC distribution and isozyme corresponded to altered enzyme activity, PKC activity was measured in membrane and cytosolic fractions obtained from cells treated as described for Figs. 2–4. To determine total PKC activity, the fractions were assayed in the presence of DAG and PS with 400 μM calcium, whereas calcium-independent activity was determined with 5 mM EGTA and no calcium (Fig. 5A). Calcium-dependent activity was calculated as the difference between total and calcium-independent...
activity (Fig. 5B). With each treatment, the activity in membrane fractions was 4–5-fold higher than cytosolic activity, which agreed with the predominant localization of PKC-δ (the most abundant isozyme) in membrane fractions. Calcium-independent and -dependent activity increased in the membrane fraction from cells incubated in new medium for 30 min. In agreement with the appearance of PKC-δ in the cytosol only after the change to new medium, incubation in new medium increased the activity in the cytosol; calcium-independent activity entirely accounted for this increase (Fig. 5A). Cytosolic calcium-dependent activity was undetectable under the conditions examined.

To determine whether the inability of PMA to activate PKC in conditioned medium (Figs. 2–4) extended to a biological response, two other PKC-related activities were examined in J774 cells. The rate of superoxide generation of cells in new medium containing 5 μM PDBu was found to be 1.21 nmol/min/10^7 cells, whereas the rate was 0.17 nmol/min/10^7 cells in conditioned medium (or 7-fold lower than in new medium). Additionally, [14C]glucose oxidation was approximately 2-fold higher in new medium. PMA stimulated a 4.4-fold increase in [14C]CO₂ release from cells in new medium, but only a 2.6-fold increase from cells in conditioned medium. Thus, conditioned medium may contain various factors that blunt PKC-activation (including, but not limited to, ammonia).

Changes in DAG in New Medium—Since there is a seemingly paradoxical increase in both cytosolic and membrane PKC when cells were incubated in new culture medium (Fig. 5), it appeared that other modulators of PKC might contribute to some of these changes. For example, changes in DAG might account for the reduction in [3H]PDBu binding, since phorbol esters are analogs of DAG (46). As shown in Fig. 6A, DAG mass did rise 60% (to 0.388 ± 0.046 pmol/mg of cell protein) within 30 min following the change from conditioned to new medium and remained elevated (0.346 ± 0.035 pmol/mg of protein) after 60 min. However, unlike sphingosine, the increase in DAG was not blocked, and sometimes rose, upon adding NH₄Cl (Fig. 6B). Additionally, 25 μM chloroquine had no effect on DAG mass. Thus, changes in DAG mass are not likely to be responsible for the alterations in [3H]PDBu binding that occurred with the change to new medium.

Effect of New Medium on Gene Expression—Since changing the culture medium has profound effects on numerous bioactive lipids (sphingosine, DAG, and ceramide (this study and Ref. 25), we explored whether this might have an impact on gene expression. For this analysis, we selected two genes that are TNF-inducible, JE and MnSOD (29, 40, 58, 59), since sphingolipids have been implicated as mediators for this cytokine (54–
could be evaluated separately, since NH₄Cl alters intracellular sphingosine levels and FB₁ affects primarily sphinganine mass. Both agents increase ['H]PDBu binding and each sphingoid base can apparently alter PDBu binding and PKC. These results agree with in vitro studies, which demonstrated that sphingosine and sphinganine are equally effective in inhibiting PKC in mixed micellar assays and in cells (16). The effects of FB₁ are particularly interesting, because Huang et al. (60) have reported that FB₁ causes dose-dependent repression of PKC in CV-1 cells. Although that study did not determine whether FB₁ caused sphingoid base accumulation, this has been seen in every other cell system examined to date (61); therefore, this result is consistent with our finding that increases in endogenous sphingosine and sphinganine can affect PKC.

As we are aware, this is the first analysis of the PKC isozymes in J774 cells, with the finding that PKC-δ is the major species plus smaller amounts of PKC-α and PKC-βII and βII; although, a previous study detected a polypeptide that reacted with antibodies directed against the C-terminal region of the regulatory domain of PKC-δ (62). PKC-δ is located primarily in the membrane fraction of J774 cells, as well as in many other cell types (34, 63, 64). Thus, translocation of PKC-δ to the membrane would not seem to be a determinant of the activation of this PKC isoyyme. One would predict, nonetheless, that the shift in the distribution of PKC-δ from the membrane to the cytosol by endogenous sphingoid bases would in some way affect its activity and/or the types of protein substrates that are phosphorylated.

The change to new medium alters cellular levels of several bioactive lipids: DAG (this study), sphingoid bases, ceramide, and phospholipids (25), all of which may influence PKC or other signal transduction pathways directly or indirectly. The complexity of these changes make this difficult to study in detail; however we have found that changing the cells to new medium affected the induction of JE and MnSOD. These effects of new and conditioned media should be borne in mind in signaling studies using J774 cells and other cell lines since similar "bursts" of sphingolipid metabolism have been seen in many systems (26, 27).

It is not known if there is any physiological significance to these changes in endogenous sphingoid bases and their effects on PKC. It has been proposed that sphingoid bases might establish a "set point" for PKC activation by positive effector stimuli (65). Even if this is not the case, the elevation of sphingoid bases (and aberrant regulation of PKC) in diseases caused by FB₁ (61) and in some sphingolipidoses (13, 66) may be significant contributors to the pathophysiology associated with these diseases.

Acknowledgments—We gratefully acknowledge and appreciate the technical assistance and advice of Drs. Edward Bowman and David Uhlinger for PKC isolation, Dr. Shiv-Raj Tyagi for DAG measurements, Dr. Xiang-Xi Xu for immunoblot analysis, and Drs. James Riley and Joseph Schroeder for RNA isolation and Northern blot analysis.

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