Inhibition of Phorbol Ester-dependent Differentiation of Human Promyelocytic Leukemic (HL-60) Cells by Sphinganine and Other Long-chain Bases*

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The effects of long-chain (sphingoid) bases on the phorbol ester-dependent differentiation of HL-60 cells were investigated since these molecules are potent inhibitors of protein kinase C (Hannun, Y. A., Loomis, C. R., Merrill, A. H., Jr., and Bell, R. M. (1986) J. Biol. Chem. 261, 12604-12609). After 24 h, low concentrations of sphinganine (1-5 μM) blocked both cell adherence and the inhibition of growth in response to phorbol 12-myristate 13-acetate, as measured by cell number and acid phosphatase activity. Sphinganine and sphingosine decreased adherence by 50% at 1-3 μM; other long-chain bases were effective in parallel to their inhibition of protein kinase C. Sphinganine decreased the binding of [3H]phorbol dibutyrate by the phorbol receptor of HL-60 cells, protein kinase C, and inhibited the response of HL-60 cells to diacylglycerol, a cell permeable activator of this enzyme. Long-chain base uptake by HL-60 cells was demonstrated with [3-3H]sphinganine and within 1-3 days much had been converted to ceramides. By day 3, most of the cells had recovered the ability to adhere and exhibited macrophage characteristics, whereas cells in suspension did not differentiate. The level of free sphinganine in HL-60 cells was determined to be 12.3 ± 1.2 pmol/10^6 cells. These results establish that sphingoid bases inhibit protein kinase C in HL-60 cells and may function physiologically as negative effectors of this enzyme.

Diverse biochemical and cellular changes occur in response to extracellular agents that stimulate phosphatidylinositol hydrolysis, mobilization of Ca^{2+}, and activation of a lipid-dependent protein kinase termed protein kinase C (1-3). Diacylglycerols produced from phosphatidylinositol are naturally occurring activators of protein kinase C, but cellular processes have been investigated using the cell permeable activators 1-oleoyl-2-acetylglycerol, 1,2-dioctanoylglycerol, and phorbol diesters, which are structurally related (1-5). Responses to these compounds range from acute metabolic effects, such as activation of the oxidative burst in neutrophils, to much more protracted responses, including stimulation or inhibition of growth and differentiation.

The mechanism of activation of protein kinase C by phoshoiipids, Ca^{2+}, and diacylglycerols (phorbol esters) has been extensively investigated in vitro and in vivo. In contrast, the ways in which protein kinase C is regulated negatively are less well defined. Sphinganine and other long-chain (sphingoid) bases have been recently discovered to be potent inhibitors of protein kinase C in vitro and in human platelets (6) and to block the oxidative burst in human neutrophils (7). These molecules have the potential of serving as natural inhibitors in vivo because they, like diacylglycerols, could be mobilized from membrane lipids.

The possible effects of sphinganine on protein kinase C-dependent differentiation of HL-60 cells into macrophage- and monocyte-like cells was investigated. HL-60 cells are a human promyelocytic cell line that matures into a variety of cell types depending on the stimulant (8-10). Since the phorbol ester receptor has been found to be protein kinase C (11) and differentiation can be effected by 1,2-dioctanoylglycerol (12), it is likely that protein kinase C is involved in differentiation, although other effects of phorbol esters cannot be excluded (13, 14).

When HL-60 cells were treated with sphinganine, there was pronounced inhibition of cell adherence and other markers of differentiation in response to phorbol 12-myristate 13-acetate (PMA). The characteristics of the inhibition and the structural specificity suggested that these effects were due to inhibition of protein kinase C. Furthermore, sphinganine inhibited phorbol dibutyrate binding by HL-60 cells and differentiation of the cells in response to dioctanoylglycerol. These results suggest that long-chain bases can inhibit protein kinase C physiologically.

EXPERIMENTAL PROCEDURES

Materials—RPMI 1640 medium was purchased from Gibco (Grand Island, NY) and defined bovine calf serum was from HyClone Laboratories (Logan, UT). Fatty acid-free bovine serum albumin and all other tissue culture reagents were obtained from Sigma. The phospholipid standards: erythro-dihydrosphingosine (sphinganine), sphingosine, N-palmitoyldihydrosphingosine, and ceramides (from bovine brain sphingomyelin) were purchased from Sigma; N-acetylsphinganine and 3-ketosphinganine were synthesized according to Gaver and Sweeney (15). The other homologs were provided by Dr. Dennis Liotta at Emory University. PMA was purchased from LC Services Corp. (Woburn, MA) and [3H]phorbol dibutyrate (8.3 Ci/mmol) was from Amersham Corp.; 1,2-dioctanoylglycerol was obtained from Avanti Polar Lipids (Birmingham, AL).

The [3-3H]sphinganine was synthesized by the reduction of N-acetyl-3-ketosphinganine (15) by NaB{eq}H_4{eq} (Amersham Corp.) followed by acid hydrolysis, and purified by silica gel column chromatography (Unisal, Clarkson Chemical Co., Williamsport, PA). The product

* This work was supported by National Institutes of Health Grants GM33369 (A. H. M.), CA22294 (J. M. K.), and AM20205 (R. M. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: PMA; phorbol 12-myristate 13-acetate; GM, NeuAcO\textsubscript{2}→βGalβ1→4Glcβ1→ Cer.
yielded a single spot coincident with sphinganine when examined by TLC with Silica Gel H plates developed in CHCl<sub>3</sub>/methanol/2 N NH<sub>4</sub>OH (40:10:1). The specific activity was adjusted to 17,000 cpm/nmol by quantitating the amount of sphinganine as the 2,4,6-trinitrobenzenesulfonic acid derivative (16).

Cell Culture—The HL-60 cells (obtained from the American Type Culture Collection, ATCC CCL240) were grown at 37°C as a suspension culture in 175 cm<sup>2</sup> Nunc tissue culture flasks (Vangard International, Neptune, NJ). The cells were subcultured at a density of 0.25 × 10<sup>5</sup> cells/ml and used between passage numbers 30 and 40.

Isolation of HL-60 Cells—Cells were centrifuged at 300 × g for 3 min, and 1 ml of HL-60 cells/ml was added to 1 × 10<sup>6</sup> cells/well in 12-well culture dishes. Medium, PMA, and long-chain bases (prepared as the 1:1 molar complex with fatty acid-free bovine serum albumin) at the indicated concentrations were added for a total volume of 2.0 ml. After the desired times, the cells in suspension were counted and checked for viability (trypan blue exclusion) with a hemacytometer. The attached cells were quantitated by measuring the DNA content (17) and by assaying for acid phosphatase (18).

When 1,2-dioctanoylglycerol was used, the cells were treated essentially as described by Ebeling et al. (12). Sphinganine was added to the cells, then dioctanoylglycerol in 1 μl of ethanol to yield 100 μM, followed by additional maintenance additions of 20 μM diacylglycerol every 2 h for 12 h.

Displacement of Phorbol by Sphinganine—Competitive binding assays were conducted as described by Goodwin and Weinberg (19) and Ebeling et al. (19). Approximately 1 × 10<sup>6</sup> cells/ml were incubated with 12 nM [*H]phorbol dibutyrate (8.3 Ci/mmol) and varying concentrations of sphinganine (1:1 with bovine serum albumin) for 1 h at 37°C. The cells were recovered on Millipore filters, washed, and counted. The data were corrected for nonspecific binding by subtracting the counts/mim obtained in the presence of 300 nM PMA.

Kinetics of [*H]Sphinganine Uptake by HL-60 Cells—Approximately 1 × 10<sup>5</sup> HL-60 cells in 1 ml of medium were mixed with an equal volume of medium containing 2.5 μM [*H]sphinganine (equimolar with bovine serum albumin). After varying time intervals, an aliquot of the cells was removed and counted, and a portion was extracted as described below. The extracts were applied to silica gel H plates and developed in CHCl<sub>3</sub>/methanol/2 N NH<sub>4</sub>OH (40:10:1, v/v/v), air dried, sprayed with Amplify (Amersham Corp.), and subjected to fluorography. Radiolabel was observed in only three regions coincident with ceramides near the solvent front, sphinganine with an RF of approximately 0.45, and in a region near the origin that encompassed spingomyelin and other more polar complex sphingolipids (RF of 0.1–0.2).

Analysis of Long-chain Bases—From 1–4 × 10<sup>6</sup> cells were recovered by centrifugation, washed twice with phosphate-buffered saline, and extracted immediately by a minor modification of the procedure of Dyer and Snell (21). Cells were suspended in 1 ml of chloroform/methanol (1:2) and mixed thoroughly; 1 ml each of chloroform and water were added and the two phases were separated by centrifugation; the upper phase was discarded, and the chloroform phase was washed twice with water and dried by passage through a small column containing NaSO<sub>4</sub>. The extracts were evaporated as described below. The extracts were injected onto a 0.5 mm silica gel plate (ISCO) and eluted isocratically with this same solvent mixture (10:1:10, v/v/v) for 3 h. The lipids were dissolved in 50 μl of methanol/ether (1:1), then 0.5 ml of 0.2% fluorodinitrobenzene (Sigma) in methanol/ether (1:1) and 0.5 ml of 2 M KOH (pH 9.6) were added. After incubating for 1 h at 37°C, 2 ml of ether and 2 ml of water were added. The ether was collected and the aqueous layer was reextracted with an additional 1 ml of ether. The combined ether extracts were washed with 2 ml of water and dried through NaSO<sub>4</sub>, and the solvent was removed under a stream of N<sub>2</sub>. The extract of [*H]sphinganine (60%) was used to correct for losses during extraction. For each experiment, the 2,4-dinitrophenyl derivatives of standard sphinganine, sphinganine (dihydrophosphosine), and phytosphingosine (Sigma) were also prepared.

The derivatives were dissolved in 50 μl of methanol, 5 mM potassium phosphate (pH 7.0) (90:10) and 10 μl were injected onto a 0.5 × 25-cm C<sub>18</sub> column (ISCO) and eluted isocratically with the same solvent. The derivatives were detected at 360 nm with an ISCO V<sub>1</sub> Detector. The standard sphinganine eluted at 11.2 min and sphingosine at 9 min, and both were well resolved from other species.

Statistical Methods—Data given in tables and figures are results typical for several different experiments. The results are expressed as means ± S.D. and the significance of differences between groups was evaluated with the Student's t test for unpaired data.

**RESULTS**

Effect of Sphinganine on Cell Growth—The effect of sphingamine on HL-60 cell viability and growth was investigated because sphingamine has been reported to alter growth and to be cytotoxic for Chinese hamster ovary cells (23). Sphingamine was chosen over sphingosine because the former is available commercially as a homogeneous compound whereas the latter is a mixture of various homologs.

Untreated cells doubled during the first 24 h (Fig. 1), and this was not changed by 1 μM sphingamine, but both 2.5 and 5 μM limited growth. None of the cells exhibited a loss of cell viability for the first 24 h. By the second day, all concentrations of sphingamine were still somewhat inhibitory; 1 and 2.5 μM inhibited growth without cytotoxicity whereas 5 μM resulted in significant cell death. The change in total cell numbers between days 2 and 3 indicated that growth inhibition had ceased for the cells in 1 and 2.5 μM sphingamine. This may have been due to removal of sphingamine by metabolism (see below).

These effects depended on both the cell number, which probably reflected surface dilution (6), and the sphinganine to albumin ratio (data not shown); therefore, these parameters were kept constant except where noted.

Effects on PMA-induced Adherence and Growth Inhibition—Upon adding 9 nM PMA, growth of the HL-60 cells was inhibited by 70% within 24 h and the majority of the cells (61%) attached to the Petri dish (Table 1), which is typical for this cell line (10). When 1 μM sphingamine was also added, the cells continued togrow and only 26% of the total adhered. These data establish that sphingamine prevented PMA-induced growth inhibition at a concentration where sphinganine itself did not affect growth (cf Table 1 and Fig. 1).

Because the cells in sphingamine continued to grow, the number available for adherence in response to PMA was higher. This result in similar numbers of adherent cells (i.e. 0.51 × 10<sup>5</sup> for PMA plus sphingamine versus 0.76 × 10<sup>5</sup> for PMA alone, or a 33% difference) while the adherent cells as a percent of the total was much lower (i.e. a 74% difference). Adherence has been expressed as the percent of the total viable cells to normalize for differences in growth.

Adherence was further limited to 17.6 ± 6.4% and 15.0 ± 2.1% by 2.5 and 5 μM sphingamine, respectively, without

**FIG. 1. Effect of sphingamine on HL-60 cell growth.** Sphingamine at varying concentrations was added to 1 × 10<sup>6</sup> cells and incubated for the times shown. The closed circles are viable cells (all groups had >90% viability, except where otherwise indicated) and the open circles reflect both viable and nonviable cells.
the dish. Sphinganine caused a concentration-dependent increase in the activity remaining in suspension and a decrease in the adherent activities, which reflect inhibition of cell adherence.

Effects of sphinganine (Sa) on the response of HL-60 cells to PMA and sphinganine

Cells were incubated for 24 h with 8 nM PMA and varying concentrations of sphinganine and the acid phosphatase activities of the cells in suspension and attached to the Petri dish were determined.

| Period of incubation | Treatment | None | Sa | PMA | PMA + Sa |
|----------------------|-----------|------|----|-----|---------|
| h                    |           |      |    |     |         |
| 0                    |           | 1.6 ± 0.1 | 1.91 ± 0.14 | 1.25 ± 0.05* | 1.95 ± 0.15 |
| 24                   |           | 1.78 ± 0.18 | 3.7 ± 0.7 | 61.3 ± 1.0 | 25.5 ± 7.7 |
| % adherence          |           | 125% | 65% | 93% | 80%     |
| % viability          |           | 95%  | 80% | 80% | 92%     |

*p < 0.05 compared to all other groups.

Acid phosphatase activities of HL-60 cells after treatment with PMA and sphinganine

Cells were incubated for 24 h with 8 nM PMA and varying concentrations of sphinganine and the acid phosphatase activities of the cells in suspension and attached to the Petri dish were determined.

| Treatment          | Acid phosphatase activity | Adherence | % total |
|--------------------|---------------------------|-----------|---------|
|                    | Suspended | Adherent |         |
| No PMA             |           |          |         |
| 0 µM sphinganine   | 1.57 ± 0.98 | 0.06 ± 0.02 | 4 |
| 8 nM PMA           | 0.11 ± 0.05 | 0.52 ± 0.01 | 83 |
| 1 µM sphinganine   | 1.53 ± 0.01 | 0.06 ± 0.03 | 4 |
| 25 µM sphinganine  | 1.25 ± 0.16 | 0.06 ± 0.02 | 5 |
| 5 µM sphinganine   | 0.91 ± 0.16 | 0.04 ± 0.01 | 4 |
| 8 nM PMA           | 0.11 ± 0.05 | 0.52 ± 0.01 | 83 |
| 20 µM sphingamine  | 0.22 ± 0.07 | 0.44 ± 0.11 | 67 |
| 2.5 µM sphingamine | 0.42 ± 0.28 | 0.30 ± 0.04 | 38 |
| 5 µM sphingamine   | 0.52 ± 3.23 | 0.14 ± 0.04 | 21 |

* The activity of the suspended cells on day 0 was 0.84 ± 0.05 nmol/h/dish.
* Percent viability of suspended cells.

This concentration of sphingamine was higher than that resulting in 50% inhibition of attachment, but a higher cell number and shorter incubation time was used for binding. Therefore, less sphingamine would have been taken up by the cells in the binding experiment, and the effective concentration in the membrane would probably also be lower.

To test this possibility, effects of sphingamine on growth, viability, and adherence were evaluated with higher cell numbers used in the binding experiments. Beginning with 10^6 cells/ml, 25 µM sphingamine had no effect on cell growth (after 24 h, cell numbers in the absence and presence of sphingamine were 1.71 ± 0.18 and 1.75 ± 0.15 × 10^6 cells/ml, respectively), and 50 µM was only slightly inhibitory (1.49 ± 0.27 × 10^6 cells/ml). All groups had viabilities >97%. Adherence was reduced by 30% at 25 µM sphingamine and 65% at 50 µM. Therefore, as predicted by the surface dilution model of Hannun et al. (6), the requirement of higher concentrations of sphingamine to inhibit phorbol dibutyrate binding at 10^6 cells/ml can be attributed to the higher cell number.

Inhibition of Diacylglycerol-induced Cell Attachment by Sphinganine—Diacylglycerol, a cell-permeant activator of protein kinase C, also induces HL-60 cell differentiation (12). Diacylglycerol was added at 100 µM with or without sphingamine in an initial loading dose, and additional diacylglycerol was given to the cells in maintenance doses (20 µM) every 2 h for 16 h total. Sphingamine reduced adherence by 50% at approximately 5 µM (Table III). This trend was observed in three separate experiments; however, more precise comparison were thwarted by variability in the response of
the cells to dioctanoylglycerol, which must be added as described above to elicit differentiation (12).

Structural Specificity of the Inhibition—The concentration dependence of sphinganine inhibition of PMA-induced attachment is shown in Fig. 3; results of similar experiments using other long-chain bases are also shown. Sphinganine at 3 μM caused 50% inhibition and sphingosine, the predominant long-chain base found in mammalian sphingolipids (24), caused 50% inhibition at 1 μM. Stearylamine, which is structurally related but lacks the 1,3-dihydroxy groups, effected similar inhibition at 10 μM. Other evidence for the minimal involvement of the 3-hydroxyl was similar inhibition by 3-ketosphinganine (not shown).

Both the free amino and the long alkyl chain were important. Ceramides from bovine brain and N-palmitoylsphinganine were not inhibitory, nor was N-acetylsphinganine at up to 500 μM. Octylamine did not inhibit, nor did another short-chain analog of sphinganine, 1,3-dihydroxy-2-amino-3-phenylpropane.

Effects of Sphinganine on Cell Morphology and Histochemical Parameters—Expression of most other signs of HL-60 cell differentiation requires longer time periods after treatment with PMA. For these experiments, the cells were examined on day 3 for adherence and acid phosphatase activity (Table IV) and morphology and the marker enzymes α-naphtholacetate esterase and acid phosphatase (Table V).

![Fig. 3. Effect of different long-chain bases on HL-60 cell attachment. The different compounds were added to the cells with 8 nM PMA and the percent attachment determined as described under “Experimental Procedures.” The compounds used were sphingosine (So), sphinganine (Sa), stearylamine (St), octylamine (Oct), and ceramide (Cer) (similar results were obtained with bovine brain ceramides and N-palmitoylsphinganine).](image)

**TABLE IV**

Adherence and acid phosphatase activities of HL-60 cells after treatment with PMA and sphinganine for 72 h

| Treatment activity | Adherent cells % | Acid phosphatase activity | Percent |
|--------------------|------------------|---------------------------|---------|
|                    |                  | Suspended | Adherent |            |
| No PMA             |                  |            |          |            |
| 0 μM sphinganine   | 8                | 2.31 ± 0.40 | 0.08 ± 0.03 | 3         |
| 1 μM sphinganine   | 9                | 2.77 ± 0.25 | 0.05 ± 0.03 | 2         |
| 2.5 μM sphinganine | 11               | 2.35 ± 0.11 | 0.05 ± 0.02 | 2         |
| 5 μM sphinganine   | 6                | 2.24 ± 0.01 | 0.04 ± 0.01 | 14        |
| 8 μM PMA           |                  |            |          |            |
| 0 μM sphinganine   | 96               | 0.05 ± 0.01 | 2.20 ± 0.09 | 98        |
| 1 μM sphinganine   | 85               | 0.05 ± 0.01 | 2.95 ± 0.62 | 98        |
| 2.5 μM sphinganine | 74               | 0.06 ± 0.01 | 3.10 ± 0.30 | 98        |
| 5 μM sphinganine   | 70               | 0.12 ± 0.08 | 0.62 ± 0.38 | 87        |

By day 3, most of the viable cells and acid phosphatase activities were adherent (Table IV). The majority of the cells had lost promyelocyte morphology, and resembled macrophages with visibly higher α-naphtholacetate esterase activity (Table V). Acid phosphatase activities were higher for adherent cells when expressed as activity per 10^6 cells, increasing 3-fold upon addition of PMA with and without sphinganine. This is a typical response of HL-60 cells to PMA (25).

None of the few viable cells in suspension had clear signs of differentiation.

**Table V**

Markers of HL-60 cell differentiation after treatment with PMA and sphinganine

| Parameter            | [Sphinganine] (μM) |
|----------------------|--------------------|
| No PMA, suspended cells |                  |
| Promyelocyte morphology | +++ + ++ +++ +    |
| Macrophage morphology | − ± − +             |
| ANA activity         | + + + +             |
| Acid phosphatase     | 0.20 0.25 0.22 0.16  |
| 8 nM PMA, suspended cells |              |
| ANA activity         | NA + + + +           |
| Acid phosphatase     | 0.30 0.19 0.12 0.06  |
| 8 nM PMA, adherent cells |              |
| Promyelocyte morphology | ++ ++ + + +++ +    |
| Macrophage morphology | + + + + + + + + +   |
| ANA activity         | ++ ++ ++ + + + + +  |
| Acid phosphatase     | 0.60 0.64 0.60 0.64  |

*a Too few viable cells to score.

*b Activity in nmol/h/10^6 cells.

![Fig. 4. Kinetics of sphinganine uptake by HL-60 cells. Approximately 1 × 10^6 cells were suspended in medium containing 1 μM [3H]sphinganine and after different intervals of incubation at 37 °C, aliquots were removed for measurement of the radiolabel in the medium and associated with the cells.](image)
proportionate level of long-chain bases in HL-60 cells was quantitated by high performance liquid chromatography. Sphingosine was the major free long-chain base detected (i.e. >80% of the total) and was present at 12.3 ± 1.2 pmol/10⁶ cells. Since leukocytes contain approximately 5 nmol of sphingolipids/10⁶ cells, this corresponds to about 0.2% of the total long-chain bases present in the cells (26). Previous studies have found that free long-chain bases are not artifacts of the isolation or derivatization procedures (22).

**DISCUSSION**

Naturally occurring long-chain (sphingoid) bases inhibit the phorbol ester- and diacylglycerol-induced adherence of HL-60 cells. Since sphinganine has been found to inhibit protein kinase C in vitro, and the structural specificity (6) was similar to the inhibition of differentiation, it appears that the effects of long-chain bases on HL-60 cells are due to inhibition of this enzyme. Further evidence for this was the displacement by sphinganine of phorbol dibutyrate from its receptor, protein kinase C (11).

Inhibition of protein kinase C by other “lipoidal amines,” which include palmitoylcarnitine, polyamines, and CP-46,665-1, an antineoplastic compound, have been reported (27, 28). Treatment of intact HL-60 cells with palmitoylcarnitine blocked PMA-induced cell adhesion but not acid phosphatase activity (29), which suggests that only some of the effects of PMA on HL-60 cells are mediated via protein kinase C. A similar conclusion has been drawn from the different effects of PMA and 1-oleoyl-2-acetylglycerol on HL-60 cells, since both activate the C kinase but only the former induced differentiation (13, 14).

In contrast to these findings, dioctanoylglycerol acts as a good analog of naturally occurring diacylglycerol activators of protein kinase C (12) and caused differentiation of HL-60 cells much like PMA (8). This suggests that PMA is inducing differentiation via protein kinase C activation. A possible explanation for the differences is that PMA and dioctanoylglycerol may be accessible to protein kinase C in all cellular compartments (phosphoproteins are found in cytoplasm and in or around the nucleus) (30) whereas 1-oleoyl-2-acylglycerol is not. Precedents for such intracellular sorting of lipids have been seen in the work of Pagano and Sleight (31). Ebeling et al. (12) found that dioctanoylglycerol displaced all of the phorbol dibutyrate from its receptor in HL-60 cells, whereas 1-oleoyl-2-acetylglycerol only displaced part of it.

Our results do not establish whether or not all of the effects of PMA involved protein kinase C. Since adherent cells differentiated in dishes containing sphinganine, protein kinase C may be responsible for adherence and growth inhibition and the other phenotypic markers arise from other effects of PMA. Alternatively, the lack of differentiation of cells in suspension may indicate that growth inhibition and adherence are prerequisites for these changes. If so, once cells have adhered, sphinganine would have no further effect on differentiation.

Since several amine inhibitors are now known, the relevance of these molecules to the normal in vivo regulation of protein kinase C is worth considering. The levels of free long-chain bases in HL-60 cells suggest that these molecules are endogenous inhibitors of protein kinase C in vivo. Unfortunately, we do not know the subcellular localization of the sphinganine which makes further comparison of intracellular and extracellular concentrations ambiguous.

We have recently demonstrated that free long-chain bases are not detected early in the biosynthesis of sphingolipids from [14C]serine, but appear at later times. This indicates that they arise from sphingolipid breakdown, rather than as intermediates of long-chain base formation. Furthermore, the majority of the newly synthesized long-chain bases were degraded with a half-life of approximately 8 h, which is much more rapid than the presumed rate of sphingolipid turnover (22).

The source of the free long-chain bases of HL-60 cells is unknown, but might be GM₃ ganglioside, which increases during differentiation of HL-60 cells and affects differentiation when added exogenously (32, 33). Since the level of free long-chain bases for normal, immature leukocytes is not known, it is possible that the amount observed in HL-60 cells is high and accounts for its arrested differentiation.

Sphingolipids provide a logical counterbalance to the activation of protein kinase C by diacylglycerols because they are primarily found in the plasma membrane, often interact with receptors, and are well known to undergo changes with differentiation and transformation (34). Long-chain bases may additionally provide an endogenous inhibitor to prevent the “accidental” activation of the C kinase by diacylglycerols that arise from biosynthetic or degradatory pathways and thereby influence the level of diacylglycerol necessary to overcome inhibition.

Acknowledgments—We thank Drs. J. David Lambeth, Carson Loomis, and Barry Ganong, and Emily Wilson for helpful discussions concerning this work, and Dr. Lee Winton for the histological scoring of the cells. We also thank Marion Little for help in preparing this manuscript.

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3 Inhibition of protein kinase C by sphinganine and stearaline may also explain the toxicity of these compounds (23, 35).
Long-chain (Sphingoid) Bases and HL-60 Cell Differentiation

1. Long-chain (Sphingoid) Bases and HL-60 Cell Differentiation

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3. Biologically active long-chain (sphingoid) bases are also synthesized in HL-60 cells during differentiation. These bases may play a role in the regulation of cell differentiation. For example, sphingosine has been shown to inhibit cell division in HL-60 cells, suggesting that it may be involved in the control of cell cycle progression.

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5. These findings have implications for our understanding of the mechanisms underlying cell differentiation and may have applications in the development of new therapeutic strategies for the treatment of disorders associated with altered cell differentiation.

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25. These studies contribute to our understanding of the molecular mechanisms underlying cell differentiation and the role of sphingoid bases in this process.

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