A Mediator Role of Ceramide in the Regulation of Neuroblastoma Neuro2a Cell Differentiation*

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Current studies indicate that ceramide is involved in the regulation of important cell functions, namely cell growth, differentiation, and apoptosis. In the present study, the possible role of ceramide in the differentiation of neuroblastoma Neuro2a cells was investigated. The following results were obtained. (a) Ceramide content of Neuro2a cells, induced to differentiate by retinoic acid (RA) treatment rapidly increased after addition of RA, was maintained at high levels in RA-differentiated cells and returned to the starting levels with removal of RA and reversal of differentiation; under the same conditions, the sphingosine content remained unchanged. (b) After a short pulse with [3H]sphingomyelin or [3H]sphingosine or L-[3H]serine, the metabolic formation of ceramide was markedly higher and more rapid in RA-differentiated than undifferentiated cells. (c) Inhibitors of ceramide biosynthesis (Fumonisin B1, β-chloroalanine and L-cycloserine) diminished the extent of the differentiating effect of RA and concomitantly Cer content decreased. (d) The activity of neutral sphingomyelinase increased after addition of RA, maintained high levels in RA-differentiated cells, and returned to the initial levels with removal of RA. (e) Experimental conditions that cause an elevation of ceramide content (treatment with sphingosine or ceramide or C2-ceramide or bacterial sphingomyelinase) inhibited cell proliferation and stimulated neurite outgrowth; dihydro-analogs of sphingosine, ceramide, and C2-ceramide had no effect on differentiation. (f) Treatment with Fumonisin B1 completely inhibited sphingosine-induced differentiation. These data suggest a specific bioregulatory function of ceramide in the control of Neuro2a cell growth and differentiation and pose the general hypothesis of a mediator role of ceramide in the differentiation of cells of neural origin.

Increasing evidence indicates important roles for molecules of sphingoid nature in the modulation of cell response to different extracellular signals. These molecules include sphingosine, ceramide, and some derivatives of them, N-methylated forms of sphingosine, sphingosine-1-phosphate, and ceramide-1-phosphate (2, 3). Ceramide (N-acetyl-erythro-sphingosine) has been shown to possess bioeffecter properties and to act as a key molecule in a new signal transduction pathway, the sphingomyelin pathway or cycle (4–7). In fact, in several cell lines, especially of the immune system, the activation of certain growth factor receptors by vitamin D3 and cytokines (tumor necrosis factor α, interleukin-1β, and γ-interferon) induces sphingomyelin hydrolysis by activation of sphingomyelinase, resulting in the elevation of the intracellular levels of ceramide. This, in turn, acts as mediator of the elicited physiological effects, presumably by controlling the activity of specific protein kinases and protein phosphatases. In particular, ceramide has emerged as a candidate for regulatory roles in biological processes that are intimately connected to each other, including cell proliferation, oncogenesis, differentiation, and apoptosis (reviewed in Refs. 4–10).

A process that is based on the regulation of proliferation/differentiation and differentiation/apoptosis is neural development. Several cell systems (neurons, glial cells, neurotumoral cells) that undergo morphological and functional differentiation in culture are available to study this process in vitro. Some studies suggest that sphingolipids and sphingoid molecules may be involved in the regulation of neural development. In fact, exogenously added glycosphingolipids are capable to affect differentiation of neurons in primary culture and to induce differentiation of neuroblastoma cells in vitro (for a review, see Ref. 11). Moreover, in cultured hippocampal neurons, sphingolipid biosynthesis is necessary for axonal outgrowth (12), and inhibition of sphingolipid biosynthesis and degradation causes opposite effects on axonal branching (13). Furthermore, induced expression of C17, and/or b-series gangliosides is followed by differentiation of Neuro2a cells (14). Finally, in T9 glioma cells, addition of a cell-permeable ceramide analog (C2-ceramide) causes growth inhibition and formation of processes, in analogy with nerve growth factor, which produced the same effects with a concomitant increase of the cellular level of ceramide (15).

On these premises, we decided to carry out a systematic investigation on the involvement of ceramide as a bioregulator in neural differentiation and the associated processes of proliferation and apoptosis. In the present study, we investigated the role played by ceramide in the differentiation of neuroblastoma Neuro2a cells. Initially, we determined the ceramide concentration and the metabolic routes leading to ceramide in Neuro2a cells induced to differentiate by treatment with retinoic acid, under strictly standardized conditions. Then, the ceramide levels of Neuro2a cells were increased by different treatments, and the effects on differentiation were observed. The data obtained strongly suggest that ceramide is involved in the regulation of Neuro2a cell differentiation.

EXPERIMENTAL PROCEDURES

Materials—All reagents were of analytical grade, and solvents were redistilled before use. Dulbecco’s modified Eagle’s medium (DMEM)1

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1 The abbreviations used are: DMEM, Dulbecco’s modified Eagle’s medium; RA, retinoic acid; Cer, ceramide; sphingosine-1-phosphate, SP; sphingomyelinase, SMase; sphingomyelinase, SMase; C17, C2-ceramide; β-chloroalanine, β-CA; L-cycloserine, L-CS; and Fumonisin B1, FB1.
and FCS (heat-inactivated before use) were from Seromed (Biochrom KG, Berlin). Crystalline bovine serum albumin, N-acetylenuraminic acid, bovine brain sphingomyelin (SM), D-erythrophosphingosine (C18-Sph), O-3-hydroxyphosphingosine (C18-Sph), retinoic acid (RA), FUMONISIN B1, STAPHYLOCOCCUS AUREUS Sph-D-erithrosphingosine, L-serine or dihydro-Ceramide, or Bacterial SM-ase (12, 28) were added to the culture medium just before use. In parallel experiments, 1 μM DL-threo-sphingosine or dihydro-Cer or C2-dihydro-Cer, dissolved as D-erythro-Sph, or C2-Cer, was used.

Effect of Inhibitors of Sphingosine and Ceramide Synthesis on Neuro2a Differentiation—The involvement of the natural endogenous enzyme, SM-ase (100 milliunits/ml), or C2-Cer (1–10 μM) or bacterial SM-ase (C2-Cerase) was assessed running experiments in the presence of Fumonisin B1 (12, 28), the known inhibitor of this enzyme. Morphological Differentiation and Measurement of Neurite-like Processes Outgrowth—The degree of morphological differentiation was assessed by phase-contrast microscopy. In particular, 200–300 cells in 4–5 random fields in each dish were counted, and cells bearing neurite-like processes longer than the major cell body diameter (after treatments not exceeding 8 h), or bearing a neurite with a length at least double that of cell diameter (after treatment longer than 8 h), were scored as differentiated. Data were expressed as neurite-bearing cells (short or long neurites) as percentage of total cells counted. Cell aggregates were not scored, and cells with more than one neurite were only counted once. Cell viability was assessed by the trypan blue exclusion method.

[3H]Thymidine Incorporation—5 × 10^5 cells were grown in 35-mm Falcon dishes in 10% FCS-DMEM. 24 h after plating cells were cultured in 2% FCS-DMEM containing different sphingoids or bacterial SM-ase (as described above) for 24 h. The medium was removed and replaced with 1 ml of DMEM containing 0.5 μCi of [3H]thymidine (29). After 2 h at 37°C, cells were harvested with phosphate-buffered saline and treated with 10% trichloroacetic acid. The insoluble residue, filtered on microfiber glass filters, was counted after fluorography, and radiochromatograms were scanned (Camag TLC densitometer) (23) after visualization with a diphenylamine reagent (37), or a diphenylamine reagent (37), respectively. Statistical significance of differences was determined by the Student t test.
undifferentiated and fully differentiated Neuro2a cells rapidly differentiated cells had a lower content of GM3 and higher glycosphingolipid pattern were recorded; particularly, RA-grown cells. Moreover, and in agreement with previous studies, the inactivation of serine-palmitoyltransferase (a key enzyme in sphingolipid biosynthesis) on RA-induced differentiation was reported. Sph and neutral glycosphingolipids (as percent of the total) is also reported. For details, see “Experimental Procedures.”

**Table I**

| Ganglioside distribution | Control | RA-differentiated |
|--------------------------|---------|-------------------|
| %                        |         |                   |
| G₃₃₃                     | 52.8 ± 5 | 34.8 ± 3.9**      |
| G₃₃₂                     | 40.2 ± 3 | 53.2 ± 5.4**      |
| G₃₃₁                     | 0.8 ± 0.1| 1.4 ± 0.1**       |
| G₀₁₆a                    | 6.2 ± 0.7| 10.6 ± 1.1**      |
| Neutral glycolipid distribution |     |                   |
| Glc-Cer                  | 62.9 ± 6.7| 62.0 ± 7.0        |
| Lac-Cer                  | 29.3 ± 2.8| 26.5 ± 3.1        |
| GgOse₂₃-Cer              | 5.8 ± 0.5| 8.8 ± 0.8**       |
| GbOse₂₃-Cer              | 2.0 ± 0.3| 2.7 ± 0.5         |

**Fig. 1.** Content of endogenous Cer and Sph in Neuro2a cells during RA-induced differentiation. For details on the culture conditions, see “Experimental Procedures.” In some experiments (−RA, dotted line), RA was removed after 24 h, and incubation continued for a further 24 h. Data are the mean values ± S.D. of three experiments in duplicate. ○, control; *, +RA; Δ, −RA.

**Fig. 2.** Incorporation of radioactivity into the total lipid extract, Sph, and water after feeding undifferentiated (control) and differentiated (RA-treated) Neuro2a cells with 40 nM [³H]Sph for different times. Data are the mean values ± S.D. of three experiments in duplicate. White bars, control; stippled bars, RA-treated.

Ceramide and Sphingosine Levels during RA-induced Differentiation of Neuro2a Cells—After RA treatment in low (2%) FCS medium, Neuro2a cells underwent differentiation, which started to be appreciable after 60–120 min and resulted in a very elaborated network of processes after 24–48 h, in agreement with reported findings (22). As shown in Fig. 1, the content of endogenous Cer (1.02 ± 0.11 nmol/mg protein, in undifferentiated cells) increased rapidly with time in Neuro2a cells after RA addition until about 8 h (1.55 ± 0.46 nmol/mg protein). This level remained almost constant in differentiated cells (1.69 ± 0.33 and 1.74 ± 0.36 nmol/mg protein at 24 and 48 h, respectively). The increase of Cer content could be acknowledged at the first investigated time after RA treatment (30 min), indicating that the cell response to RA, in terms of Cer production, was very prompt. The removal of RA from the medium caused reversal of differentiation, as expected (22), with a parallel decrease of the Cer content to the starting level. It is noteworthy that the Sph content (100 ± 12 pmol/mg protein) appeared to be unaffected by RA treatment. Concomitant treatment of Neuro2a cells with RA and 25 μM Fumonisins B1, the inhibitor of Cer synthase (12, 28), reduced but did not block the process of differentiation (Fig. 7, lanes RA and RA + Fumonisins B1) and caused a concomitant decrease of Cer content (1.34 ± 0.12 nmol/mg protein). It is noteworthy that upon RA plus Fumonisins B1 treatment, the Cer content remained substantially higher than that of undifferentiated cells.

Metabolic Source of Ceramide in RA-differentiated Neuro2a Cells—The pathways of Cer generation in RA-differentiated cells were inspected by pulsing Neuro2a cells with [³H] precursors of Cer, namely [³H]Sph or L-[³H]serine or [Sph-³H]SM, and following the formation of [³H] metabolites. As shown in Fig. 2, undifferentiated and fully differentiated Neuro2a cells rapidly incorporated and metabolized [³H]Sph in a time-dependent fashion. The radioactivity present in the total lipid extract, as well as tritiated water released in the culture medium (volatile radioactivity), produced by complete Sph degradation, was a little higher in differentiated than undifferentiated cells and higher correlated with pulse time in both cell types. Conversely, [³H]Sph diminished with pulse time and represented only a minor portion of total incorporated radioactivity. Radioactivity in the total lipid extract plus volatile radioactivity at all the investigated times (about 5% at 30 min and 2% at 120 min) was a minor product of [³H]Sph metabolism, since after 30 and 120 min of pulse it accounted only for about 8% and 12%, respectively, of the total incorporated radioactivity in both undifferentiated and RA-differentiated cells. [³H]Cer represented by far the major radiolabeled metabolite of exogenous [³H]Sph, accounting for more than half of total incorporated radioactivity in both undifferentiated and RA-differentiated cells (Fig. 3). It was produced more rapidly and at a higher extent in differentiated cells, whereas its utilization for the biosynthesis of complex sphingolipids (Glc-Cer, SM, and gangliosides) was higher in undifferentiated cells.

In pulse-chase experiments with L-[³H]serine (Table II), control and RA-differentiated Neuro2a cells incorporated similar amounts of radioactivity into total sphingolipids (mainly Cer, SM, gangliosides, and neutral glycosphingolipids). At all investigated times [³H]Cer represented the major [³H]-sphingolipid (from 52 to 87% of total sphingolipids) and was produced in significantly higher amounts by differentiated than undifferentiated cells. On the basis of these results, the effect of inhibitors of serine-palmitoyl transferase (a key enzyme in sphingolipid biosynthesis) was then investigated. As shown in Table III, treatment of Neuro2a cells with 2.5 mM β-chloroalanine or L-cycloserine resulted in a time-dependent, substantial, although not complete, inhibition of
Ceramide and Neuro2a Cell Differentiation

The feeding experiments with \( \text{Sph}^{-3} \text{H} \)SM provided similar results (Fig. 4). After 2 h of feeding with 4 \( \mu \text{M} \) \( \text{Sph}^{-3} \text{H} \)SM, followed or not by 4 h chase, \( [\text{H}] \)Cer represented the major metabolite in both undifferentiated and RA-differentiated cells, but its metabolic formation was significantly higher in the differentiated ones (2.4-fold and 1.8-fold at 0 and 4 h chase, respectively) (Fig. 4). Conversely, other \( ^3 \text{H} \)metabolites produced during \( \text{Sph}^{-3} \text{H} \)SM metabolism (mainly GLc-Cer, gangliosides, Sph, and water) were markedly lower in differentiated than control cells (Fig. 4). Also in these experiments \( ^3 \text{H} \text{O} \) (which could be properly measured after 4 h chase) constituted a very minor metabolite (about 6.5 and 2.5% of total incorporated radioactivity in undifferentiated and RA-differentiated cells, respectively). When \( \text{Sph}^{-3} \text{H} \)SM was administered under conditions that block endocytosis or lysosomal degradation, \( [\text{H}] \)Cer formation was only partially reduced in both control and RA-differentiated cells (Table IV). This indicates that only a portion of \( \text{Sph}^{-3} \text{H} \)SM is internalized into cells and processed in the lysosomes, the remainder being produced at an extralysosomal level (possibly the plasma membrane). The amount of \( [\text{H}] \)Cer produced in the presence of chloroquine or at 4°C was much higher (2.7-fold and 1.9-fold, respectively) in RA-differentiated than undifferentiated cells. On these premises, and since neuroblastoma cells are known to contain an Mg\(^{2+}\)-stimulated N-SM-ase (41, 42), we investigated the possible role of this enzyme in Cer formation during RA-induced Neuro2a cell differentiation. As shown in Fig. 5, the activity of Mg\(^{2+}\)-stimulated N-SM-ase, increased during RA-induced differentiation, was maximal in the fully differentiated cells and decreased upon removal of RA, paralleling reversal of cell differentiation. Fumonisin B1, at the concentrations used with cells in culture, did not affect the in vitro assay of N-SM-ase.

Table III

| Effect of inhibitors of serine-palmitoyl transferase on RA-induced differentiation of Neuro2a cells | Neurite-bearing cells | Percentage versus control |
|-----------------------------------------------|----------------------|--------------------------|
| \( \text{RA} \) (control) | 31.8 ± 3.6 | 100 |
| \( \text{RA} + \beta\text{-chlooroalnine} | 22.0 ± 2.5 | 69 |
| \( \text{RA} + \text{l-cycloserine} | 20.4 ± 2.5 | 64 |
| 8-h treatment | \( \text{RA} \) (control) | 79.8 ± 7.0 | 100 |
| \( \text{RA} + \beta\text{-chlooroalnine} | 48.2 ± 5.0 | 60 |
| \( \text{RA} + \text{l-cycloserine} | 43.0 ± 4.5 | 54 |

Fig. 3. Incorporation of radioactivity into different metabolites after feeding undifferentiated (control) and differentiated (RA-treated) Neuro2a cells for different times with 40 nM \( [\text{H}] \text{Sph} \). Data are the mean values ± S.D. of three experiments in duplicate. Asterisk, \( p < 0.01 \), RA-treated (stippled bar) versus control (white bar) at the same pulse time.

Table II

| Incorporation of radioactivity (nCi/mg protein) into total sphingolipids | Pulse/chase |
|-------------------------------|-------------|
| Control | RA-differentiated |
| Total sphingolipids | 16.4 ± 1.4 | 18.3 ± 1.9 |
| 2/0 | 21.6 ± 2.6 | 26.8 ± 3.0 |
| 1/2 | 16.1 ± 1.7 | 18.0 ± 1.6 |
| 1/4 | 15.8 ± 1.4 | 16.5 ± 1.2 |
| Ceramide | 12.1 ± 0.9 | 16.0 ± 1.4** |
| 2/0 | 14.2 ± 1.7 | 23.0 ± 2.0** |
| 1/2 | 10.4 ± 0.9 | 14.2 ± 1.2** |
| 1/4 | 8.2 ± 0.8 | 11.3 ± 0.9** |

Fig. 4. Metabolism of exogenous SM in control and RA-differentiated Neuro2a cells. Cells were fed with 4 \( \mu \text{M} \) \( \text{Sph}^{-3} \text{H} \)SM for 2 h followed or not by 4 h chase in the absence of exogenous SM. The radioactivity incorporated into Cer, GLc-Cer, gangliosides, Sph, and water was measured. Data are the mean values ± S.D. of three experiments in duplicate. Asterisk, \( p < 0.01 \), RA-treated versus control at the same chase time.
ent manner (Fig. 6, b and c, and Fig. 8, B and C). When Neuro2a cells were treated with 25 mM Fumonisin B1, the differentiating effect of exogenous Sph was not detectable (Fig. 7, lanes Sp and Sp + Fumonisin B1). It is noteworthy (and as reported above) that treatment with Fumonisin B1 inhibited the differentiating effect of RA as well, but only partially. Treatment of Neuro2a with 1 mM natural Cer, 1 mM C2-Cer, or bacterial SM-ase (100 milliunits/ml) for 2–4 h also resulted in the stimulation of neurite outgrowth (up to 2.5-fold with bacterial SM-ase and natural Cer and 3.5-fold with C2-Cer) (Figs. 8, D–F, and 9). In contrast to the marked inhibitory effect exerted on Sph and RA-induced differentiation, Fumonisin B1 did not affect the differentiation promoted by bacterial SM-ase after 4 and 24 h (106 and 93% with respect to SM-ase treated cells, respectively). As shown in Fig. 10, the administration of exogenous Sph, or natural Cer or C2-Cer or bacterial SM-ase, at the conditions stimulating neuritogenesis, caused a marked diminution of [3H]thymidine incorporation into DNA, indicating inhibition of cell proliferation.

Specificity of Ceramide-induced Differentiation of Neuro2a Cells—To evaluate the specificity of ceramide-induced differentiation, we compared the effects of equimolar concentrations of different sphingoids on this process. As shown in Table V, under the experimental conditions used, where Sph, natural Cer, and C2-Cer were active, either a racemic mixture of threo-dihydro-Sph or C2-dihydro-Cer or dihydro-Cer did not exert any effects on Neuro2a cell differentiation.

DISCUSSION

The first piece of evidence provided by this study is that enhanced levels of Cer are characteristic of RA-differentiated Neuro2a cells. In fact, Cer (but not Sph) content increases during RA-induced differentiation, is maintained at high levels in differentiated cells, and returns to the basal values upon reversal of differentiation. At least two metabolic pathways, de novo Cer biosynthesis and SM degradation, seem to contribute to increasing the Cer content in differentiated cells. Both pathways appear to be more efficient in differentiated cells. Particularly, it is surprising the rapidity and efficiency by which exogenous Sph is acylated to Cer in RA-differentiated cells, with a concomitant lesser degree of Cer metabolic progression to more complex sphingolipids (gangliosides, SM), thus resulting in Cer accumulation. A similar situation has been reported to occur in GH3.C1 cells, where treatment with RA, at concentrations able to inhibit cell proliferation, causes a significant
and prolonged increase of cellular Cer content as a result of increased Sph N-acylation (43). Since Sph content is main-
tained constant during RA differentiation, an increased replen-
ishment of Sph pool either by neosynthesis or sphingolipid
degradation is requested in differentiated cells. The data here
presented on L-[3H]serine metabolism and on the effects of two
inhibitors of serine palmitoyltransferase demonstrate that an
increased neosynthesis of Cer does occur in RA-differentiated
cells. This evidence suggests that the activity of serine palmi-
toyltransferase, a rate-limiting enzyme in de novo Cer biosyn-
thesis (44), is enhanced in RA-differentiated cells.

The results obtained with the [Sph-3H]SM feeding experi-
mements showed that also SM degradation contributes to enhance
the Cer level in differentiated Neuro2a cells. Cer formation in
differentiated cells remains markedly elevated also when en-
docytosis or lysosomal degradation are inhibited, especially in
RA-differentiated cells. This indicates that an extralysosomal,
possibly plasma membrane-bound SM-ase is mainly respon-
sible for the increased SM degradation in RA-differentiated cells.
Consistent with this interpretation is the evidence, here provided, that the activity of the Mg$^{2+}$-dependent N-SM-ase, an enzyme especially concentrated in neuronal tissues (45) and in cells of neuronal origin (41, 42), increases during RA-induced differentiation of Neuro2a cells. It is also worth noting that previous studies have shown that N-SM-ase increases in rat brain parallelly with neuronal maturation (46). A further support to the notion that Cer increase concomitant to RA-induced differentiation is due to stimulation of both de novo biosynthesis and SM degradation comes from the observation that treatment with Fumonisin B1, which inhibits ceramide synthase (50) in neuroblastoma cells, seemingly contrasting our results, can be explained on the basis of the different culture and general experimental conditions used by those investigators. Moreover, no evidence was provided by these authors for any increase of Cer levels under the adopted experimental conditions.

In conclusion, this work provides solid evidence for a bioregulatory implication of ceramide in the differentiation of Neuro2a cells and poses the general question of a mediator role of ceramide in the control and maintenance of differentiation in cells of neural origin.

**REFERENCES**

1. Svennerholm, L. (1980) Adv. Exp. Med. Biol. 125, 11–21
2. Hannun, Y. A., and Bel, R. M. (1993) Adv. Lipid Res. 25, 27–41
3. Kolesnick, R., and Golde, D. W. (1994) Cell 77, 325–328
4. Kolesnick, R. (1992) Trends Cell Biol. 2, 232–236
5. Hannun, Y. A., and Linardic, C. M. (1993) Biochim. Biophys. Acta 1154, 223–236
6. Hannun, Y. A. (1994) J. Biol. Chem. 269, 3125–3129
7. Kolesnick, R. (1994) Mod. Chem. Neuroph. 21, 287–297
8. Merrill, A. H., Jr., and Jr. (1994) in Current Topics in Membranes (Hoekstra, D., ed.) Vol. 40, pp. 361–380, Academic Press Inc., New York
9. Hannun, Y. A., and Obeid, L. M. (1995) Trends Biochem. Sci. 20, 73–77
10. Hakomori, S. (1990) J. Biol. Chem. 265, 17113–17116
11. Tettamanti, G., and Riboni, L. (1994) Proc. Brain Res. 101, 77–100
12. Hard, R., and Futerman, A. H. (1993) J. Biol. Chem. 268, 14476–14481
13. Schwartz, A., Rapaport, E., Trucksis, M., Schirchberg, K., and Futerman, A. H. (1995) J. Biol. Chem. 270, 10990–10998
14. Kojima, N., Kurosawa, N., Nishi, T., Hanai, N., and Tsuji, S. (1994) J. Biol. Chem. 269, 30451–30477
15. Dobrowsky, R. T., and Werner, M. H., Castellino, A. M., Chao, M. V., and Hannun, Y. A. (1994) Science 265, 1596–1599
16. Iwamori, M., Moser, H. W., and Kishimoto, Y. (1975) J. Lipid Res. 16, 322–336
17. Takedomi, T., and Kawamura, N. (1970) J. Biochem. (Tokyo) 68, 475–485
18. Riboni, L., Prinetti, A., Bassi, R., and Tettamanti, G. (1994) FEBS Lett. 352, 323–326
19. Riboni, L., and Tettamanti, G. (1991) J. Neurochem. 57, 1931–1939
20. Riboni, L., Bassi, R., Soninno, S., and Tettamanti, G. (1992) FEBS Lett. 300, 188–192
21. Riboni, L., Caminiti, A., and Tettamanti, G. (1995) J. Neurochem. 64, 451–454
22. Matsumoto, S., Nishida, K., Nakamura, H., and Inagaki, Y. (1994) Adv. Lipid Res. 27, 77–100
23. Riboni, L., Prinetti, A., Pitto, M., and Tettamanti, G. (1993) J. Neurochem. 60, 1175–1183
24. Deedan, R. W., Yu, R. K., and Eng, L. F. (1973) J. Neurochem. 21, 629–839
25. Ji, L., Zhang, G., Uematsu, S., Akahori, Y., and Hiraibayashi, Y. (1995) FEBS Lett. 358, 211–214
26. Sundaram, K. S., and Lev, M. (1984) J. Neurochem. 27, 4197–4198
27. Medlock, K. A., and Merrill, A. H., Jr. (1980) Biochemistry 19, 7079–7084
28. Merril, A. H., Jr., and Jr. (1993) J. Biol. Chem. 268, 27397–27306
29. Colgan, J., E. Krusebeck, A. M., Margules, D. H., Shesvach, E. M., Strober, W. (eds.) (1991) Current Protocols in Immunology, Vol. 1, pp. 152–160, Greene Publishing and Wiley-Interscience, New York
30. Press, J., Loomis, C. R., Bishop, W. R., Stein, R., Niedel, J. E., and Bel, R. M. (1986) J. Biol. Chem. 261, 8597–8600
31. Ohta, H., Yatomi, Y., Sweeney, E. A., Hakomori, S., and Igarashi, Y. (1994) FEBS Lett. 355, 267–270
32. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
33. Mooibroek, M. J., Cook, H. W., Clarke, J. T. R., and Spence, M. W. (1985) J. Neurochem. 44, 1551–1558
34. Svennerholm, L. (1957) Biochim. Biophys. Acta 24, 604–611
35. Vance, D. L., and Sweeney, C. C. (1967) J. Lipid Res. 8, 621–630
36. Partridge, S. M. (1948) Biochem. J. 42, 238–248
37. Harris, G., and MacWilliam, Y. C. (1954) Chem. Ind. 39, 249–250
38. Dodge, J. T., and Phillips, G. B. (1967) J. Lipid Res. 8, 667–675
39. Bartlett G. R. (1959) J. Biol. Chem. 234, 466–468
40. Kadowaki, H., Evans, J. E., Rys-Sikora, E., and Koff, R. (1990) J. Neurochem. 54, 2125–2137
41. Spence M. W., Wakkary, J., Clarke, J. T. R., and Cook, H. W. (1982) Biochim. Biophys. Acta 719, 162–164
42. Das, D. V. M., Cook, H. W., and Spence, M. W. (1984) Biochim. Biophys. Acta 777, 339–342
43. Kalén, A., Borchardt, R. A., and Bell, R. M. (1992) Biochim. Biophys. Acta 1125, 90–96
44. Merrill, A. H., Jr., and Jones, D. (1990) Biochim. Biophys. Acta 1044, 1–12
45. Gatt, S. (1976) Biochem. Biophys. Res. Commun. 68, 235–241
46. Spence, M. W., and Burgess, J. K. (1978) J. Neurochem. 30, 917–919
47. Sugiyama, E., Uemura, K., Hara, A., and Taketomi, T. (1993) J. Biochem. (Tokyo) 113, 467–472
48. Okazaki, T., Bell, R. M., and Hannun, Y. A. (1989) J. Biol. Chem. 264, 19076–19080
49. Tsuji, S., Yamashita, T., Tanaka, M., and Nagai, Y. (1988) J. Neurochem. 50, 414–423
50. Uemura, K., Hara, A., and Taketomi, T. (1993) J. Biochem. 114, 610–614