1-Methylthiodihydroceramide, a Novel Analog of Dihydroceramide, Stimulates Sphinganine Degradation Resulting in Decreased de novo Sphingolipid Biosynthesis*

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1-Methylthiodihydroceramide (10 µM) decreased de novo ceramide biosynthesis by about 90% in primary cultured cerebellar neurons. Accordingly, de novo formation of sphingomyelin and of glycosphingolipids, all of which contain ceramide in their backbone, was reduced in a time- and concentration-dependent manner by up to 80%. Complex sphingolipid synthesis was restored upon addition of dihydroceramide or ceramide, in micromolar concentrations, to the culture medium, suggesting that none of the glycosyltransferases involved in glycosphingolipid biosynthesis is inhibited by this analog. Assays of the enzymes catalyzing sphinganine biosynthesis, as well as its N-acylation to form dihydroceramide, revealed that they were also not affected. In contrast, there was a 2.5-fold increase in the activity of sphinganine kinase. Reduction of de novo sphingolipid biosynthesis by 1-methylthiodihydroceramide is therefore due to its ability to deplete cells of newly formed free sphinganine. As a consequence of depletion of sphinganine levels, 1-methylthiodihydroceramide disrupted axonal growth in cultured hippocampal neurons in a manner similar to that reported for direct inhibitors of sphingolipid synthesis; thus, there was essentially no axon growth after incubation with 1-methylthiodihydroceramide between days 2 and 3, and co-incubation with short acyl chain analogs of ceramide (5 µM) antagonized the inhibition of growth. Interestingly, the β-erythro and the β-threo isomers were equally effective, but the corresponding free base as well as other structurally related compounds did not affect either sphingolipid biosynthesis or neuronal growth.

Sphingolipids (SLs) are found in all eukaryotic cells, where they are primarily components of the plasma membrane. SLs contain a ceramide backbone, which anchors them in the outer leaflet of the lipid bilayer. The ceramide backbone can be modified by attachment of phosphorylcholine, to form sphingomyelin (SM), or by attachment of one or more sugar residues, to form glycosphingolipids (GSLs). GSLs form cells- and species-specific profiles known to change characteristically during development, differentiation, and transformation, suggesting that they play a role in cell-cell interactions and in cell adhesion (1). Gangliosides, the sialic acid containing GSLs, are particularly abundant in neuronal cells, and their involvement in neuritogenesis and possibly synaptogenesis has been extensively studied (2, 3). During the last few years studies have also been initiated to examine the role of endogenous SLs in neuronal growth (4–7). In these studies inhibitors of SL biosynthesis, such as fumonisin B₁ (FB₁) and PDMP (N-\(\text{threo}\)-1-phenyl-2-decanoylamino-3-morpholino-1-propanol), as well as inhibitors of lysosomal GSL degradation, like conduritol B-epoxide (5), have been employed. In addition, evidence has emerged during the last few years that SL metabolites such as ceramide (Cer), sphingosine, and sphingosine 1-phosphate (SPP) play an important role as intracellular signaling molecules for a variety of different targets. Sphingosine and SPP, originally proposed as negative regulators of protein kinase C (9), were shown to play alternative signaling roles as mitogenic second messengers (8). Cer is involved in what has become known as the “sphingomyelin cycle” (10). For example, Cer serves as a mediator of cellular senescence (11), apoptosis, and differentiation in many cell types (12, 13). The increasing amount of data concerning the role of SL metabolites in cellular signal transduction strongly suggests that SL metabolism is tightly regulated. The identification of factors interfering with SL metabolism and the examination of their mode of action is therefore of great importance.

Much of the current knowledge on SL metabolism has been derived from studies with compounds specifically inhibiting defined steps of SL biosynthesis (14). Taking into consideration that dihydroceramide (DH-Cer) does not mimic the effects of Cer in signaling pathways (15, 16), we are studying the effects of DH-Cer analogs on SL biosynthesis and on neuronal growth. We now demonstrate that treatment of cerebellar neurons with 1-methylthiodihydroceramide (1-MSDH-Cer) strongly interferes with de novo Cer synthesis, and hence SL formation, by stimulating the catabolism of sphinganine, a vital precursor of medium; FB₁, fumonisin B₁; GlcCer, glucosylceramide; GSLs, glycosphingolipids; HPLC, high performance liquid chromatography; MEM, minimum essential medium; PDMP, N-\(\text{threo}\)-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; SPP, sphingosine 1-phosphate; the term sphinganine is used for dihydrosphingosine.
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SL biosynthesis. Furthermore, this analog significantly reduces the rate of axonal growth in cultured hippocampal neurons, in a manner similar to that reported for compounds that directly inhibit Cer synthesis (4, 5, 13, 17).

EXPERIMENTAL PROCEDURES

Materials—Six-day-old NMRI (Naval Marine Research Institute) mice were obtained from Dr. Brigitte Schmitz from the Institut für Anatomie und Physiologie der Haustiere of the University of Bonn, Germany. Embryonic day 18 Wistar rats were from the Weizmann Institute Breeding Center, Rehovot, Israel.

The DH-Cer analogs, 1-MSDHCer (the 2-erythro and the 3-threo stereoisomer), as well as the corresponding free base, 1-deoxy-DH-Cer and pyrrolidine-DH-Cer (see Fig. 1), were synthesized in our laboratory as described (18). 2-3H-Sphinganine was synthesized in our laboratory according to Clasen (19). Semi-truncated 3H-Cer labeled Cer and DH-Cer were obtained by N-acetylation of sphingosine and sphinganine, respectively, with 1-14C-octanoic acid (162.8 GBq/mol) as described (18). 3-14CSerine (2.0 GBq/mmol) was purchased from Amersham-Buchler (Braunschweig, Germany). Fumonisin B1, trypsin, L-serine, and 1,2-3H-sphingosine were from Cytogen (Berlin, Germany). DNase was from Boehringer Mannheim (Mannheim, Germany). L-Cholesterol RP-18 and Silica Gel 60 were purchased from Merck (Darmstadt, Germany). Ultima Gold was from Packard (Groningen, Netherlands). All other chemicals were of analytical grade and obtained from Sigma (Deisenhofen, Germany) or Merck (Darmstadt, Germany).

Sphingosine and Sphinganine Mass—The values obtained for sphingosine and sphinganine were corrected for free sphingosine and free sphinganine, as determined above, to give the complex SL content (total minus free).

Enzyme Assays—Cells homogenates obtained by sonication of the cell pellets and Granule cells—Cells were cultured from cerebella of 6-day-old mice according to the method of Trenkner and Sidman (20). Cells were obtained by mild trypsinization (0.05%, w/v), and dissociated by repeated passage through a constricted Pasteur pipette in a DNase solution (0.1%, w/v). The cells were then suspended in MEM containing 10% heat-inactivated horse serum, and plated onto poly-L-lysine-coated 35-mm-diameter Petri dishes (Costar) (6 × 10^5 cells/dish). 24 h after plating, cytosine arabinoside was added to the medium (4 × 10^-3 M) to arrest the division of non-neuronal cells (21).

Hippocampal neurones were cultured at low density as described (22) with some modifications (5). In brief, the dissected hippocampi of embryonic day 18 rats (Wistar) were dissociated by trypsinization (0.025%, w/v, for 15 min at 37 °C). The tissue was washed in Mg2+/Ca2+-free Hank's balanced salt solution (Life Technologies, Inc.) and dissociated by repeated passage through a constricted Pasteur pipette. Cells were plated in MEM with 10% horse serum, at a plating density of 6,000 cells per 13-mm glass coverslip that had been precoated with poly-L-lysine (1 mg/ml). After 3–4 h, coverslips were transferred into 100-mm Petri dishes (Nunc) containing a monolayer of astroglia. Cultures were main-
**RESULTS**

1-MSDH-Cer Decreases de Novo Ceramide and More Complex Sphingolipid Formation in Primary Cultured Cerebellar Neurons—The effect of four different short chain DH-Cer analogs (Fig. 1) on de novo Cer biosynthesis in primary cultured cerebellar neurons was studied by examining the incorporation of L-[3-14C]serine into cellular Sls. After 48 h preincubation with 10 μM each analog and an additional 24 h of labeling, a drastic reduction of Cer formation (>80%) was observed only in the presence of 1-MSDH-Cer (Fig. 1). As illustrated in Fig. 1 the inhibitory effect of 1-MSDH-Cer on Cer formation was not stereospecific since both stereoisomers were equally effective. The L-erythro- and the L-threo isomer caused an 82% and 87% decrease of Cer labeling, respectively (compounds 1 and 2 in Fig. 1). In contrast, Cer formation was almost unchanged (about 15% decrease) in the presence of pyrrolidine

-1-MSDH-Cer and with the free base (the deacylated amine). Fig. 2 depicts the results obtained with both stereoisomers of 1-MSDH-Cer and with the free base (the decylated 1-MSDH-Cer). Both L-threo- and D-erythro-1-MSDH-Cer strongly reduced incorporation of [14C]serine into cellular Sls (by 78 and 73%, respectively), whereas the free base was much less effective (32% reduction of overall SL labeling). The other two DH-Cer analogs (1-deoxy-DH-Cer and pyrrolidine-DH-Cer) were even less, if at all, effective (not shown). Taken together our results indicate that the various effects of the respective compounds on Cer biosynthesis (see Fig. 1) were paralleled by analogous effects of these compounds on ongoing SL formation. This is not surprising since Cer is the direct biosynthetic precursor of cellular Sls.

I-MSDH-Cer Decreases [14C]Serine Incorporation into Cellular Sphingolipids in a Dose- and Time-dependent Manner—Newly synthesized Sls were labeled for 24 h with L-[3-14C]serine in the presence of increasing concentrations (up to 50 μM) of 1-MSDH-Cer, after 48 h preincubation with the DH-Cer analog. A drastic decrease of radioactive labeling of Sls (∼60%) was observed with concentrations as low as 5 μM 1-MSDH-Cer (Table I). In the presence of 10 μM 1-MSDH-Cer, levels of radiolabeled SM were reduced by ∼90%, but labeling of GSL was less affected, by ∼80% for GlcCer and ∼50% for ganglioside GQ1b. The radiolabeled lipid, SX (see Fig. 2), was affected even less (by ∼40%, Table I). A concentration of 50 μM 1-MSDH-Cer was cytotoxic after 72 h of treatment, with fragmentation of neurites and of the plasma membrane.

Studies of the time course of the effect of 10 μM 1-MSDH-Cer on the incorporation of radiolabeled serine into cellular Sls...
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Primary cultured cerebellar neurons were incubated in the absence (control) or presence of 1-MSDH-Cer (10 μM). Medium was renewed every 24 h. After 72 h cells were harvested and enzyme activities determined in the cell homogenate as described under “Experimental Procedures.” Results are means of three different experiments with at least double determinations.

| Lipid         | 1-MSDH-Cer (10 μM) | Control |
|---------------|--------------------|---------|
| 3-Dehydroceramide synthase | 181 ± 18.2 | 150 ± 21.7 |
| Sphinganine N-acetyltransferase | 51 ± 12.2 | 54 ± 15.8 |

It seems unlikely that this slight reduction of SPT activity (by about 17%) is responsible for the reduction of [14C]serine incorporation into SLs (see Fig. 2 lane 3).

These values are from one experiment with triple determinations.

...treated controls. Results not shown).

1-MSDH-Cer Does Not Inhibit Biosynthetic Enzymes—The de novo biosynthetic pathway of SLs begins with the condensation of serine and palmitoyl-CoA by serine palmitoyltransferase to form 3-dehydroceramide. Reduction of 3-dehydroceramide by a NADPH-dependent reductase leads to formation of sphinganine, which is N-acetylated to form DH-Cer. Preincubation of cells with 1-MSDH-Cer for 72 h had no effect on any of these enzyme activities when measured in vitro compared with untreated controls (Table II). Furthermore, direct addition of 1-MSDH-Cer to the in vitro enzyme assay, in concentrations up to 100 μM, also had no effect on any of the enzyme activities (not shown).

As shown above, addition of 10 μM 1-MSDH-Cer reduced de novo biosynthesis of all SLs in cerebellar neurons, but to a different extent, using [14C]serine to label SLs. To determine whether these results were due to alteration of Cer metabolism, neurons were labeled with 28 mCi [14C]ceramide and processed to the characteristic profile of GSl (Fig. 3), demonstrating that 1-MSDH-Cer does not interfere with any of the glycosyltransferases involved in GSL biosynthesis. Furthermore, the formation of labeled SM and dihydrosphingomyelin was not altered when Cer or DH-Cer was added to the culture medium. As illustrated in Fig. 3, SM migrated as a double band only when labeled DH-Cer was used as a biosynthetic precursor of cellular SLs, indicating that, as previously suggested (29), some desaturation might also occur at the level of SM.

1-MSDH-Cer Depletes Cells of Free Sphinganine by Stimulating Its Catabolism—There are two potential mechanisms to decrease SL biosynthesis as follows: (i) by inhibition or down-regulation of biosynthetic enzymes, and (ii) by stimulation of the degradation of a vital precursor. As shown above, the first possibility can be excluded as a means to explain the mechanism of action of 1-MSDH-Cer. We therefore examined whether 1-MSDH-Cer enhances the rate of SL degradation. The precursor for (DH)Cer formation in the de novo biosynthetic pathway is sphinganine, and the rate-limiting enzyme for sphinganine degradation is sphinganine kinase. We measured sphinganine kinase activity in vitro in the presence and absence of 1-MSDH-Cer, as well as in the cytosol of cells cultured in the presence of the analog for various periods. In the in vitro assay, 100 μM (10-fold the concentration used in the cell culture experiments) 1-MSDH-Cer caused only a slight stimulation of enzyme activity to 138 ± 16% (data not shown). However, pretreatment of cells with 10 μM 1-MSDH-Cer from 5 min up to 72 h clearly stimulated sphinganine kinase activity in a time-dependent...
DH-Cer metabolism. [14C]st-Cer, cannot explain at present the heavy labeling of this band as a result of the band marked st-SX 6 lanes 5 saturated SL species formed from semi-truncated DH-Cer (cated ceramide (RF hydrophobicity, the st
90% reduction of the free sphinganine concentration when addition of 1-MSDH-Cer antagonized this effect causing a degradation by inhibiting its phosphorylation. Simultaneous controls, demonstrating that it strongly interfered with its cellular level of free sphinganine about 2.5-fold compared with

- C12-sphinganine alone increased the intracellular level of free sphinganine, cells were preincubated for 24 h with 25 µM FB1 (erythro-C12-sphinganine) and L-t-Sa. Data are means ± S.E. from two different experiments with double determinations. 100% free sphinganine equals 1.2 nmol/mg protein.

Figure 3. Effect of 1-MSDH-Cer on the metabolism of exogenous (dihydro)ceramide in primary cultured neurons. Cerebellar neurons were incubated for 48 h in the absence (lanes 2, 3, and 5) or presence of 10 µM 1-MSDH-Cer (lanes 1, 4, and 6). Medium was renewed every 24 h. [14C]Serine (lanes 1 and 2), 28 µM [14C]semi-truncated ceramide (lanes 3 and 4), or [14C]semi-truncated dihydroceramide (lanes 5 and 6) were subsequently added to the culture medium. After an additional 24 h, cells were harvested and lipids analyzed as described under “Experimental Procedures.” The RP values of authentic SLs and semi-truncated (st) SLs are given. Note that due to lower hydrophobicity, the RP values of semi-truncated SLs are lower than those of their physiologic counterparts, and the RP values of the desaturated semi-truncated SLs biosynthesized from exogenous semi-truncated Cer (lanes 3 and 4) are slightly lower than those of the respective saturated SL species formed from semi-truncated DH-Cer (lanes 5 and 6) as well. Cer, ceramide; for other abbreviations see Table I.

Figure 4. Effect of 1-MSDH-Cer on sphinganine kinase activity from primary cultured neurons. Primary cultured cerebellar neurons were incubated for the indicated times in the absence (C, control) or presence of 10 µM 1-MSDH-Cer. Medium was renewed every 24 h. Cells were harvested, and sphinganine kinase was measured as described under “Experimental Procedures.” Data are means ± S.E. from three different experiments. 100% sphinganine kinase activity of untreated controls corresponds to ~20 pmol/min/mg.

Figure 5. Effect of 1-MSDH-Cer, FB1, and L-threo-C12-sphinganine on the level of free sphinganine in cultured neurons. Primary cultured cerebellar neurons were incubated for 24 h in MEM containing 25 µM FB1. Then the medium was discarded, and the cells were rinsed three times and chased in DMEM containing no further addition (C, controls), 25 µM FB1, or 20 µM L-threo-C12-sphinganine (C12-sphinganine) in the absence (white bars) or presence (black bars) 10 µM 1-MSDH-Cer, as indicated. After an additional 24 h, cells were harvested and free sphinganine determined in the cellular lipid extracts as described under “Experimental Procedures.” The retention times of the o-phthalaldehyde derivative of L-threo-C12-sphinganine (C12-sphinganine) and C12-sphinganine used as an internal standard were 2.4, 8.3, and 13.9 min, respectively. Data are means ± S.E. from two different experiments with double determinations. 100% free sphinganine equals 1.2 nmol/mg protein.
neurons were incubated with 10 μM of 1-MSDH-Cer. Medium was renewed every 24 h. After the indicated periods cells were harvested and lipids extracted. Sphingosine and sphinganine released after acid hydrolysis were determined as described under "Experimental Procedures."

![Image](image-url)

**Fig. 6.** Morphological characteristics of hippocampal neurons. Camera lucid drawings of representative cells at 72 h in culture after addition at 48 h of either 10 μM L-threo-1-MSDH-Cer or 10 μM L-threo-1-MSDH-Cer together with 5 μM Ceramide. The bar corresponds to 50 μm.

**TABLE III**

| Treatment (long chain base) | Period of incubation | 24 h | 72 h | 96 h |
|----------------------------|---------------------|------|------|------|
| Control                    |                     | 212 ± 27 | 211 ± 39 | 215 ± 34 |
| Sphingosine                | 1-MSDH-Cer          | 18 ± 3.5 | 15 ± 3.2 | 17 ± 1.9 |
| Sphinganine                | 1-MSDH-Cer          | 216 ± 31 | 181 ± 39 | 181 ± 28 |
| Sphinganine                | 1-MSDH-Cer          | 16 ± 4.0 | 14 ± 3.6 | 14 ± 2.2 |
*Mean ± range of two experiments with double determinations each.

**TABLE IV**

| DH-Cer analog (10 μM) | C6-NBD-Cer (5 μM) | Branch points per axon |
|-----------------------|-------------------|------------------------|
| Control               | –                 | 1.22 ± 0.12            |
| L-threo-1-MSDH-Cer    | –                 | 0.61 ± 0.07            |
| L-erythro-1-MSDH-Cer  | +                 | 1.26 ± 0.13            |
| D-erythro-1-MSDH-Cer  | –                 | 0.73 ± 0.08            |
| 1-Deoxy-DH-Cer        | –                 | 1.39 ± 0.14            |
| 1-Deoxy-DH-Cer        | +                 | 1.06 ± 0.11            |
| Pyrrolidine-DH-Cer    | –                 | 1.29 ± 0.13            |
| Pyrrolidine-DH-Cer    | +                 | 1.20 ± 0.14            |

**DISCUSSION**

In this study, we have described an SL analog that stimulated the activity of sphinganine kinase in vivo, resulting in increased rates of sphinganine degradation and, as a consequence, significantly reduced rates of SL synthesis. This novel analog, 1-MSDH-Cer, may provide an important new means of manipulating levels of SL synthesis without accumulation of toxic SL intermediates, such as sphinganine and sphingosine that accumulate upon incubation with FB1.

Previous studies from our laboratory have shown that exogenous sphingosine homologs of different chain length as well as the biosynthetically stable azidosphingosine (23, 27) and the cis-configured 4-methylsphingosine (35) cause a decrease of de novo SL biosynthesis in primary cultured neurons by specifically interfering with serine palmitoyltransferase activity. Our results revealed a striking correlation of the relative order and magnitude of the sphingosine analogs in terms of the percentage of the phosphorylated metabolites in cultured cells and the inhibitory effect of the respective compound on serine palmitoyltransferase activity, supporting the idea that 1-phosphates are the link between sphingosine metabolites and serine palmitoyltransferase regulation (35).

In the present study we examined the effects of truncated DH-Cer analogs (with a chain length corresponding to 12 carbons) on neuronal morphology and trypan blue exclusion, cells were still viable after these long times of treatment with 1-MSDH-Cer (10 μM).

**1-MSDH-Cer Blocks Axonal Growth—**Although cerebellar neurons cultured according to the methods described above are useful for biochemical analysis (23, 31), they are less useful for accurate determination of parameters of neuronal growth, for which cultured hippocampal neurons (22) have proved an invaluable tool (4, 5, 13, 17). It has been previously demonstrated that the synthesis of GlcCer from Cer is required to sustain normal axonal growth (4, 5, 13) and also axonal growth stimulated by growth factors (17) in hippocampal neurons; these studies were performed using FB1, an inhibitor of (DH)- Cer synthesis (32), and PDMP, an inhibitor of GlcCer synthesis (33, 34). Incubation with either inhibitor at 48 h in culture resulted in a decrease in the length of the axonal plexus and a reduction in the number of axonal branch points compared with untreated cells at 72 h in culture. Addition of C6-NBD-Cer together with the inhibitors at 48 h reversed the inhibitory effect of FB1 on axonal growth (4, 13, 17) but not of PDMP (13, 17).

We have now examined the effect of four different DH-Cer analogs (see Fig. 1) on axonal growth (Table IV). Hippocampal neurons were incubated with 10 μM each analog between 48 and 72 h in culture. The number of axonal branch points was measured at 72 h. Both stereoisomers of 1-MSDH-Cer completely blocked axonal growth between 48 and 72 h, as indicated by the 50% reduction of the number of axonal branch points per cell at 72 h compared to control cells; however, the other two analogs exhibited no effect (Table IV and Fig. 6).

**Similar to results obtained with FB1 (4, 13, 17), the effect of 1-MSDH-Cer could be antagonized by the simultaneous addition of C6-NBD-Cer to the medium, confirming that both stereoisomers of 1-MSDH-Cer decrease levels of DH-Cer and Cer biosynthesis (Fig. 6 and Table IV).**
bon atoms in the sphingoid and in the fatty acid moiety, respectively) with changed polar head groups on SL metabolism as well as on neuronal growth using primary cultured neuronal cells. All of the analogs tested lack the 1-hydroxyl group and are thus resistant to glycosylation as well as to phosphorylation at this position. However, only 1-MSDH-Cer was found to exhibit a pronounced effect on both de novo SL biosynthesis and consequently on neuronal growth. The effect of 1-MSDH-Cer was not stereospecific. Both, the D-erythro and the L-threo isomer exerted similar effects on de novo SL biosynthesis and on axonal growth in cerebellar and hippocampal neurons. The corresponding free base (1-methylthiodihydrosphingosine) had almost no effect. Together, these results suggest that several structural requirements are essential for the observed effect of 1-MSDH-Cer.

In contrast to the sphingoid bases previously studied, reduction of de novo SL biosynthesis by 1-MSDH-Cer was not due to a decrease of serine palmitoyltransferase activity, the rate-limiting enzyme of SL biosynthesis, but rather to the stimulation of sphinganine kinase, the rate-limiting enzyme of long chain base degradation. Both, DH-Cer and sphingosine are intermediates of SL metabolism, but the former is primarily a biosynthetic metabolite, whereas the latter is exclusively a degradation product (29, 36). It therefore appears that there might exist two different mechanisms for regulation of SL biosynthesis. First, accumulation of an SL degradation product (SPP) leads to down-regulation of serine palmitoyltransferase (27, 35); alternatively, accumulation of a biosynthetic intermediate (DH-Cer) stimulates the catabolism of its biosynthetic precursor (Fig. 7). An unusual degradation of sphinganine also occurs in the presence of FB1, which causes an accumulation of sphinganine, a biosynthetic intermediate, by inhibiting sphinganine N-acyltransferase (37) (see also Fig. 7). In that study, however, sphinganine degradation was indirectly evaluated by its utilization for phosphatidylethanolamine synthesis and not by direct measurements of sphinganine kinase activity. The fact that 1-MSDH-Cer does not directly stimulate sphinganine kinase upon addition to the in vitro enzyme assay but only after preincubation of the cultured neurons suggests that it does not directly interact with the enzyme on molecular level. More likely, up-regulation of sphinganine kinase activity by 1-MSDH-Cer seems to be a complex process which requires cell integrity and longer incubation. Stimulation of sphingosine kinase of human erythroleukemia cells by phorbol 12-myristate 13-acetate also required preincubation of cells for at least 18 h with the phorbol ester (38). Unlike growth factors (39) or G_{M1} (40) which rapidly and transiently increase sphingosine kinase activity, stimulation of sphingosine kinase by phorbol ester appeared to be dependent on transcriptional as well as on translational events (38).

Reduction of SL biosynthesis by about 80% as well as some reduction of total SL mass after long incubation times (72–96 h) caused by 1-MSDH-Cer (10 μM) is quite similar to the results obtained in cerebellar neurons with FB1 (25 μM), known to specifically inhibit sphinganine (sphingosine) N-acyltransferase (41). In contrast to FB1 which caused a 20-fold increase of the amount of free sphinganine, 1-MSDH-Cer almost completely depleted the cells of sphinganine within 24 h. Moreover, when both compounds, FB1 and 1-MSDH-Cer, were simultaneously supplied, the latter completely prevented sphinganine accumulation caused by the former.

It is intriguing to note that 1-MSDH-Cer has a similar effect on axonal growth of hippocampal neurons as that reported for FB1 (4, 5). Both reduce axonal length and the number of axonal branch points. In addition, the effect of both compounds on axonal growth can be fully reversed by the addition of C_{6-NBD}-Cer to the culture medium, supporting the idea that endogenous SLs in general (4) or Cer (6) and GlcCer (5, 13) in particular are involved in neuronal growth. These results therefore confirm the usefulness of 1-MSDH-Cer as an investigative tool for the manipulation of endogenous SL pathways. The carcinogenicity of fumonisins which most probably is due to the mitogenic effect of the sphingoid bases (42), known to accumulate as a result of the inhibited sphinganine (sphingosine) N-acyltransferase (32), could be overcome with 1-MSDH-Cer. Moreover, this compound might be clinically useful in the therapy of SL storage diseases.

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**Fig. 7. Pathway of sphinganine metabolism.** Circled triangle, up-regulation; ○, inhibition.
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