Sphingosine-1-phosphate, a Metabolite of Sphingosine, Increases Phosphatidic Acid Levels by Phospholipase D Activation*

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Sphingosine and sphingosine-1-phosphate, metabolites of membrane sphingolipids, have recently been shown to stimulate release of calcium from internal sources and to increase proliferation of quiescent Swiss 3T3 fibroblasts (Zhang, H., Desai, N. N., Olivera, A., Seki, T., Brooker, G., and Spiegel, S. (1991) J. Cell Biol. 114, 155–167). The present study demonstrates that mitogenic concentrations of sphingosine induce early increases in sphingosine-1-phosphate levels which precede the increase in the potent mitogen, phosphatidic acid. Sphingosine-1-phosphate itself induces a more rapid increase in phosphatidic acid, thus suggesting that it may mediate the effects of sphingosine on phosphatidic acid accumulation. The concentration dependence for the formation of phosphatidic acid induced by sphingosine-1-phosphate correlates with its effect on DNA synthesis. Similar to sphingosine, sphingosine-1-phosphate also stimulates the activity of phospholipase D, although a significant effect is observed at a much lower concentration. However, in contrast to previous reports with sphingosine, sphingosine-1-phosphate does not inhibit the phosphatidic acid phosphohydrolase activity in cell homogenates. Thus, in addition to its effect on mobilization of calcium, sphingosine-1-phosphate can increase the level of phosphatidic acid, most likely via activation of phospholipase D. We suggest that sphingosine-1-phosphate mediates the effect of sphingosine on phosphatidic acid accumulation in Swiss 3T3 fibroblasts and may regulate cellular proliferation by affecting multiple transmembrane signaling pathways.

Long chain sphingoid bases are the basic building units of sphingolipids, which are important membrane lipid constituents. Recently, sphingolipids have been implicated in the regulation of diverse cellular functions (reviewed in Sweeney, 1986; Hakomori, 1990; Merrill, 1991; Hannun and Bell, 1987). Sphingosine (4-trans-sphingenine), a naturally occurring sphingoid breakdown product of cellular sphingolipids (Wilson et al., 1988), has recently emerged as a bioregulatory molecule (Hannun and Bell, 1989; Merrill and Stevens, 1989). Sphingosine was found to inhibit protein kinase C (Hannun et al., 1986; Wilson et al., 1986; Merrill et al., 1986), a pivotal regulatory enzyme in many cellular processes, and therefore has been implicated as a negative modulator of transmembrane signaling, opposing the action of diacylglycerol which stimulates protein kinase C (Hannun and Bell, 1987, 1989; Merrill and Stevens, 1989). Further studies demonstrated that sphingosine has complex biological effects, many of which seem to be protein kinase C-independent (Faucher et al., 1988; Davis et al., 1988; Jefferson and Schulman, 1988; Winicov and Gershengorn, 1988; Krishnamurthi, et al., 1988; Igarashi et al., 1989; Arnold and Newton, 1991; Zhang et al., 1990a). We have previously reported that sphingosine stimulates proliferation of quiescent Swiss 3T3 fibroblasts acting in a fundamentally different, protein kinase C-independent pathway (Zhang et al., 1990a). The mitogenic effect of sphingosine was accompanied by an increase in the levels of phosphatidic acid (PA) (Zhang et al., 1990b), which is a potent mitogen for a variety of cell types (Yu et al., 1988; Moolenaar et al., 1986; Zhang et al., 1990b) and may function as an intracellular second messenger (Exton, 1990; Bocckino et al., 1991). Recently, we found that, in addition to phosphatidic acid, mitogenic concentrations of sphingosine also induced rapid and sustained increases in sphingosine-1-phosphate levels (Zhang et al., 1991). Sphingosine-1-phosphate has long been known to be produced from sphingosine by the action of a specific kinase in the major sphingolipid degradation pathway (Stöffler et al., 1970, 1973). Sphingosine-1-phosphate is rapidly degraded to trans-2-hexadecenal and phosphorylethanolamine by the action of a microsomal lyase (Stöffler et al., 1970), reported to be located in the endoplasmic reticulum (Van Veldhoven and Mannetta, 1991). Recently, sphingosine-1-phosphate has been proposed as a putative messenger in the release of calcium from intracellular stores (Ghosh et al., 1990; Zhang et al., 1991). In viable 3T3 fibroblasts, we found sphingosine-1-phosphate to be a potent mitogen by itself and to mediate calcium release (Zhang et al., 1991). Thus, sphingosine-1-phosphate may be an important component of the intracellular signalling system that is involved in calcium release and in the regulation of cell growth induced by sphingosine. In this paper, we present evidence that sphingosine-1-phosphate markedly increases phosphatidic acid levels in quiescent cultures of Swiss 3T3 fibroblasts primarily through activation of phospholipase D.

**EXPERIMENTAL PROCEDURES**

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1 The abbreviations used are: PA, phosphatidic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; DAG, diacylglycerol; BSA, bovine serum albumin.

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Phosphatidylpropanol appears to be exclusively mediated by phospholipase D (Pai et al., 1989). Briefly, sphingosine-1-phosphate was prepared by enzymatic digestion of sphingosylphosphorylcholine with phospholipase D as previously described (Van Veldhoven et al., 1989). After various times, the incubations were terminated, cellular lipids were analyzed by TLC using the isooctane solvent system.

Other phospholipids were analyzed by TLC using hexane/diethyl ether/methanol/concentrated HCl (100:20:1, v/v) and 0.2 ml of balanced salt solution containing 10 mM EDTA (Dressler and Kolesnick, 1990). The organic phase of the mixture of isooctane/ethyl acetate/acetic acid/water (50:110:20:100, v/v) was well separated from the silica gel (CCL 92). Cells were routinely cultured as previously described (Spiegel, 1989; Zhang et al., 1991).

Culture and Labeling with ^32P—Swiss 3T3 fibroblasts were from the American Type Culture Collection (CCL 92). Cells were routinely cultured as previously described (Spiegel, 1989; Zhang et al., 1991). Confluent quiescent cultures of 3T3 cells were washed with phosphate-free Dulbecco’s modified Eagle’s medium and incubated with this buffer containing[^32P] (40 μCi/ml) for 24 h. In some experiments, confluent cultures were labeled with [2-^3H]glycerol (4 μCi/ml) for the last 3 days of culture, and[^32P] (40 μCi/ml) was added for the final 24 h. The cells were treated with sphingosine, sphingosine-1-phosphate, or vehicle alone for various times, the medium was rapidly removed, and the cells were scraped from the dish in 1 ml of 0.1 M HCl (Zhang et al., 1990a, 1990b).

Extraction and Analysis of Lipids—Lipids were extracted with chloroform/methanol/concentrated HCl (100:200:1, v/v), and the phospholipid extract containing 150,000 cpm. The lipids in the lower chloroform phase were analyzed by two dimensional TLC on Silica Gel 60 G. The plates were developed in chloroform/methanol/concentrated HCl (100:200:1, v/v), and the lipid standards were eluted from the silica with chloroform/methanol (1:1, v/v) and treated with 6 N HCl/1-butanol (1:1, v/v) for 1 h at 100 °C to produce ^32P-labeled sphingosine-1-phosphate which was further characterized as previously described (Zhang et al., 1991).

RESULTS

The Formation of Sphingosine-1-phosphate in Response to Sphingosine Precedes the Accumulation of Phosphatidic Acid—Sphingosine significantly stimulated incorporation of ^32P into phosphatidic acid and sphingosine-1-phosphate in cells metabolically prelabeled with[^32P] to isotopic equilibrium (Zhang et al., 1990b, 1991 and also Fig. 1). Consistent with our previous study (Zhang et al., 1990b), sphingosine caused a dose-dependent increase in[^32P] phosphatidic acid levels (Fig. 1A). The accumulation of sphingosine-1-phosphate in

FIG. 1. Dose-response and time course for sphingosine-induced accumulation of[^32P]-labeled phosphatidic acid and[^32P]-labeled sphingosine-1-phosphate. Quiescent cultures of 3T3 cells were washed with phosphate-free Dulbecco’s modified Eagle’s medium and incubated with this buffer containing[^32P] (40 μCi/ml) for 24 h. The cells were treated with increasing concentrations of sphingosine-BSA complex for 1 h (A) or with sphingosine-BSA complex (10 μM) for the indicated time periods (B). Lipids were extracted, separated by TLC, and phospholipids were located by autoradiography as described under “Experimental Procedures.” The silica gel areas containing the labeled phosphatidic acid (□) and sphingosine-1-phosphate (●) were scraped and counted by liquid scintillation spectrometry. The results are expressed as fold increase relative to untreated controls. The incorporation of[^32P] into phosphatidic acid and sphingosine-1-phosphate in untreated cells were 60 ± 80 and 150 ± 60 cpm, respectively, determined from an aliquot of the phospholipid extract containing 150,000 cpm.
response to sphingosine was also concentration-dependent, with a maximal effect at a significantly lower concentration (20 \( \mu M \)). Concentrations of sphingosine greater than 50 \( \mu M \) were not studied since they were highly cytotoxic (Zhang et al., 1990a, 1990b). The time courses of \(^{32}\)P-labeled sphingosine-1-phosphate and phosphatidic acid accumulation in response to an optimal mitogenic concentration of sphingosine are shown in Fig. 1B. A significant increase in \(^{32}\)P-sphingosine-1-phosphate was detected within 5 min after exposure of the cells to sphingosine. This preceded the increase in formation of \(^{32}\)P-phosphatidic acid which could be detected only after 20 min. Thus, the formation of sphingosine-1-phosphate was rapid, reaching nearly maximal levels within 60 min, when 6-fold stimulation over the basal value was observed. The maximum accumulation of phosphatidic acid also occurred at approximately the same time. The rapid increase in sphingosine-1-phosphate levels appears to precede the increase in accumulation of phosphatidic acid, suggesting that sphingosine-1-phosphate could be mediating the effect of sphingosine on phosphatidic acid accumulation (Zhang et al., 1990b).

Two different approaches were used to further investigate the possibility that sphingosine-1-phosphate mediated the sphingosine-induced accumulation of phosphatidic acid. Cellular levels of ATP were depleted by pretreatment of the cells for 15 min with antimycin A (0.5 \( \mu M \)) and oligomycin (1.2 \( \mu M \)) which block the mitochondrial respiratory chain and ATP synthetase, respectively (Mohr and Fewtrell, 1990). These inhibitors completely blocked the phosphorylation of sphingosine (20 \( \mu M \)) and also prevented the sphingosine-dependent increase in \(^{32}\)P phosphatidic acid levels without affecting the labeling of other phospholipids. However, this approach will result in inhibition of any pathway that utilizes ATP as a phosphorylating agent. To further substantiate these effects of ATP depletion, we used an inhibitor of sphingosine kinase, DL-threo-dihydrosphingosine (10 \( \mu M \)), which has been shown to inhibit the production of sphingosine-1-phosphate in isolated platelets (Buehrer and Bell, 1992). The production of sphingosine-1-phosphate from sphingosine was decreased 63 \( \pm \) 2% by threo-dihydrosphingosine, and the sphingosine-induced production of phosphatidic acid was completely eliminated.

The Effect of Sphingosine-1-phosphate on Phosphatidic Acid Accumulation—Addition of pure sphingosine-1-phosphate to confluent 3T3 fibroblasts resulted in a rapid increase in \(^{32}\)P phosphatidic acid accumulation. An increase of 2-fold in \(^{32}\)P phosphatidic acid levels was detected within 5 min after exposure of the cells to sphingosine-1-phosphate (Fig. 2). After 30–60 min, the levels decrease and nearly return to the basal value by 2–4 h (data not shown). Since the cells were metabolically labeled to isotopic equilibrium, it is most probable that these increases reflect changes in phosphatidic acid mass rather than enhanced radioactive labeling. To further substantiate this point, the cells were double-labeled with both \(^{3}H\)glycerol and \(^{32}\)P, to isotopic equilibrium. Mitogenic concentrations of sphingosine-1-phosphate induced identical stimulations of incorporation of \(^{3}H\)glycerol and \(^{32}\)P into phosphatidic acid, indicating that the changes reflect increases in phosphatidic acid mass. The dose-response of phosphatidic acid accumulation induced by sphingosine-1-phosphate is shown in Fig. 3. A significant effect was detected at a concentration of sphingosine-1-phosphate as low as 0.1 \( \mu M \), and the maximum effect of more than 2-fold stimulation over the control was found at a concentration of 2 \( \mu M \). Interestingly, when compared to the effects of sphingosine on the increase in phosphatidic acid levels, a much lower concentra-

![Fig. 2. Time course of phosphatidic acid accumulation in response to sphingosine-1-phosphate.](image)

![Fig. 3. Dose-response of sphingosine-1-phosphate-induced formation of \(^{32}\)P-labeled phosphatidic acid.](image)
Phosphate was the only labeled lipid detected and there was elicited by sphingosine-1-phosphate. The hydrolysis of phosphatidic acid activity was sphingosine-1-phosphate itself and was not due to rapid reversion to sphingosine by the action of a membrane phosphatase.

**Effect of Sphingosine-1-phosphate on Phospholipase D Activity**—We next investigated the source of phosphatidic acid elicited by sphingosine-1-phosphate. The hydrolysis of phospholipids by phospholipase D is a major pathway by which sphingosine increases the levels of phosphatidic acid in 3T3 fibroblasts (Lavie and Liscovitch, 1990; Zhang et al., 1990b; Kiss and Anderson, 1990). Phospholipase D has transphosphatidyldiacylglycerolase activity with a large variety of alcohol acceptors, producing the corresponding phosphatidyl alkyl ester (Pai et al., 1988). We utilized the production of phosphatidylpropanol, when 1-propanol was the phosphatidyl group acceptor, as a measure of phospholipase D activity. Sphingosine-1-phosphate treatment markedly increased phospholipase D activity (Figs. 4 and 5). Addition of sphingosine-1-phosphate in the presence of 1-propanol induced the synthesis of [32P]phosphatidylpropanol, in addition to [32P]PA (Fig. 4). The formation of both was rapid and occurred in parallel, reaching a maximum within 5–10 min (Fig. 5A). Sphingosine-1-phosphate-induced [32P]PA accumulation decreased in the presence of 1-propanol, consistent with a redistribution of phosphatidyl moieties to the synthesis of phosphatidylpropanol. The dose-response for the increase in phosphatidylpropanol is shown in Fig. 5B and was comparable to the dose-response for the increase in PA levels induced by sphingosine-1-phosphate found in the absence of 1-propanol (compare Fig. 3 and Fig. 5B). A significant accumulation of [32P]PA and [32P]phosphatidylpropanol was detected at 0.1 µM sphingosine-1-phosphate and reached a maximum at 5 µM. In contrast, higher concentrations of sphingosine (10 µM) only resulted in a very small increase (Fig. 4), and, even at a concentration of 50 µM, the formation of both [32P]PA and [32P]phosphatidylpropanol did not reach a plateau level (data not shown).

**Effect of Sphingosine-1-phosphate on Phosphohydrolase Activity**—Recently, it has been shown that in addition to stimulation of phospholipase D, sphingosine also inhibits the activity of phosphatidic acid phosphohydrolase (Lavie et al., 1990; Mullmann et al., 1991). Phosphatidic acid phosphohydrolase catalyzes the hydrolysis of phosphatidic acid to produce diacylglycerol. However, in cells which were double-labeled with both [3H]glycerol and 32P, to isotopic equilibrium, sphingosine-1-phosphate induced significant increases in the levels of phosphatidic acid without corresponding decreases in the levels of [H]diacylglycerol. There was even an apparent trend toward increases in the levels of [H]diacylglycerol (Fig. 6). This increase in [H]diacylglycerol lagged behind the elevation in phosphatidic acid levels. Interestingly, the levels of [H]triaclyglycerols significantly decreased after treatment with sphingosine-1-phosphate, while the level of [H]monoaclglycerol remained unchanged (data not shown).

Previously, it had been demonstrated that sphingosine inhibited phosphatidic acid phosphohydrolase activity in several other cell types measured in vitro (Lavie et al., 1990; Mullmann et al., 1991). It was thus of interest to examine the effects of sphingosine-1-phosphate on phosphatidic acid phosphohydrolase activity in 3T3 cell homogenates. When mixed liposomes containing [14C]phosphatidic acid were incubated with 3T3 cell homogenates, [14C]diacylglycerol was formed in a time- and dose-dependent manner. Boiled homogenates had no measurable activity. Sphingosine-1-phosphate increased diacylglycerol formation with an IC50 value of 140 µM (Fig. 7) which is a much higher concentration than that required for all of the reported biological effects observed in intact cells (Zhang et al., 1991).

**Effect of Sphingosine-1-phosphate on Diacylglycerol Kinase**—While increases in phosphatidic acid levels are a direct consequence of the hydrolysis of phospholipids by phospholipase D and, in some cases, combined with inhibition of phosphatidic acid phosphohydrolase activity (Mullmann et al., 1991), phosphatidic acid can also be formed by alternative pathways, such as diacylglycerol phosphorylation by diacylglycerol kinase or by acylation of glycerol 3-phosphate. Recently, it has been demonstrated that sphingosine activates the 80-kDa isoenzyme form of diacylglycerol kinase in vitro leading to an increase in phosphatidic acid levels (Sakane et al., 1989; Kanoh et al., 1990). We utilized the compound R-59022, a specific inhibitor of this diacylglycerol kinase isoenzyme form (IC50, 10 µM), in an attempt to assess the role of this kinase in the formation of phosphatidic acid in response to sphingosine-1-phosphate. Surprisingly, R-59022, at relatively low concentrations (1–10 µM), potentiated, rather than inhibited, the accumulation of phosphatidic acid induced by sphingosine-1-phosphate (Fig. 8). Furthermore, R-59022 also potentiated long-term cellular proliferation induced by sphingosine-1-phosphate or sphingosine (Fig. 8). The concentration range of R-59022 that synergized with sphingosine-1-phosphate and sphingosine was narrow and resembled the dose-response observed previously for bombesin-induced DNA synthesis in these cells (Morris et al., 1988). Strong inhibition of cellular proliferation was observed at concentrations of R-59022 greater than 10 µM, which is a concentration within the range used by other workers to inhibit diacylglycerol kinase activity (Sakane et al., 1989; Kanoh et al., 1990). At higher concentrations (≥20 µM), R-59022 also inhibited the accumulation of phosphatidic acid induced by sphingosine-1-phosphate.
Sphingosine-1-phosphate and Phosphatidic Acid Levels

**FIG. 5.** Time course and dose-response of sphingosine-1-phosphate-stimulated formation of \[^{32}P\]phosphatidylpropanol and \[^{32}P\]phosphatidic acid in the presence of 1-propanol. 3T3 cells were prelabeled with \[^{32}P\]I for 24 h and were treated with sphingosine-1-phosphate (5 μM) for the indicated periods (A) or with increasing concentrations of sphingosine-1-phosphate for 30 min (B) in the presence of 1-propanol (134 mM), and the formation of \[^{32}P\]PA (○) and \[^{32}P\]phosphatidylpropanol (□) were determined. The data are expressed as fold increase over unstimulated cells and are the means of duplicate determinations. In untreated control cells, the amounts of PA and phosphatidylpropanol were 0.53 ± 0.07 and 0.08 ± 0.01% of the total phospholipids, respectively.

**DISCUSSION**

The turnover of phosphoglycerolipids, often related to the phosphoinositide cycle and more recently to phosphatidylcholine hydrolysis, plays a pivotal role in the initiation of a variety of cellular responses (Billah and Anthes, 1990; Dennis et al., 1991). Agonist-induced activation of phospholipase C, phospholipase D, and phospholipase A2 produces the crucial intracellular second messengers, diacylglycerol, phosphatidic acid, and arachidonic acid, respectively. A cycle of sphingolipids, comparable to the phosphoglycerolipid cycles, with its own messenger transducing products (sphingosine or ceramide) has been proposed recently (Hannun and Bell, 1989; Merrill and Stevens, 1989; Merrill, 1991). Sphingosine, a breakdown product of cellular sphingolipids, has appropriate properties that make it a suitable candidate to function as an intracellular second messenger (reviewed in Merrill, 1991): 1) sphingosine elicits different responses in a wide variety of cell types; 2) the structural properties of this molecule allow for its incorporation and rapid mobility in membranes and makes it accessible to different effector systems; 3) the level of free sphingosine in cells is very low and can be regulated by some physiological stimuli (Wilson et al., 1988); and 4) the turnover of sphingosine by phosphorylation to form sphingosine-1-phosphate followed by cleavage to a long chain aldehyde and phosphorylethanolamine is extremely rapid (Stoffel et al., 1970).

Although in several systems some of the biological effects of sphingosine appear to be a consequence of the inhibition of protein kinase C, it seems clear that it also has other biochemical targets. Previous studies from our laboratory have shown that sphingosine may play an important role in cell growth regulation acting in a protein kinase C-independent pathway (Zhang et al., 1990a). We have shown that the mitogenic effect of sphingosine is accompanied by an increase in phosphatidic acid (Zhang et al., 1990b), a potent mitogen for Swiss 3T3 cells (Yu et al., 1988; Moolenaar et al., 1986; Zhang et al., 1990b) which has recently been implicated as an intracellular second messenger (Bocckino et al., 1991). This
The mechanism by which sphingosine-1-phosphate stimulates formation of phosphatidic acid was investigated. An increase in cellular phosphatidic acid levels can occur through several known pathways, such as stimulation of phospholipase D, inhibition of the degradation of existing phosphatidic acid pools by phosphatidic acid phosphohydrolase, acylation of glycerol 3-phosphate, and increased phosphorylation of DAG catalyzed by DAG kinase. Recently, sphingosine has been shown to stimulate phospholipase D in several cell types (Lavie and Liscovitch, 1990). It has been observed that sphingosine decreases phosphatidylcholine levels in NG108-15 neuroblastoma-glioma hybrid cells (Lavie and Liscovitch, 1990) and in Swiss 3T3 fibroblasts (Zhang et al., 1990b) and also stimulates the hydrolysis of phosphatidylethanolamine in NIH 3T3 fibroblasts (Kiss and Anderson, 1990). Furthermore, recent evidence demonstrates that sphingosine has the additional effect of inhibiting phosphatidic acid phosphohydrolase activity in cell lysates (Lavie et al., 1990; Mullmann et al., 1991). Thus, it has been suggested that sphingosine has a dual action on the regulation of phosphatidic acid levels (Lavie et al., 1990).

We observed that sphingosine-1-phosphate induced a rapid increase in the activity of phospholipase D, as measured by the formation of phosphatidylpropanol. The time course was similar for the effect of sphingosine-1-phosphate on phosphatidic acid formation. This indicates that a rapid activation of phospholipase D is responsible for the sphingosine-1-phosphate-induced phosphatidic acid accumulation. It should be emphasized that sphingosine-1-phosphate stimulated phospholipase D activity at much lower concentrations than sphingosine and reached the maximum response more rapidly. Therefore, it appears that sphingosine-1-phosphate is more potent than sphingosine in stimulating phospholipase D activity, suggesting that it could mediate the effect of sphingosine on phospholipase D activation. In contrast to earlier reports that the increase in phosphatidic acid induced by sphingosine in other cell types was accompanied by a decrease in the levels of [3H]diacylglycerol (Lavie et al., 1990; Mullmann et al., 1991), we found that sphingosine-1-phosphate slightly increased the levels of [3H]-labeled diacylglycerol in Swiss 3T3 cells, which lagged behind the elevation in phosphatidic acid levels. This result indicates that sphingosine-1-phosphate, in contrast to sphingosine, did not inhibit phosphatidic acid phosphohydrolase. Indeed, in contrast to previous reports that sphingosine inhibits phosphatidic acid phosphohydrolase activity in vitro, optimal mitogenic concentrations of sphingosine-1-phosphate had no measurable effects on the activity of this enzyme. We only found an effect in Swiss 3T3 lysates at very high concentrations of sphingosine-1-phosphate (>100 µM), where it stimulated the activity rather than inhibiting it. These concentrations are much higher than required for the other effects of sphingosine-1-phosphate in cells, such as increased DNA synthesis, calcium release, or increases in phosphatidic acid levels (Zhang et al., 1991). Taken together, these results indicate that phosphatidic acid phosphohydrolase probably does not contribute to the increase in the levels of phosphatidic acid in Swiss 3T3 fibroblasts induced by sphingosine-1-phosphate.

Another possibility that we examined is that sphingosine-1-phosphate stimulates DAG kinase-catalyzed phosphorylation of DAG. Previous studies demonstrated that sphingosine activates the 80-kDa isoenzyme form of DAG kinase while inhibiting the 150-kDa form (Sakane et al., 1989). Therefore, the effect of sphingosine on DAG kinase could be different depending on the type of DAG kinase isozyme present in a particular cell type. Using high concentrations of R-59022, an
inhibitor of the 80-kDa isozyme (Sakane et al., 1989), we found that it inhibits the accumulation of phosphatidic acid and mitogenesis induced by sphingosine-1-phosphate. However, at low concentrations, R-59022 stimulated both responses. Although the nature of the R-59022 effects are unclear, nonspecific actions of this compound have been described (Nunn and Watson, 1987; Mahadevappa, 1988). Furthermore, it is still not clear which isoenzyme form of DAG kinase is present in Swiss 3T3 fibroblasts (Kanoh et al., 1990; MacDonald et al., 1988). Results using R-59022 should also be interpreted with caution since it may have an effect on phosphatidic acid-dependent pathways.

Recently, phospholipase D has emerged as a key element of stimulus-response coupling in diverse cellular systems (reviewed in Billah and Anthes, 1990; Dennis et al., 1991; Shukla and Halenda, 1991). In this study, we have shown that sphingosine-1-phosphate can increase the level of phosphatidic acid, most likely via activation of phospholipase D. This effect may be unrelated to its ability to mobilize calcium from internal stores (Ghosh et al., 1990; Zhang et al., 1991). Thus, sphingosine-1-phosphate could mediate the effect of sphingosine-1-phosphate accumulation in Swiss 3T3 fibroblasts (Zhang et al., 1990b) and may regulate cellular proliferation by affecting multiple transmembrane signaling pathways. It still remains to be established by further experiments whether mitogenic stimuli regulate intracellular levels of sphingosine-1-phosphate. If this is the case, sphingosine-1-phosphate could function not only as a novel intracellular second messenger inducing the release of calcium from internal stores but also to amplify the cascade of events leading to mitogenic stimulation via its effect on phosphatidic acid levels.

REFERENCES