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EDG3 Is a Functional Receptor Specific for Sphingosine 1-Phosphate and Sphingosylphosphorylcholine with Signaling Characteristics Distinct from EDG1 and AGR16

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AGR16/H218/EDG5 and EDG1 are functional receptors for lysosphingolipids, whereas EDG2 and EGD4 are receptors for lysophosphatidic acid (LPA). The present study demonstrates that EDG3, the yet poorly defined member of the EDG family G protein-coupled receptors, shows identical agonist specificity, but distinct signaling characteristics, compared to AGR16 and EDG1. Overexpression of EDG3 conferred a specific [^{32}P]S1P binding, which was displaced by S1P and sphingosylphosphorylcholine (SPC), but not by LPA or other related lipids. In cells overexpressing EDG3, S1P induced inositol phosphate production and $[Ca^{2+}]_i$ increase in a manner only partially sensitive to pertussis toxin (PTX), which was similar to the case of AGR16, but quite different from the case of EDG1, in which the S1P-induced responses were totally abolished by PTX. EDG3 also mediated activation of mitogen-activated protein kinase (MAPK) in PTX-sensitive and Ras-dependent manners, as in the cases of EDG1 and AGR16, although EDG3 and EDG1 were more effectively coupled to activation of MAPK, compared to AGR16. Additionally, EDG3 mediated a decrease in cellular cyclic AMP content, like EDG1, but contrasting with AGR16 which mediated an increase in cyclic AMP. These and previous results establish that EDG1, AGR16 and EDG3 comprise the lysosphingolipid receptor subfamily, each showing distinct signaling characteristics.

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The lysosphingolipids sphingosine 1-phosphate (S1P) and sphingosylphosphorylcholine (SPC) evoke cell type-specific diverse cellular responses in various cell types, including mitogenesis (1, 2), inhibition of migration (3, 4), microfilament reorganization (5, 6) and cell shape change (2, 5). Stimulation of cells with the lysosphingolipids were demonstrated to trigger the activation of multiple intracellular signaling molecules including phospholipase C (4, 7, 8), phospholipase D (2, 8), protein kinase C (PKC) (9), MAPK (4, 10) and K^+ channel (muscarinic K^+ current) (11). Many, though not all, of the lysosphingolipid-induced responses were shown to be inhibited by pertussis toxin (PTX) pretreatment (4, 8, 12).

The EDG (endothelial differentiation gene) receptor family belongs to the G protein-coupled receptor superfamily, and consists of the five known members, EDG1 (13), EDG2 (14), EDG3 (15), EDG4 (16) and AGR16/H218/EDG5 (17, 18). Many of cell lines usually used for expression of exogenous genes, including COS, NIH3T3 and HEK293 cells, express endogenous receptors for and respond to lysosphingolipids (12). By employng selected cell types with a very low or no background response to lysosphingolipids for expression of the EDG genes, we have successfully demonstrated that both EDG1 and AGR16 are functional receptors specific for S1P and SPC; EDG1 is linked via Gi to multiple signaling pathways including phospholipase C activation, Ca^{2+} mobilization, mitogen-activated protein kinase(MAPK)/extracellular signal-regulated protein kinase(ERK) activation, and adenylate cyclase inhibition (21). AGR16 shows the identical agonist specificity as EDG1, but its signaling mechanisms are distinct from EDG1 in that AGR16 is linked via Gq/11, Gi and Gs to phospholipase C activation, MAPK activation and adenylate cyclase stimulation, respectively (22). On the other hand, EDG2 and EDG4 have been

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Abbreviations used: S1P, sphingosine 1-phosphate; SPC, sphingosylphosphorylcholine; LPA, lysophosphatidic acid; EDG, endothelial differentiation gene; $[Ca^{2+}]_i$, cytoplasmic free Ca^{2+} concentration; PTX, pertussis toxin; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; PDBu, phorbol-12, 13-butyrate, PKC, protein kinase C.

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demonstrated to serve as receptors specific for lysophosphatidic acid (LPA) (16, 23).

An et al. (24) first reported that overexpression of EDG3 in Jurkat T-cells and *Xenopus* oocytes conferred the ability to respond to both S1P and SPC, with stimulation of serum-response element-driven transcriptional activity and Ca^{2+} efflux, respectively. In contrase to this, Van Brocklyn et al. (25) reported very recently that SPC did not compete with [^{32}P]S1P binding to EDG3 in HEK293 cells. Sato et al. (26) showed that EDG3 mediated S1P-induced phospholipase C activation and Ca^{2+} mobilization in CHO cells. However, they did not address to the agonist specificity of EDG3. Thus, the agonist specificity of EDG3 is yet to be established, and intracellular signaling mechanisms of EDG3 are still poorly understood.

By using selected mammalian cell expression systems in the present study, we revealed the agonist specificity and the charateristics of signaling mechanisms of EDG3, as compared to EDG1 and AGR16. Our results demonstrate that EDG3, like EDG1 and AGR16, is a receptor for S1P and SPC, but not for LPA or other related lipids, and is coupled to phospholipase C-Ca^{2+} mobilization, MAPK activation and adenylate cyclase inhibition with differential sensitivity to PTX. These signaling characteristics of EDG3 are distinct from those of EDG1 and AGR16.

MATERIALS AND METHODS

Cells. CHO-K1(CHO) and human erythroleukemia HEL cells, obtained from RIKEN Cell Bank and the Japanese Cancer Research Resources Bank (Tokyo, Japan), respectively, were grown in Ham's F-12 (CHO) and RPMI (HEL) media supplemented with 10% fetal calf serum (Equitech-Bio, Ingram, TX), 100 units/ml penicillin, and 100 μg/ml streptomycin (Wako Pure Chemicals, Osaka, Japan). Before each experiment, cells were switched to the respective low serum (1%) medium.

Plasmids and transfections. Human EDG3 gene containing the entire coding region was cloned by PCR amplification from human genomic DNA with the sense primer 5′-CGGAATTCATGCCAAGTGATGGCAACTGCCCTCCCGCCGCGTCT-3′ and the antisense primer 5′-CGGAATTCTCAGTTGCAGAAGATCCCATTCTGAAGTGCTGCGTT-3′, both of which have *Eco* RI recognition sites created near the 5′ ends. The nucleotide sequences of the cloned EDG3 were confirmed by sequencing. EDG3 DNA was ligated into a mammalian expression vector pME18S (obtained from Dr. K. Maruyama at Tokyo Medical and Dental College) at the *Eco* RI site downstream of the SRα promoter (27). EDG1 and AGR16 cDNAs were described previously (21, 22). Transfections were carried out by using LipofectAMINE (Life Technologies, Inc.). Stable transfectants were selected with G418 as described (27).

[^{32}P]-S1P binding, measurements of [Ca^{2+}]i, inositol phosphate production, MAPK activation and determination of cyclic AMP content. The procedures for these assays were described in detail previously (21, 22). For *in vitro* measurements of activities of immunoprecipitated MAPK from transiently transfected cells, CHO-EDG3 cells on 35-mm dishes were co-transfected with an expression vector for Myc epitope-tagged MAPK (pME18S-Myc-ERK) (28, 29) and either an expression vector for a dominant negative form of H-Ras (pME18S-Asn^{17}-H-Ras) (21, 22) or an empty vector pME18S. Myc-tagged MAPK was immunoprecipitated by using a mouse monoclonal anti-Myc epitope antibody (clone 9E10). MAPK activity associated with the immune complex was assayed *in vitro* using myelin basic protein (Sigma) as substrate, as described (21, 22, 28). The band shift of endogenous p42ERK2 in CHO cells stably expressing EDG1, EDG3 and AGR16 was detected by Western blot analysis of total cell lysate with a mouse monoclonal anti-ERK antibody (clone 03-6600, Zymed Laboratories Inc.) (21, 22), and quantitated as described (22).

Materials. S1P, SPC and ceramide (C8)-1-phosphate were obtained from Biomol (Plymouth Meeting, PA, USA). Sphingosine, lysophosphatidylserine (purified from bovine brain), lysophosphatidylcholine (C18), lysophosphatidylethanolamine (C18), ceramide (C2, C6 and C18), phosphatidic acid (diC18), LPA (C18) and glucosylsphingosine were purchased from Sigma. Lysophosphatidylinositol (purified from bovine liver) was purchased from Avanti (Birmingham, AL, USA). Fura-2/AM solution was purchased from Dojin (Kumamoto, Japan). S1P was dissolved in dimethylsulfoxide at 2×10^{-3} M, aliquoted and stored at −80°C. Other lipids were dissolved in methanol. Final solvent concentrations did not exceed 0.1%.

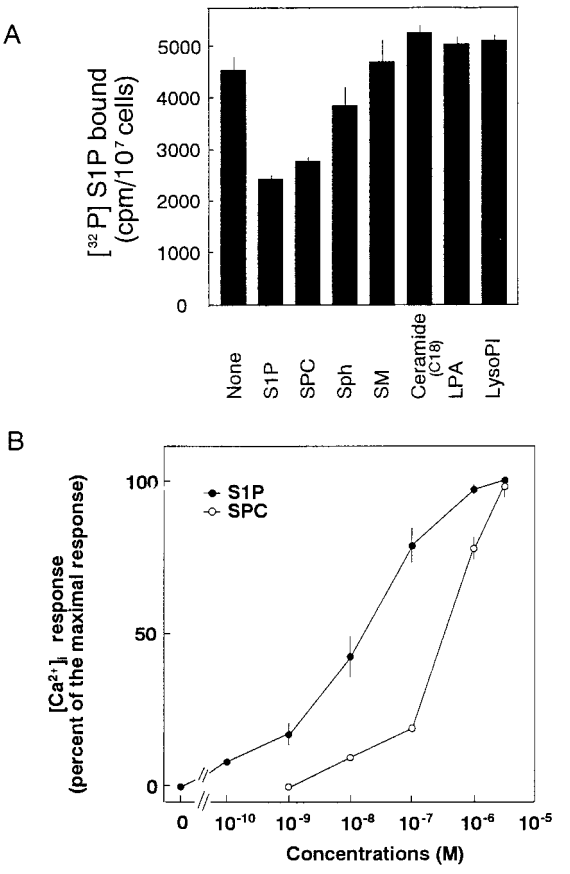

FIG. 1. Competition of [^{32}P]S1P binding to intact HEL-EDG3 cells by unlabelled S1P and related lipids, and dose- dependent effects of S1P and SPC on the $[Ca^{2+}]_i$ in HEL-EDG3 cells. A, HEL-EDG3 cells were incubated with [^{32}P]S1P in the presence of unlabelled S1P or related lipids (10^{-6} M), as described in the Materials and Methods section. Sph, sphingosine; SM, sphingomyelin; PA, phosphatidic acid; LysoPI, lysophpsphatidylinositol. Values are means ± S. E. of three determinations. B, HEL-EDG3 cells were stimulated with various concentrations of lipids. The maximal increase in the $[Ca^{2+}]_i$ with 3×10^{-6} M S1P was 192 ± 12 nM (n = 3). Values are means ± S. E. of three determinations.

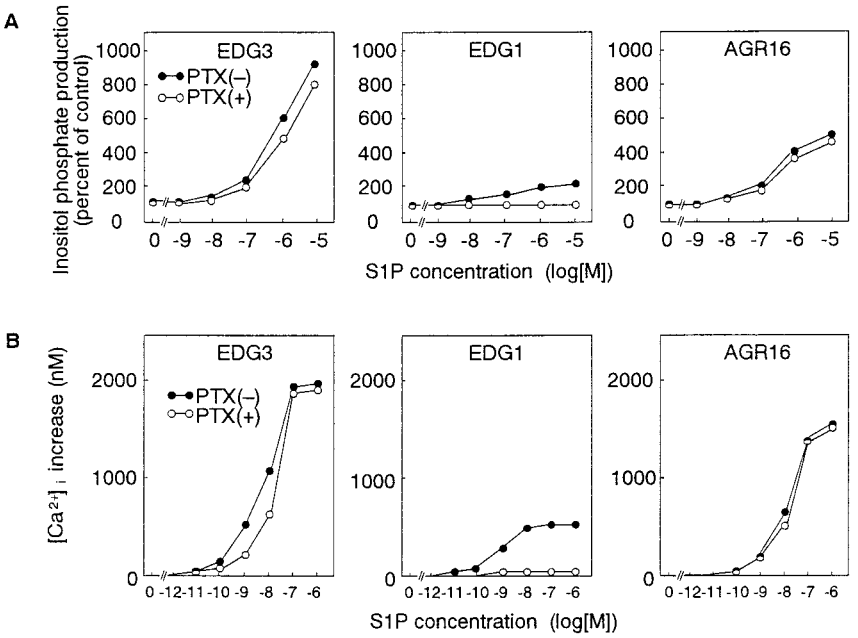

FIG. 2. Dose-dependent stimulation of inositol phosphate production (A) and $[Ca^{2+}]_i$ increase (B) by S1P in CHO-EDG3, CHO-EDG1 and CHO-AGR16 cells pretreated or not with PTX. A, Cells prelabelled with myo-[2-^{3}H] inositol were pretreated or not with 100 ng/ml PTX for 24 hours, and then stimulated with various concentrations of S1P for 60 min in the presence of 10 mM LiCl. Results were expressed as a percent of an unstimulated control, and means of duplicate determinations. B, Cells pretreated or not with PTX as in A were stimulated with various concentrations of S1P. Values are means of duplicate determinations.

RESULTS

We demonstrated in our previous publication (21) that HEL cells do not have significant, specific binding sites for [^{32}P]S1P, do not express detectable levels of transcripts of any of the EDG members, or do not respond to S1P, SPC, LPA or other related lipids with an increase in the $[Ca^{2+}]_i$. We first established a HEL cell clone stably expressing EDG3 (HEL-EDG3). We detected a specific binding of [^{32}P]S1P to HEL-EDG3 cells, which was inhibited dose-dependently by the addition of unlabeled S1P with a half-maximally inhibitory concentration (IC_{50}) value of approximately 10^{-8} M. Then, the specificity of binding to EDG3 for a variety of related lipids were examined. Similar to the cases of EDG1 and AGR16 (21, 22), we found that only S1P and SPC competed with [^{32}P]S1P for binding to HEL-EDG3 cells (Fig. 1A).

We next examined Ca^{2+}-mobilizing effects of S1P, SPC and other lipids in HEL-EDG3 cells. S1P increased the $[Ca^{2+}]_i$ dose-dependently with a half-maximally effective (EC_{50}) concentration value of approximately 10^{-8} M (Fig. 1B). SPC was a less potent agonist with an EC_{50} value of approximately 5×10^{-7} M. In agreement with the binding data, other lipids

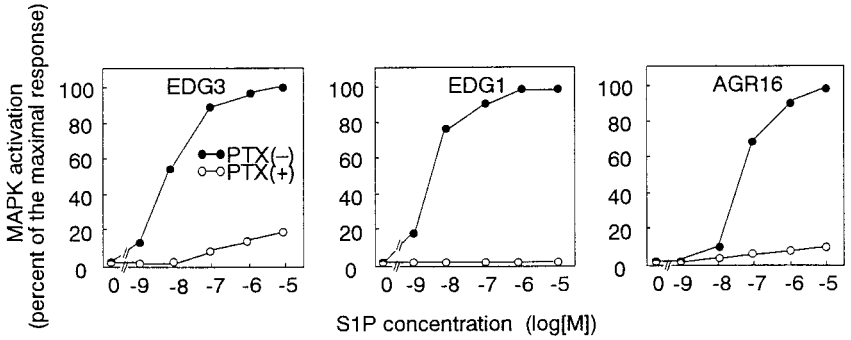

FIG. 3. Dose-dependent activation of MAPK by S1P in CHO-EDG3, CHO-EDG1 and CHO-AGR16 cells preteated or not with PTX. Cells pretreated or not with 100 ng/ml PTX for 24 hours were stimulated with various concentrations of S1P for 5 min. MAPK activation was evaluated by detection of band shift of p42 ERK2 as described in the Materials and Methods section. Values are means of duplicate determinations.

examined, which included sphingosine, sphingomyelin, ceramide, ceramide-1-phosphate, glucosylsphingosine, phosphatidic acid, lysophosphatidylinositol, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylserine and LPA, up to 10^{-6} M did not increase the $[Ca^{2+}]_i$. Thus, it is concluded that EDG3 is a functional receptor specific for S1P and SPC. We also observed in CHO cells stably expressing EDG3 (CHO-EDG3 cells) that S1P increased the $[Ca^{2+}]_i$ dose-dependently with a maximal increase of approximately 2000 nM (Fig. 2B). In agreement with S1P-induced Ca^{2+}-mobilization, S1P stimulated inositol phosphate production in a dose-dependent manner with an approximately 9.5-fold stimulation at 10^{-5} M of S1P (Fig. 2A). Pertussis toxin (PTX) pretreatment (100 ng/ml for 24 hours) partially (20–50%) inhibited increases in both inositol phosphate production and the $[Ca^{2+}]_i$ over a wide concentration of S1P (Fig. 2 A and B left). We showed recently that both EDG1 and AGR16 also mediated inositol phosphate production and $[Ca^{2+}]_i$ increase (21, 22). In an attempt to clarify possible differences in the signaling mechanisms between EDG3 and the other two lysosphinolipid receptors, we comparatively examined these S1P-induced responses to a full range of S1P concentrations and their PTX sensitivity in CHO clones stably expressing EDG1 or GR16. In CHO-EDG1 and CHO-AGR16 cells, S1P at maximal doses caused approximately 2- and 5-fold stimulation over an unstimulated value of inositol phosphate production, and 500 and 1600 nM increases in the $[Ca^{2+}]_i$, respectively (Fig. 2 middle and right). The EC_{50} value (10^{-8} M) for the $[Ca^{2+}]_i$ increase in CHO-EDG3 cells was similar to that in CHO-AGR16 cells and higher than in CHO-EDG1 cells (10^{-9} M). Several other clones stably expressing each of S1P receptor subtypes showed similar results on the maximal responses and EC_{50} values. Thus, stimulation of EDG3 and AGR16 causes robust phospholipase C activation and Ca^{2+} mobilization, while stimulation of EDG1 induces modest activation of the Ca^{2+} signaling pathway. PTX pretreatment nearly totally abolished EDG1-mediated inositol phosphate production and $[Ca^{2+}]_i$ increase, but only slightly (10–30%) inhibited AGR16- mediated responses (Fig. 2 middle and right). These observations indicate that EDG3 and AGR16 are coupled to phospholipase C activation and Ca^{2+} mobilization via both PTX-insensitive and -sensitive G proteins, whereas EDG1 is coupled to the signaling pathway exclusively via PTX-sensitive Gi protein.

We next examined the coupling of EDG3 to the MAPK/ERK pathway. In CHO-EDG3, S1P activated MAPK dose-dependently with the EC_{50} value of 5×10^{-9} M, as evaluated by detection of the band shift of p42 ERK2 (Fig. 3 left). Differently from S1P-induced phospholipase C activation, PTX pretreatment completely inhibited MAPK activation at lower S1P concentrations and more than 80% even at its higher

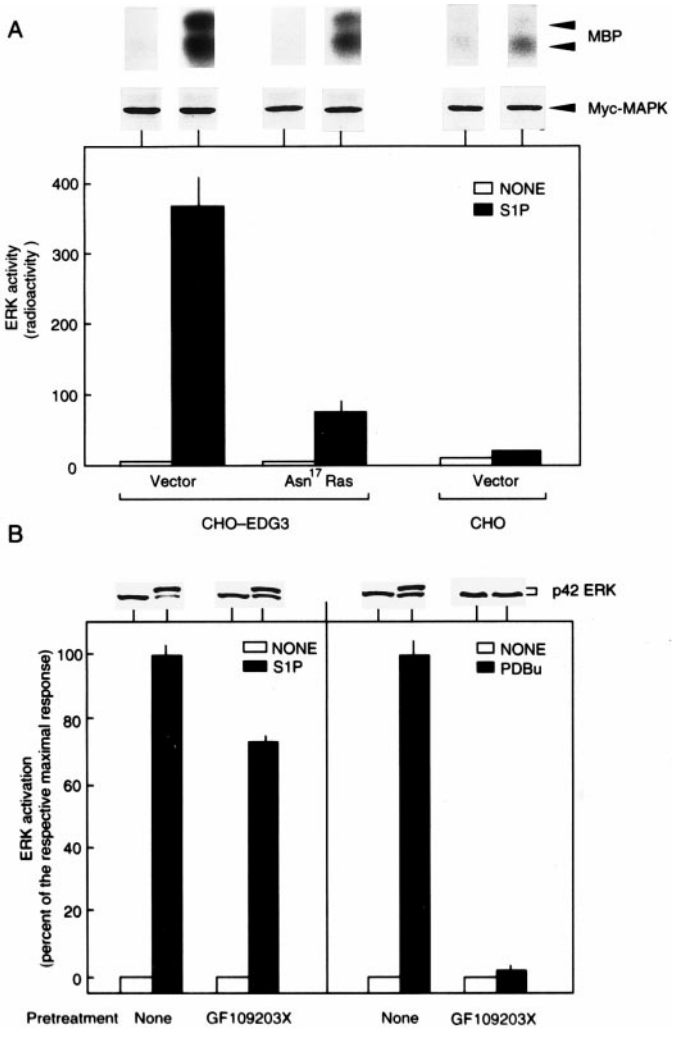

FIG. 4. Dependence of S1P-induced MAPK activation on Ras and PKC. A, CHO-EDG3 cells and CHO cells were transiently co-transfected with a Myc-tagged MAPK expression vector and either an Asn^{17}-H-Ras expression vector or an empty vector, and then stimulated with 10^{-7} M S1P for 5 min. Autoradiograms of myelin basic protein (MBP) and Western blots of Myc-MAPK are shown above the bar graphs. Note that the levels of expression of Myc-tagged MAPK were similar between the transfection groups. B, CHO-EDG3 cells were treated or not with 3 μM GF109203X for 10 min, and then stimulated with either 10^{-7} M S1P for 5 min or with 10^{-7} M PDBu for 5 min. Cell lysates were separated on a 10% polyacrylamide gel, followed by Western blotting using an anti-ERK antibody. Values are means ± S. E. of three determinations.

concentrations. S1P activated MAPK in CHO-EDG1 and CHO-AGR16 cells as well. The potency of S1P was lower in CHO-AGR16 cells (the EC_{50} value of 5×10^{-8} M) compared to CHO-EDG3 and CHO-EDG1 cells (the EC_{50} value of 5×10^{-9} M). Similar to the case of CHO-EDG3 cells, PTX nearly totally abolished S1P-induced MAPK activation in CHO-EDG1 and CHO-AGR16 cells. We demonstrated previously that EDG1- and AGR16- mediated MAPK activation was abrogated by expression of a dominant negative form of Ras

TABLE 1

S1P Inhibits Forskolin-Induced Increase in the Cellular Cyclic AMP Content in CHO-EDG3 Cells

Cells	Pretreatment	Simulation	Cyclic AMP content	
			pmol/well	
CHO	None	None	0.77 ± 0.05	
		Forskolin	7.32 ± 0.19	NS
		Forskolin + S1P	7.25 ± 0.18	
	PTX	None	0.55 ± 0.04	
		Forskolin	4.96 ± 0.10	NS
		Forskolin + S1P	4.94 ± 0.08	
CHO-EDG3	None	None	0.87 ± 0.09	
		Forskolin	7.28 ± 0.38	$p < 0.05$*
		Forskolin + S1P	4.32 ± 0.03	
	PTX	None	0.81 ± 0.08	
		Forskolin	4.40 ± 0.12	NS
		Forskolin + S1P	4.59 ± 0.12	

Note. Cells pretreated or not with PTX (100 ng/ml for 24 h) were stimulated with forskolin (2×10^{-7} M) for 5 min and then incubated with or without S1P (3×10^{-8} M) for further 5 min in the presence of 0.2 mM 3-isobutyl-1-methylxanthine. Cellular cyclic AMP contents were determined by radioimmunoassay. Results are the means ± S. E. of three determinations. NS, statistically not significant.

* Statistically significant by Student's t test.

(Asn^{17}-H-Ras) (21, 22). The expression of Asn^{17}-H-Ras inhibited S1P-induced MAPK activation by more than 70% (Fig. 4A), in CHO-EDG3 cells as well. In contrast, the specific PKC inhibitor GF109203X only partially (25%) inhibited S1P-induced MAPK activation (Fig. 4B). GF109203X at the dose employed completely abrogated PDBu-induced MAPK activation (Fig. 4B).

Since S1P-induced MAPK activation was sensitive to PTX in CHO-EDG3 cells, we examined whether S1P decreased cellular cyclic AMP content via a PTX-sensitive G protein. As shown in Table 1, S1P reduced the folskolin-stimulated increase in the cyclic AMP content by approximately 40% in CHO-EDG3 cells. Pretreatment of cells with PTX totally abolished this effect of S1P. S1P was without effect on the cyclic AMP content in parental CHO cells.

DISCUSSION

The present study, by employing HEL cells which do not respond to either S1P or other related lipids, establishes that EDG3 is a functional receptor specific for S1P and SPC (Fig. 1). Thus, EDG1, AGR16 and EDG3 are the lysosphingolipid receptors with the identical agonist apecificity (Fig. 1 and our previous studies (references 21 and 22). It is of note that LPA does not act as an agonist on any of these three lysophospholipid receptors (21, 22). Conversely, S1P does not act on the LPA receptors EDG2 or EDG4 (16, 23). Thus, the EDG family is clearly separated into the two subgroups, i.e. the lysosphingolipid receptors consisting of EDG1, AGR16 and EDG3, and the LPA receptors consisting of EDG2 and EDG4.

In any of the three lysosphingolipid receptors, S1P is more than one order of magnitude more potent than SPC in causing Ca^{2+} mobilization (Fig. 1B and references 21 and 22). The relative agonist potencies of the lysosphingolipids for the cloned EDG family receptors are consistent with previous results on the relative activities of S1P and SPC reported in several cell types, including vascular endothelial and smooth muscle cells as wells as HEK293 cells (4, 12, 30). However, our [^{32}P] S1P binding results (Fig. 1A) are different from those by Van Brocklyn et al. (25). Although the reason for the discrepancy between our results and theirs is not clear at present, a difference in the cell types employed for the studies may be responsible for this. We reported recently that S1P is present in the serum at the concentration as high as 10^{-7} M order (31). On the other hand, very little is known about concentrations in body fluids, biosynthesis and metabolism of SPC. It was shown that the SPC content in brain and extraneural tissues is much elevated in patients with Niemann-Pick disease, a lipid storage disorder (32), compared to normal individuals. The observations raise the possibility that SPC could play a pathological role by acting through the lysosphingolipid receptors.

We have shown that all members of the three lysosphingolipid receptors are coupled to phospholipase C activation- Ca^{2+} mobilization, but through the differential coupling mechanisms: EDG3 and AGR16 are coupled to phospholipase C via both PTX-insensitive and, to a lesser extent, -sensitive G proteins, most likely Gq/11 and Gi, whereas EDG1 is coupled exclusively via PTX-sensitive Gi protein to phospholipase C (Fig. 2 and reference 21). Consistent with this, the lysosphingolipids cause a prominent increase in the $[Ca^{2+}]_i$ in cells expressing each of EDG3 and AGR16, whereas they cause only a modest increase in the $[Ca^{2+}]_i$ in cells expressing EDG1. On the other hand,

the coupling of the three lysosphingolipid receptors to the MAPK is uniformly largely via PTX-sensitive Gi and Ras (Fig. 3). However, the efficiency of the coupling to the MAPK is different among the three lysosphingolipid receptors: in cells expressing EDG3 or EDG1 low concentiations of S1P effectively activates MAPK activation, whereas in cells expressing AGR16 higher concentrations of S1P are required for MAPK activation. Finally, EDG3 is similar to EDG1 in the action on cellular cyclic AMP level, but contrasts with AGR16, which mediates an increase in cellular cyclic AMP (21, 22).

In summary, we have shown in the present study that EDG3 is a third member of the lysosphingolipid receptors that have the specificity for S1P and SPC. EDG3 is distinct from either of EDG1 and AGR16, in that EDG3 is efficiently coupled to robust activation of both phospholipase C-Ca^{2+} mobilization and the MAPK. It is important to explore how the functional differences in the signalling of the receptor subtypes affect their biological roles, since the three lysosphingolipid receptors are widely expressed in most tissues in an overlapping manner.

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REFERENCES

1. Desai, N. N., and Spiegel, S. (1991) *Biochem. Biophys. Res. Commun.* **181,** 361–366.
2. Zhang, H., Desai, N. N., Olivera, A., Seki, T., Brooker, G., and Spiegel, S. (1991) *J. Cell Biol.* **114,** 155–167.
3. Sadahira, Y., Ruan, F., Hakomori, S., and Igarashi, Y. (1992) *Proc. Natl. Acad. Sci USA* **89,** 9686–9690.
4. Bornfeldt, K. E., Graves, L. M., Rained, E. W., Igarashi, Y., Wayman, G., Tamamura, S., Yatomi, Y., Sidhu, J. S., Krebs, E. G., Hakomori, S., and Ross, R. (1995) *J. Cell Biol.* **130,** 193–206.
5. Yatomi, Y., Ruan, F., Hakomori, S., and Igarashi, Y. (1995) *Blood.* **86,** 193–202.
6. Postma, F. R., Jalink, K., Hengeveld, T., and Moolenaar, W. H. (1996) *EMBO J.* **15,** 2388–2837.
7. Chao, C. P., Laulederkind, S. J. F., and Ballou, L. R. (1994) *J. Biol. Chem.* **269,** 5849–5856.
8. Goodemote, K. A., Mattie, M. E., Berger, A., and Spiegel, S. (1996) *J. Biol. Chem.* **270,** 10272–10277.
9. Seufferlein, T., and Rozengurt, E. (1995) *J. Biol. Chem.* **270,** 24334–24342.
10. Wu, J., Spiegel, S., and Sturgill, T. W. (1995) *J. Biol. Chem.* **270,** 11484–11488.
11. Büneman, M., Liliom, K., Brandt, B. K., Pott, L., Tseng, J. L., Desiderio, D. M., Sun, G., Miller, D., and Tigyi, G. (1996) *EMBO J.* **15,** 5527–5537.
12. Van Koppen, C. J., Meyer zu Heringdorf, D., Laser, K. T., Zhang, C., Jabobs, K H., Bünemann, M., and Pott, L. (1996) *J. Biol. Chem.* **271,** 2082–2087.
13. Hla, T., and Maciag, T. (1990) *J. Biol. Chem.* **265,** 9308–9313.
14. Masana, M. I., Brown, R. C., Pu, H., Gurney, M. E., and Dubocovich, M. L. (1995) *Receptors Channels* **3,** 255–262.
15. Yamaguchi, F., Tokuda, M., Hatase, O., and Brenner, S. (1996) *Biochem. Biophys. Res. Commun.* **227,** 608–614.
16. An, S., Bleu, T., Hallmark, O. G., and Goetzl, E. J. (1998) *J. Biol. Chem.* **273,** 7906–7910.
17. Okazaki, H., Ishizaka, N., Sakurai, T., Kurokawa, K., Goto, K., Kumada, M., and Takuwa, Y. (1993) *Biochem. Biophts. Res. Commun.* **190,** 1104–1109.
18. Maclennan, A. J., Browe, C. S., Gaskin, A. A., Lado, D. C., and Shaw, G. (1994) *Mol. Cell. Neurosci.* **5,** 201–209.
19. Lee, M., Van Brocklyn, J. R., Thangada, S., Liu, C. H., Hand, A. R., Menzeleev, R., Spiegel, S., and Hla, T. (1998) *Science* **279,** 1552–1555.
20. Zondag, G. C. M., Postma, F. R., Verlaan, I., and Moolenaar, W. H. (1998) *Biochem. J.* **330,** 605–609.
21. Okamoto, H., Takuwa, N., Gonda, K., Okazako, H., Chang, K., Yatomi, Y., Shigematsu, H., and Takuwa, Y. (1998) *J. Biol. Chem.* **273,** 27104–27110.
22. Gonda, K., Okamoto, H., Takuwa, N., Yatomi, Y., Okazaki, H., Sakura, T., Kimura, S., Sillard, R., Harii, K., and Takuwa, Y. (1999) *Biochem J.* **337,** 67–75.
23. Hecht, J. H., Weiner, J. A., Post, S. R., and Chun, J. (1996) *J. Cell Biol.* **135,** 1071–1083.
24. An, S., Bleu, T., Huang, W., Hallmark, O. G., Coughlin, S. R., and Goetzl, E. J. (1997) *FEBS Lett.* **417,** 279–282.
25. Van Brocklyn, J. R., Tu, Z., Edsall, L. C., Schmidt, R. R., and Spiegel, S. (1999) *J. Biol. Chem.* **274,** 4626–4632.
26. Sato, K., Kon, J., Tomura, H., Osada, M., Murata, N., Kuwabara, A., Wattanabe, T., Ohta, H., Ui, M., and Okajima, F. (1999) *FEBS Lett.* **443,** 25–30.
27. Chang, K., Hanaoka, K., Kumada, M., and Takuwa, Y. (1995) *J. Biol. Chem.* **270,** 26152–26158.
28. Mitsui, H., Takuwa, N., Kurokawa, K., Exton, J. H., and Takuwa, Y. (1997) *J. Biol. Chem.* **272,** 4904–4910.
29. Takuwa, N., and Takuwa, Y. (1997) *Mol. Cell. Biol.* **17,** 5348–5358.
30. Meyer zu Heringdorf, D., Van Koppen, C. J., Windorfer, B., Himmel, H. M., and Jakobs, K. K. (1996) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **354,** 397–403.
31. Yatomi, Y., Lgarashi, T., Yang, L., Hisano, N., Qi, R., Asazuma, N., Satoh, K., Ozaki, Y., and Kume, S. (1997) *J. Biochem. (Tokyo)* **121,** 969–973.
32. Rodriguez-Lafrasse, C., and Vanier, M. T. (1999) *Neurochem. Res.* **24,** 199–205.