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Enhancement by Sphingosine 1-Phosphate in Vasopressin-Induced Phosphoinositide Hydrolysis in Aortic Smooth-Muscle Cells: Involvement of p38 MAP Kinase

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Abstract We previously reported that sphingosine 1-phosphate (S-1-P), a sphingomyelin metabolite, activates p44/p42 mitogen-activated protein (MAP) kinase and p38 MAP kinase in aortic smooth-muscle A10 cells. In the present study, we investigated the effect of sphingomyelin metabolites on phospholipase C-catalyzing phosphoinositide hydrolysis induced by arginine vasopressin (AVP) in A10 cells. C_2-ceramide and sphingosine had little effect on inositol phosphate (IP) formation stimulated by AVP. S-1-P, which alone slightly stimulated the IPs formation, dose-dependently amplified the AVP-induced formation of IPs. Tumor necrosis factor-α enhanced the AVP-induced formation of IPs. However, S-1-P did not enhance the formation of IPs by NaF, a heterotrimeric GTP-binding protein activator. Pertussis toxin inhibited the effect of S-1-P. PD98059, an inhibitor of the upstream kinase that activates p44/p42 MAP kinase, had little effect on the enhancement by S-1-P. SB203580, an inhibitor of p38 MAP kinase, suppressed the effect of S-1-P on the formation of IPs by AVP. SB203580 inhibited the AVP-induced phosphorylation of p38 MAP kinase. Pertussis toxin suppressed the phosphorylation of p38 MAP kinase by S-1-P. These results indicate that S-1-P amplifies AVP-induced phosphoinositide hydrolysis by phospholipase C through p38 MAP kinase in vascular smooth-muscle cells. J. Cell. Biochem. 80:46–52, 2000.† © 2000 Wiley-Liss, Inc.

Key words: sphingosine 1-phosphate; MAP kinase; phosphoinositide; vascular smooth-muscle cells

Sphingolipid and its metabolites have recently been implicated in a variety of cellular functions [Hannun, 1994; Spiegel and Merrill, 1996]. Sphingomyelin is catalyzed by sphingomyelinase, resulting in the formation of ceramide, which is subsequently metabolized to sphingosine and sphingosine 1-phosphate (S-1-P) [Hannun, 1994; Spiegel and Merrill, 1996]. Ceramide has been reported to induce apoptosis in several types of cells, whereas sphingosine and S-1-P are mitogenic. Accumulating evidence indicates that these sphingomyelin metabolites play an important role as second messengers and mediate several biological effects induced by extracellular agonists such as tumor necrosis factor-α (TNF) [Schutze et al., 1995; Westwick et al., 1995; Kanety et al., 1996]. Vascular smooth-muscle cells play a crucial role in the pathogenesis of atherosclerosis and hypertension [Ross, 1993]. TNF reportedly induces sphingomyelin hydrolysis by sphingomyelinase in vascular smooth-muscle cells [Johns and Webb, 1998]. It has been reported that S-1-P inhibits the chemotactic motility stimulated by platelet-derived growth factor (PDGF) in vascular smooth-muscle cells [Bornfeldt et al., 1995]. We have recently shown that, among sphingomyelin metabolites, S-1-P activates p44/p42 mitogen-activated protein (MAP) kinase and p38 MAP kinase in aortic smooth-muscle A10 cells, and that p38 MAP kinase is involved in the arginine vasopressin (AVP)-stimulated induction of heat shock protein 27 [Kozawa et al., 1999]. However, the

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exact role of sphingomyelin metabolites in vascular smooth-muscle cells is still poorly understood.

AVP is a potent vasoactive agent that stimulates the proliferation of aortic smooth-muscle cells [Scott-Burden et al., 1992]. As for intracellular signaling of AVP in vascular smooth-muscle cells, AVP stimulates phosphoinositide hydrolysis by phospholipase C through its binding to the V_1 receptor [Grillone et al., 1988]. It has been shown that pertussis-toxin-sensitive GTP-binding protein is involved in the phospholipase C activation by AVP in aortic smooth-muscle A10 cells [Xuan et al., 1987]. In addition, PDGF reportedly induces phosphoinositide hydrolysis in part through S-1-P in aortic smooth-muscle cells, and S-1-P alone stimulates phosphoinositide hydrolysis by phospholipase C in these cells [Bornfeldt et al., 1995]. In the present study, we investigated the effects of sphingomyelin metabolites on AVP-induced phosphoinositide hydrolysis by phospholipase C in A10 cells. We show that neither ceramide nor sphingosine but rather S-1-P amplified AVP-induced phosphoinositide hydrolysis by a p38 MAP kinase-dependent mechanism in these cells.

MATERIALS AND METHODS

Materials

Myo-[^{3}H]inositol (90 Ci/mmol) and an ECL Western blotting detection system were purchased from Amersham Japan (Tokyo, Japan). AVP was purchased from Peptide Institute, Inc. (Minoh, Japan). C_2-ceramide was obtained from BIOMOL Research Laboratories, Inc. (Plymouth, PA). Sphingosine and S-1-P were obtained from Sigma Chemical Co. (St. Louis, MO). TNF and pertussis toxin were purchased from Funakoshi Pharmaceutical Co. (Tokyo, Japan). Phospho-specific p38 MAP kinase antibodies and p38 MAP kinase antibodies were purchased from New England BioLabs, Inc. (Beverly, MA). PD98059 and SB203580 were obtained from Calbiochem (La Jolla, CA). Other materials and chemicals were obtained from commercial sources. C_2-ceramide, sphingosine, S-1-P, PD98059, and SB203580 were dissolved in dimethyl sulfoxide. The maximum concentration of dimethyl sulfoxide was 0.1%, which did not affect the measurement of inositol phosphates (IPs) formation or Western blotting analysis.

Cell Culture

Aortic smooth-muscle A10 cells were obtained from the American Type Culture Collection (Rockville, MD) and were maintained as previously described [Kozawa et al., 1999]. In brief, the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum at 37°C in a humidified atmosphere of 5% CO_2/95% air. Cells were seeded into 35-mm (1×10^5) or 90-mm (5×10^5) diameter dishes. After 5 days, the medium was exchanged for serum-free DMEM, and cells were used for experiments after 48 h. For measurements of the formation of IPs, the medium was exchanged for inositol-free DMEM. When indicated, cells were pretreated with sphingomyelin metabolites or TNF for 20 min before the stimulation of AVP or NaF. Pretreatment of pertussis toxin was performed for 24 h.

Measurement of IP formation

To determine phosphoinositide hydrolysis by phospholipase C, the cells were labeled with myo-[^{3}H]inositol (3 μCi/dish) for 48 h. Labeled cells were preincubated with 10 mM LiCl for 10 min in 1 ml of an assay buffer (5 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM KCl, 5.5 mM glucose, 0.8 mM $MgSO_4$, and 1 mM $CaCl_2$) containing 0.01% bovine serum albumin. Cells were then stimulated by AVP or NaF at 37°C for the indicated periods. The reaction was terminated by the addition of 1 ml of 30% trichloroacetic acid, and the acid supernatant was treated with diethyl ether to remove the acid and then neutralized with 0.1 M NaOH. The supernatant was applied to a 1-ml Dowex AG1-X8 (100–200 mesh, formate form) as described previously [Suzuki et al., 1996]. Radioactive IPs were then eluted from the column with 8 ml of 0.1 M formic acid containing 1 M ammonium formate. When indicated, the cells were pretreated with PD98059 or SB203580 for 60 min.

Analysis of p38 MAP Kinase by Western Blotting

Cultured cells were stimulated by S-1-P in serum-free DMEM for the indicated periods. They were washed twice with phosphate-buffered saline and then lysed, homogenized, and sonicated in a lysis buffer containing 62.5 mM Tris/Cl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 50 mM dithiothreitol, and 10%

glycerol. The cytosolic fraction was collected as the supernatant after centrifugation at 125,000 x g for 10 min at 4°C. SDS–polyacrylamide gel electrophoresis was performed by the method of Laemmli [Laemmli, 1970] in 10% polyacrylamide gels. Western blotting analysis was performed as described previously [Kozawa et al., 1999], using phospho-specific p38 MAP kinase antibodies or p38 MAP kinase antibodies, with peroxidase-labeled antibodies raised in goat against rabbit IgG being used as secondary antibodies. Peroxidase activity on the nitrocellulose sheet was visualized on X-ray film by means of the ECL Western blotting detection system. When indicated, the cells were pretreated with SB203580 for 60 min.

Statistical Analysis

Data were analyzed by analysis of variance followed by Bonferroni method for multiple comparisons between pairs, and a $P < 0.05$ was considered significant. All data are presented as the mean ± SD of triplicate determinations.

RESULTS

Effect of C_2-Ceramide, Sphingosine or S-1-P on AVP-Induced Formation of IPs in A10 Cells

We previously found that AVP induces phosphoinositide hydrolysis by phospholipase C in aortic smooth-muscle A10 cells [Tanabe et al., 1999]. We first examined the effects of sphingomyelin metabolites on the AVP-induced formation of IPs in these cells. C_2-ceramide and sphingosine had little effect on IPs formation by AVP in the range between 0.1 and 30 μM (Fig. 1). S-1-P, which alone stimulated the formation of IPs, consistent with a previous report [Bornfeldt et al., 1995], significantly enhanced the AVP-induced IPs formation. The effect of S-1-P on the IPs formation was dose dependent in the range between 0.1 and 30 μM, and the maximum effect was observed at 30 μM.

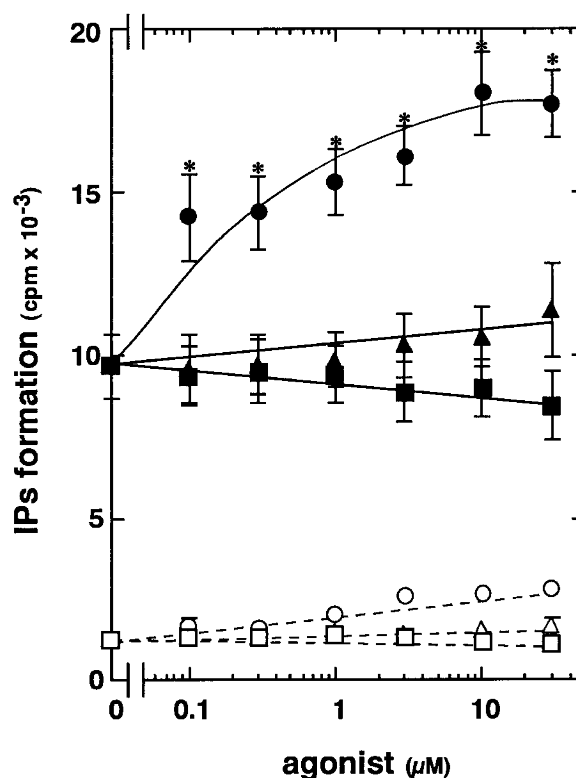

Fig. 1. Effect of C_2-ceramide, sphingosine or S-1-P on AVP-induced formation of IPs in A10 cells. The cultured cells were pretreated with various doses of C_2-ceramide (filled and open squares), sphingosine (closed and open triangles) or S-1-P (fillled and open circles) for 20 min, and then stimulated by 0.1 μM AVP (closed symbol) or vehicle (open symbol) for 30 min. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}P < 0.05$ vs. AVP alone.

Effect of TNF on AVP-Induced Formation of IPs in A10 Cells

It has been reported that TNF induces sphingomyelin hydrolysis by sphingomyelinase, resulting in the formation of sphingomyelin metabolites in vascular smooth-muscle cells [Johns et al., 1998]. Thus, to investigate whether TNF affects the AVP-induced phosphoinositide hydrolysis in A10 cells, we examined the effect of TNF on the AVP-stimulated formation of IPs. TNF significantly amplified the IPs formation by AVP (Table I).

Effect of S-1-P on NaF-Induced Formation of IPs in A10 Cells

It has been reported that pertussis-toxin-sensitive GTP-binding protein is involved in AVP-induced phosphoinositide hydrolysis by phospholipase C in A10 cells [Grillone et al., 1988]. To investigate whether or not the effect of S-1-P is exerted at a point upstream from the GTP-binding protein in these cells, we examined the effect of S-1-P on the IPs formation stimulated by NaF, a direct activator of heterotrimeric GTP-binding proteins [Gilman, 1987]. S-1-P did not enhance the NaF-induced formation of IPs (data not shown).

TABLE I. Effect of TNF on AVP-Induced Formation of IPs in A10 Cells[a]

TNF	AVP	IP formation (cpm)
−	−	1,355 ± 182
−	+	9,515 ± 932
+	−	1,097 ± 155
+	+	12,626 ± 1,075*

[a]The cultured cells were pretreated with 0.3 ng/ml TNF or vehicle for 20 min, and then stimulated 0.1 μM AVP for 30 min. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$ vs. AVP alone.

TABLE II. Effect of Pertussis Toxin (PTX) on S-1-P Amplification of AVP-Induced Formation of IPs in A10 Cells[a]

PTX	S-1-P	AVP	IP formation (cpm)
−	−	+	8,092 ± 895
−	+	+	12,168 ± 1,012
+	−	+	6,156 ± 525
+	+	+	7,356 ± 671*

[a]The cultured cells were pretreated with 1 μg/ml PTX or vehicle for 24 h, and then stimulated by 0.1 μM AVP for 30 min. The pretreatment of 10 μM S-1-P was performed 60 min before the stimulation of AVP. Values for AVP-unstimulated cells were subtracted to produce each data point. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$ vs. AVP with S-1-P.

TABLE III. Effects of PD98059 or SB203580 on S-1-P Amplification of AVP-Induced Formation of IPs in A10 Cells[a]

	S-1-P	AVP	IP formation (cpm)
—	−	+	8,277 ± 921
—	+	+	15,135 ± 1,122
PD98059	−	+	8,352 ± 855
PD98059	+	+	15,714 ± 1,035
SB203580	−	+	8,239 ± 907
SB203580	+	+	10,524 ± 936*

[a]The cultured cells were pretreated with 50 μM PD98059, 30 μM SB203580, or vehicle for 60 min, and then stimulated by 0.1 μM AVP for 30 min. The pretreatment of 10 μM S-1-P was performed 20 min before the stimulation of AVP. Values for AVP-unstimulated cells were subtracted to produce each data point. Each value represents the mean ± SD of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$ vs. AVP with S-1-P.

Effect of Pertussis Toxin on S-1-P Amplification of AVP-Induced IP Formation in A10 Cells

It has been shown that S-1-P extracellularly acts as a ligand for cell surface receptors that are coupled to pertussis-toxin-sensitive GTP-binding protein as well as intracellularly acting as a second messenger [von Koppen et al., 1996; van Brocklyn et al., 1998]. Thus, we examined the effect of pertussis toxin on the amplification by S-1-P of the AVP-induced formation of IPs in A10 cells. Pertussis toxin, which suppressed the AVP-induced IP formation consistent with a previous report [Grillone et al., 1988], significantly reduced the enhancement by S-1-P (Table II).

Effects of PD98059 or SB203580 on S-1-P Amplification of AVP-Induced Formation of IPs in A10 Cells

We have previously reported that S-1-P phosphorylates p44/p42 MAP kinase and p38 MAP kinase in A10 cells [Kozawa et al., 1999]. Thus, to explore the role of p44/p42 MAP kinase in the S-1-P amplification of AVP-induced phosphoinositide hydrolysis in A10 cells, we investigated the effect of PD98059, a specific inhibitor of the upstream kinase that activates p44/p42 MAP kinase [Dudley et al., 1995], on S-1-P amplification of AVP-induced IP formation. PD98059, which inhibited the S-1-P-induced phosphorylation of p44/p42 MAP kinase as previously described [Kozawa et al., 1999], had little effect on the amplification by S-1-P (Table III). We next examined the effect of SB203580, a specific inhibitor of p38 MAP kinase [Cuenda et al., 1995], on the amplification by S-1-P. SB203580, which alone did not affect the basal levels of IPs, significantly suppressed the amplification by S-1-P of AVP-stimulated IP formation (Table III). We found that SB203580 suppressed the AVP-induced phosphorylation of p38 MAP kinase (Fig. 2).

Effect of Pertussis Toxin on the Phosphorylation of p38 MAP Kinase by S-1-P in A10 Cells

We examined the effect of pertussis toxin on the p38 MAP kinase phosphorylation by S-1-P in A10 cells. Pertussis toxin, which alone did not affect p38 MAP kinase, significantly reduced the phosphorylation of p38 MAP kinase by S-1-P (Fig. 3).

DISCUSSION

In the present study, we showed that, among sphingomyelin metabolites, neither ceramide

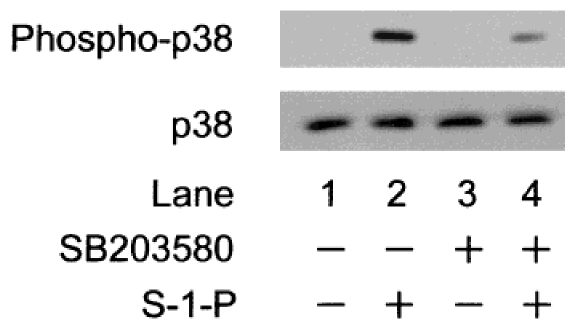

Fig. 2. Effects of SB203580 on the phosphorylation of p38 MAP kinase by S-1-P in A10 cells. The cultured cells were pretreated with 30 μM SB203580 or vehicle for 60 min, and then stimulated by 30 μM S-1-P or vehicle for 10 min. Extract of cells were subjected to SDS–polyacrylamide gel electrophoresis against phospho-specific p38 MAP kinase antibodies or p38 MAP kinase antibodies. The results are representative of triplicate independent experiments.

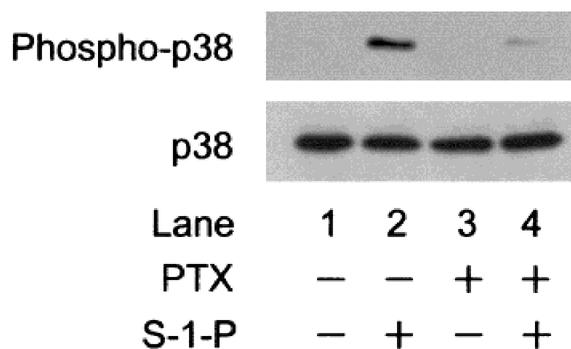

Fig. 3. Effect of pertussis toxin (PTX) on the phosphorylation of p38 MAP kinase by S-1-P in A10 cells. The cultured cells were pretreated with 1 μg/ml PTX or vehicle for 24 h, and then stimulated by 30 μM S-1-P or vehicle for 10 min. Extract of cells were subjected to SDS–polyacrylamide gel electrophoresis against phospho-specific p38 MAP kinase antibodies or p38 MAP kinase antibodies. The results are representative of triplicate independent experiments.

nor sphingosine but rather S-1-P dose-dependently enhanced the AVP-induced formation of IPs in aortic smooth-muscle A10 cells. In addition, we demonstrated that TNF, a multifunctional cytokine, amplified the IP formation by AVP as a physiological agonist in these cells. It has been shown that TNF induces sphingomyelinase-catalyzing sphingomyelin hydrolysis in vascular smooth-muscle cells [Johns and Webb, 1998]. Thus, it is probable that AVP-induced phosphoinositide hydrolysis by phospholipase C is physiologically enhanced by S-1-P in such cells. It has been shown that pertussis-toxin-sensitive GTP-binding protein is involved in the AVP-induced phosphoinositide hydrolysis by phospholipase C [Grillone et al., 1988]. In addition, we found that the NaF-stimulated IPs formation was not enhanced by S-1-P. Because NaF is well known to be a direct activator of heterotrimeric GTP-binding proteins [Gilman, 1987], it is probable that the amplifying effect of S-1-P is exerted at a point upstream from the heterotrimeric GTP-binding protein in aortic smooth-muscle A10 cells.

Accumulating evidence indicates that S-1-P is implicated as a pivotal intracellular second messenger [Hannun, 1994; Spiegel et al., 1996]. In addition, S-1-P has also been demonstrated to act extracellularly as a ligand for cell surface receptors, which are coupled to pertussis-toxin-sensitive GTP-binding protein [von Koppen et al., 1996; van Brocklyn et al., 1998]. It has been shown that S-1-P is abundantly stored in platelets and released upon the platelet activation into circulation [Yatomi et al., 1995]. In the present study, pertussis toxin markedly suppressed the effect of S-1-P. It is well known that the ADP-ribosylation of the α–subunit of GTP-binding proteins such as Gi and Go by pertussis toxin causes the uncoupling of the receptor to the GTP-binding protein [Gilman, 1987]. Therefore, it is most likely that S-1-P amplifies AVP-induced phosphoinositide hydrolysis through pertussis-toxin-sensitive GTP-binding protein-coupled receptors. However, the inhibitory effect of pertussis toxin was only partial. Taking our results into account, it is possible that S-1-P acts both intracellularly and extracellularly as a physiological agent, resulting in the amplification of the phosphoinositide hydrolysis stimulated by AVP in aortic smooth-muscle A10 cells.

The MAP kinase superfamily plays an important role in the intracellular signaling system by a variety of extracellular stimuli [Nishida and Goto, 1993; Widmann et al., 1999]. It has been reported that the MAP kinase superfamily is involved in S-1-P signaling in several types of cells [Spiegel and Merrill, 1996]. We have previously reported that S-1-P activates both p44/p42 MAP kinase and p38 MAP kinase in aortic smooth-muscle A10 cells [Kozawa et al., 1999]. To clarify the involvement of p44/p42 MAP kinase or p38 MAP kinase in the S-1-P amplification of AVP-induced IP formation, the effect of PD98059 or SB203580 was then examined. Not PD98059 but SB203580 inhibited the S-1-P amplifying

effect on phospholipase C-catalyzing phosphoinositide hydrolysis induced by AVP. We found that SB203580 actually inhibited the phosphorylation of p38 MAP kinase induced by S-1-P. Furthermore, we have reported that the phosphorylation of p44/p42 MAP kinase by AVP is suppressed by PD98059. Additionally, pertussis toxin suppressed the S-1-P-induced phosphorylation of p38 MAP kinase. Based on our findings, it is most likely that S-1-P enhanced AVP-induced phosphoinositide hydrolysis by a p38 MAP kinase-dependent manner in aortic smooth-muscle A10 cells.

An intrinsic function of vascular smooth-muscle cells is their important role in regulating vascular tone [Ross, 1993]. It is well recognized that phosphoinositide hydrolysis by phospholipase C leads to the formation of diacylglycerol, a physiological activator of protein kinase C and IPs [Nishizuka, 1992; Berridge, 1993]. As an element of the intracellular signaling system in vascular smooth-muscle cells, the activation of protein kinase C has a crucial role in the induction of vasoconstriction [Nishizuka, 1992; Walsh, 1994]. AVP, a potent vasoactive peptide, induces vasoconstriction through protein kinase C activation in vascular smooth-muscle cells [Scott-Burden et al., 1992; Nishizuka, 1992]. In the present study, we showed that the AVP-stimulated formation of IPs was amplified by S-1-P in A10 cells. Based on our findings, it is probable that S-1-P enhances AVP-induced vasoconstriction in vascular smooth-muscle cells.

In conclusion, these results strongly suggest that S-1-P enhances AVP-induced phosphoinositide hydrolysis by phospholipase C via p38 MAP kinase in vascular smooth-muscle cells.

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REFERENCES

Bornfeldt KE, Graves LM, Raines EW, Igarashi Y, Wayman G, Yamamura S, Yatomi Y, Sidhu JS, Krebs EG, Hakomori S, Ross R. 1995. Sphingosine-1-phosphate inhibits PDGF-induced chemotaxis of human arterial smooth muscle cells: spartial and temporal modulation of PDGF chemotactic signal transduction. J Cell Biol 130:193–206.

Berridge MJ. 1993. Inositol trisphosphate and calcium signalling. Nature 361:315–325.

Cuenda A, Rouse J, Doza YN, Meier R, Cohen P, Gallagher TF, Young PR, Lee JC. 1995. SB203580 is a specific inhibitor of a MAP homologue which is stimulated by cellular stresses and interleukin-1. FEBS Lett 364:229–233.

Dudley DT, Pang L, Decker SJ, Bridges AJ, Saltiel AR. 1995. A synthetic inhibitor of the mitogen-activated protein kinase cascade. Proc Natl Acad Sci USA 92:7686–7689.

Gilman AG. 1987. G proteins: transducers of receptor-generated signals. Annu Rev Biochem 56:615–649.

Grillone LR, Clark MA, Godfrey RW, Stassen F, Crooke ST. 1988. Vasopressin induces V1 receptors to activate phosphatidylinositol- and phosphatidylcholine-specific phospholipase C and stimulates the release of arachidonic acid by at least two pathways in the smooth muscle cell line, A10. J Biol Chem 263:2658–2663.

Hannun YA. 1994. The sphingomyelin cycle and the second messenger function of ceramide. J Biol Chem 269:3125–3128.

Johns DG, Webb RC. 1998. TNF-alpha-induced endothelin-independent vasodilation: a role for phospholipase A2-dependent ceramide signaling. Am J Physiol 275:H1592–H1598.

Kanety H, Hemi R, Papa MZ, Karasik A. 1996. Sphingomyelinase and ceramide suppress insulin-induced tyrosine phosphorylation of the insulin receptor substrate-1. J Biol Chem 271:9895–9897.

Kozawa O, Tanabe K, Ito H, Matsuno H, Niwa M, Kato K, Uematsu T. 1999. Sphingosine 1-phosphate regulates heat shock protein 27 induction by a p38 MAP kinase-dependent mechanism in aortic smooth muscle cells. Exp Cell Res 250:376–380.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Nishida E, Goto Y. 1993. The MAP kinase cascade is essential for diverse signal transduction pathways. Trends Biochem Sci 18:128–131.

Nishizuka Y. 1992. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607–614.

Ross R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362:801–809.

Schutze S, Wiegmann K, Machleidt T, Kronke M. 1995. TNF-induced activation of NF-kappa B. Immunobiology 193:193–203.

Scott-Burden T, Hahn AWA, Buhler FR, Resink TJ. 1992. Vasoactive peptides and growth factors in the pathophysiology of hypertension. J Cardiovasc Pharmacol 20: S55–S64.

Spiegel S, Merrill JAH. 1996. Sphingolipid metabolism and cell growth regulation. FASEB J 10:1388–1397.

Suzuki A, Shinoda J, Oiso Y, Kozawa O. 1996. Tyrosine kinase is involved in angiotensin II-stimulated phospholipase D activation in aortic smooth muscle cells: function of Ca^{2+} influx. Atherosclerosis 121:119–127.

Tanabe K, Kozawa O, Matsuno H, Niwa M, Dohi S, Uematsu T. 1999. Effects of propofol on arachidonic cascade by vasopressin in aortic smooth muscle cells. Anesthesiology 90:215–224.

van Brocklyn JR, Lee M-J, Menzeleev R, Olivera A, Edsall L, Cuvillier O, Thomas DM, Coopman PJP, Thangada S, Liu CH, Hla T, Spiegel S. 1998. Dual actions of sphingosine-1-phosphate: extracellular through the Gi-coupled receptor Edg-1 and intracellular to regulated proliferation and survival. J Cell Biol 142:229–240.

von Koppen CJ, zu Heringdorf DM, Laser KT, Zhang C, Jakobs KH, Bunemann M, Pott L. 1996. Activation of an affinity Gi protein-couple plasma membrane receptor by sphingosine-1-phosphate. J Biol Chem 271: 2082–2087.

Walsh MP. 1994. Regulation of vascular smooth muscle tone. Can J Physiol Pharmacol 72:919–936.

Westwick JK, Bielawsks AE, Dbaibo G, Hannun YA, Brenner DA. 1995. Ceramide activates the stress-activated protein kinases. J Biol Chem 270:22689–22692.

Widmann C, Gibson S, Jarpe MB, Johnson GL. 1999. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiol Rev 79:143–180.

Xuan YT, Su YF, Chang KJ, Watkins WD. 1987. A pertussis/cholera toxin sensitive G protein may mediate vasopressin-induced inositol phosphate formation in smooth muscle cells. Biochem Biophys Res Commun 146:898–906.

Yatomi Y, Ruan F, Hakomori S, Igarashi Y. 1995. Sphingosine-1-phosphate: a platelet-activating sphingolipid released from agonist-stimulated human platelets. Blood 86:193–202.