

Effects of sphingomyelinase and sphingosine on arterial vasomotor regulation

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Abstract The sphingomyelin pathway is an important signal transduction system regulating various cellular functions. However, little is known about the effect of sphingomyelin metabolites on vasomotor function. We examined the vascular effects of sphingomyelin, sphingosine, and sphingomyelinase (SPMase) in vitro. In pig coronary rings precontracted with prostaglandin F_{2α}, sphingosine and SPMase evoked initial contraction and subsequent gradual relaxation; however, sphingomyelin did not influence the tone. The initial contractions in response to either SPMase (40 μU/ml to 0.4 U/ml) or sphingosine (0.5–10 μM) treatment were abolished in rings denuded of endothelium. This initial contraction in response to sphingosine treatment was significantly attenuated by a cyclo-oxygenase inhibitor indomethacin, but not altered by either a nitric oxide synthase inhibitor, N^ω-monomethyl-L-arginine (L-NMMA), a protein kinase C (PKC) inhibitor staurosporine, or superoxide dismutase (SOD, 100 U/ml). Incubation of coronary rings with sphingosine (10 μM) or SPMase (0.4 U/ml) for 120 min significantly attenuated subsequent endothelium-dependent relaxation in response to thrombin and A23187, but did not affect endothelium-independent relaxation in response to sodium nitroprusside. In contrast, sphingomyelin (10 μM) did not alter the endothelium-dependent relaxation. **Conclusion** In conclusion, in the sphingomyelin pathway, sphingosine induces vasoconstriction in coronary arteries that seems to be mediated by the release of cyclooxygenase-sensitive vasoconstrictor prostanoids from the endothelium. Sphingosine also induced endothelial dysfunction characterized by impaired endothelium-dependent relaxation. Thus, the sphingomyelin pathway may be an important regulator of vascular function.—Murohara, T., K. Kugiyama, M. Ohgushi, S. Sugiyama, Y. Ohta, and H. Yasue. Effects of sphingomyelinase and sphingosine on arterial vasomotor regulation. *J. Lipid Res.* 1996. **37**: 1601–1608.

Supplementary key words sphingolipid • sphingomyelin • vascular endothelium • prostaglandin • endothelium-derived relaxing factor • coronary artery • vascular tone • nitric oxide

Atherosclerosis is associated with endothelial dysfunction such as impaired release of nitric oxide (NO) (1–3). Diminished endothelial NO release promotes vasoconstriction, platelet aggregation, and leukocyte adhesion to the endothelial surface (4–7). Although precise

mechanism of the impaired NO release in the atherosclerotic arteries remains unclear, there is evidence that oxidized low density lipoprotein (ox-LDL) and lysolipids play a crucial role (3, 8–11). For example, lysophosphatidylcholine contained in ox-LDL attenuates endothelium-dependent relaxation in isolated rabbit aorta and pig coronary arteries (3, 9, 10, 12).

The sphingomyelin pathway is emerging as an important regulator of membrane signal transduction and thus, a variety of cellular functions (13–18). The sphingomyelin signaling is initiated by hydrolysis with sphingomyelinase (SPMase) to yield lipids that can modulate protein kinase activities (13–18). Sphingolipids are predominantly located in the outer leaflet of plasma membrane, being components of lipoproteins (19) and of atherosclerotic plaques (20, 21). A recent study has shown that sphingolipids are increased in the aorta of the Watanabe Heritable Hyperlipidemic Rabbit (22) and, more recently, Mukhin, Chao, and Kruth (23) reported that certain glycosphingolipids accumulate in the atherosclerotic aorta. Therefore, it is now evident that accumulation of sphingolipids is another feature of atherosclerosis. Although endothelial dysfunction (i.e., reduced NO release) is considered to trigger vasospasm in atherosclerotic coronary arteries (7, 24, 25), little is known about the vasomotor effects of sphingomyelin metabolites (26).

Therefore, the main objective of the present study was to examine the effects of sphingomyelin and sphingos-

Abbreviations: K-H, Krebs-Henseleit; LDL, low density lipoprotein; L-NMMA, N^ω-monomethyl-L-arginine; NO, nitric oxide; ox-LDL, oxidized LDL; PGF_{2α}, prostaglandin F_{2α}; PGH₂, prostaglandin H₂; PKC, protein kinase C; SOD, superoxide dismutase; SPMase, sphingomyelinase; TXA₂, thromboxane A₂; VSMC, vascular smooth muscle cell.

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ine, major metabolites of the sphingomyelin pathway and its key initiating enzyme SPMase, on vasomotor regulation in isolated normal pig coronary arteries.

MATERIALS AND METHODS

Reagents

Bradykinin, thrombin, sodium nitroprusside, sphingomyelin, sphingomyelinase, sphingosine, staurosporine, calcium ionophore A23187, SOD, and indomethacin were all purchased from Sigma Chemical Co. (St. Louis, MO). *N*^ω-monomethyl-L-arginine (L-NMMA) was obtained from Calbiochem (La Jolla, CA). Prostaglandin F_{2α} was from Ono Pharmaceuticals, Osaka, Japan, and nitroglycerin was from Nihonkayaku, Tokyo, Japan. Sphingomyelin, sphingosine, and ceramide in PBS were sonicated for 30 sec before use. All concentrations expressed are the final concentrations reached in the organ chambers. All other reagents used for making physiological salt solutions were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of porcine coronary arteries

The left anterior descending and circumflex coronary arteries were isolated from domestic Yorkshire pigs within 5 min after death at a local slaughterhouse. The arteries were cleaned of fat and connective tissues, and cut into small rings 2 mm in length. Some rings were denuded of the endothelial layer by inserting a forceps into the lumen and rolling the ring on moistened filter paper. Each ring was vertically suspended between two stainless steel hooks in an organ chamber with 3 ml of Krebs-Henseleit solution (K-H solution in mM; NaCl, 118; KCl, 4.7; NaH₂PO₄, 1.2; MgSO₄, 1.2; CaCl₂, 2.0; NaHCO₃, 25; and glucose, 10.0). The solution was bubbled with mixed gas (25% O₂ + 5% CO₂ + 70% N₂) at 37°C and pH 7.4. The upper hook was connected to a force transducer UL-20GR (Minebea, Tokyo Japan) and isometric tension changes were recorded (Graphtec, Tokyo, Japan). The rings were progressively stretched over 90 min to optimum resting tone previously determined to be 3–4 g, during which K-H solution was replaced every 20 min.

All rings were first contracted with 60 mM KCl to validate vascular smooth muscle cell (VSMC) function, and rings with less than 2 g of contraction were excluded from study protocol. The rings were then precontracted with prostaglandin F_{2α} (PGF_{2α}; 5–30 μM) to give a preload of 3–4 g, and an endothelium-dependent vasodilator bradykinin (10 nM) was added to validate endothelial function and also to pharmacologically confirm the endothelial denudation (4). After wash out, the rings were reequilibrated for 15 min in the K-H solution.

Effects of sphingomyelin, SPMase and sphingosine on coronary arterial vasomotor tone

Effects of sphingomyelin, (0.3–10 μM), SPMase (40 μU/ml–0.4 U/ml) and sphingosine (0.3–10 μM) on the isometric tension changes were first examined in non-stimulated quiescent rings (rings under the resting tension). Other rings were precontracted with PGF_{2α} as mentioned above, and effects of these sphingolipid-related compounds on isometric tension change were examined during stable plateau contraction. Contraction or relaxation induced by either sphingomyelin, SPMase, or sphingosine is expressed as percent increase or decrease from the PGF_{2α}-induced contraction at the initial addition of each compound. To further clarify mechanism of sphingosine-induced vasomotion, we also examined effects of either endothelial denudation, a cyclooxygenase inhibitor indomethacin (10 μM), or *N*^ω-monomethyl-L-arginine (L-NMMA; 300 μM) an inhibitor of NO synthase on vasomotor responses induced by sphingosine (10 mM). In addition, since another lysolipid, lysophosphatidylcholine, induced endothelial dysfunction by a PKC-involving mechanism (12), we also examined effects of the PKC inhibitor staurosporine (100 nM) on sphingosine-mediated vasomotor regulation. We also examined effects of superoxide dismutase (SOD; 100 U/ml; Sigma) on sphingosine-induced vasomotor regulation. Indomethacin, L-NMMA, or staurosporine was added to the organ chamber at 30 min prior to the addition of PGF_{2α}. SOD was added to the organ chamber at 10 min prior to the addition of PGF_{2α}. These inhibitors were present in the K-H solution during the experimental protocol.

Effects of sphingomyelin, SPMase and sphingosine on endothelium-dependent vasorelaxation

In another set of experiments, we examined effects of sphingomyelin (10 μM), SPMase (0.4 U/ml), or sphingosine (10 μM) on endothelium-dependent and -independent vasorelaxation responses. Sphingomyelin, SPMase, or sphingosine was added and incubated with coronary arterial rings for 120 min. After this incubation, the rings were washed three times with K-H solution and precontracted as mentioned above. After a stable plateau contraction was obtained, endothelium-dependent relaxation in response to cumulative concentrations of thrombin (0.001–0.3 U/ml) was examined. After the response stabilized, the rings were washed and re-equilibrated for 15 min. Above procedures were repeated twice to test effects of A23187, receptor-independent endothelium-dependent vasodilator (1 nM to 3 μM), and an endothelium-independent vasodilator sodium nitroprusside (0.01–30 μM). Time control rings were incubated in K-H solution for the same incubation period (120 min), and then vascular responses to throm-

bin, A-23187, and sodium nitroprusside were examined as described above.

Data analysis

All results are expressed as means \pm standard error (SE), and *n* refers to the number of the coronary artery rings studied. Statistical evaluation of the difference of two means was performed by unpaired Student's *t* test. When more than two means were compared, one-way analysis of variance (ANOVA) followed by the Bonferroni's *t* test was used. Differences between values were considered to be statistically significant at $P < 0.05$. The entire protocol for these experiments has been accepted by the Kumamoto University School of Medicine Animal Care Committee.

RESULTS

Effects of sphingomyelin, SPMase, and sphingosine on isolated porcine coronary arterial tone

Sphingomyelin (0.3–10 μM), SPMase (40 $\mu\text{U}/\text{ml}$ –0.4 U/ml), or sphingosine (0.3–10 μM) added to organ chambers containing resting coronary artery rings did not elicit any significant changes in isometric force ($< \pm 0.1$ gram from the baseline resting tension). However, SPMase (0.04 U/ml), when added to the coronary rings precontracted with $\text{PGF}_2\alpha$, induced biphasic response, an initial contraction and a subsequent gradual relaxation (Fig. 1a). Sphingosine (10 μM) added to a precontracted coronary ring also elicited initial contraction and subsequent relaxation during stable contraction evoked by $\text{PGF}_2\alpha$ (Fig. 1c). In endothelium-denuded rings, both initial contraction and subsequent relaxation induced

by either SPMase or sphingosine were abolished (Figs. 1b and 1d). In contrast, sphingomyelin (0.3–10 μM) did not elicit any changes in isometric force during precontraction evoked by $\text{PGF}_2\alpha$ (data not shown). Figure 2 summarizes concentration–response relationship of both initial contraction and subsequent relaxation exerted by either SPMase or sphingosine. In endothelium-intact rings, both initial contraction and subsequent relaxation were induced by SPMase or sphingosine in a concentration-dependent manner. In contrast, these responses were abolished in endothelium-denuded rings. Thus, sphingosine and SPMase but not sphingomyelin affected arterial vasomotor regulation in the precontracted pig coronary arteries.

As ceramide is only an intermediate metabolite between sphingomyelin and sphingosine by action of SPMase, we also examined effects of ceramide on vasomotor regulation. However, exogenous addition of ceramide (10 μM) had no effect on vasomotion in either quiescent or precontracted pig coronary arteries ($2.8 \pm 2.1\%$ of precontraction, $n = 4$; NS).

In order to further examine whether enzymatic action of SPMase is essential for the action of SPMase on vasomotor regulation, we examined effects of heat-inactivated SPMase on pig coronary vasomotion. Heat-inactivated SPMase (60°C for 30 min) had no effect on vasomotion in either quiescent or precontracted pig coronary arteries ($0.8 \pm 0.8\%$ of precontraction, $n = 3$; NS).

Effects of inhibitors on sphingosine-induced initial vasoconstriction

We further examined mechanism(s) of the sphingosine-induced vasomotor changes in the porcine coronary

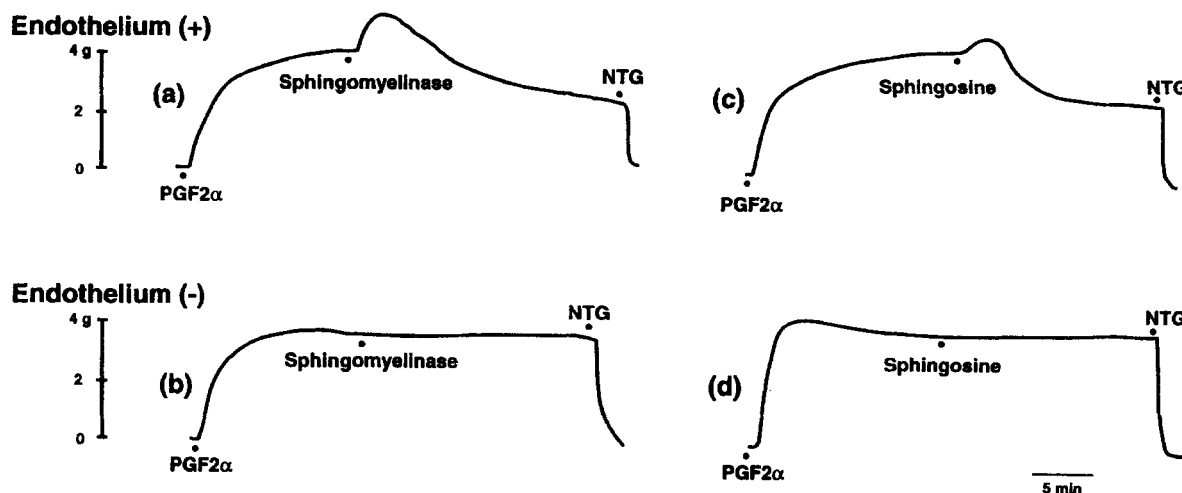


Fig. 1. Representative tracings showing the effects of either sphingomyelinase (SPMase) (0.04 U/ml) or sphingosine (10 μM) on isometric tension changes during stable contraction evoked by prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$; 5–30 μM). In a ring with endothelium, SPMase (a) and sphingosine (c) induced initial contraction and subsequent relaxation. Whereas, in a ring without endothelium, both contraction and relaxation to SPMase (b) and sphingosine (d) were virtually abolished. NTG, nitroglycerin (10 μM).

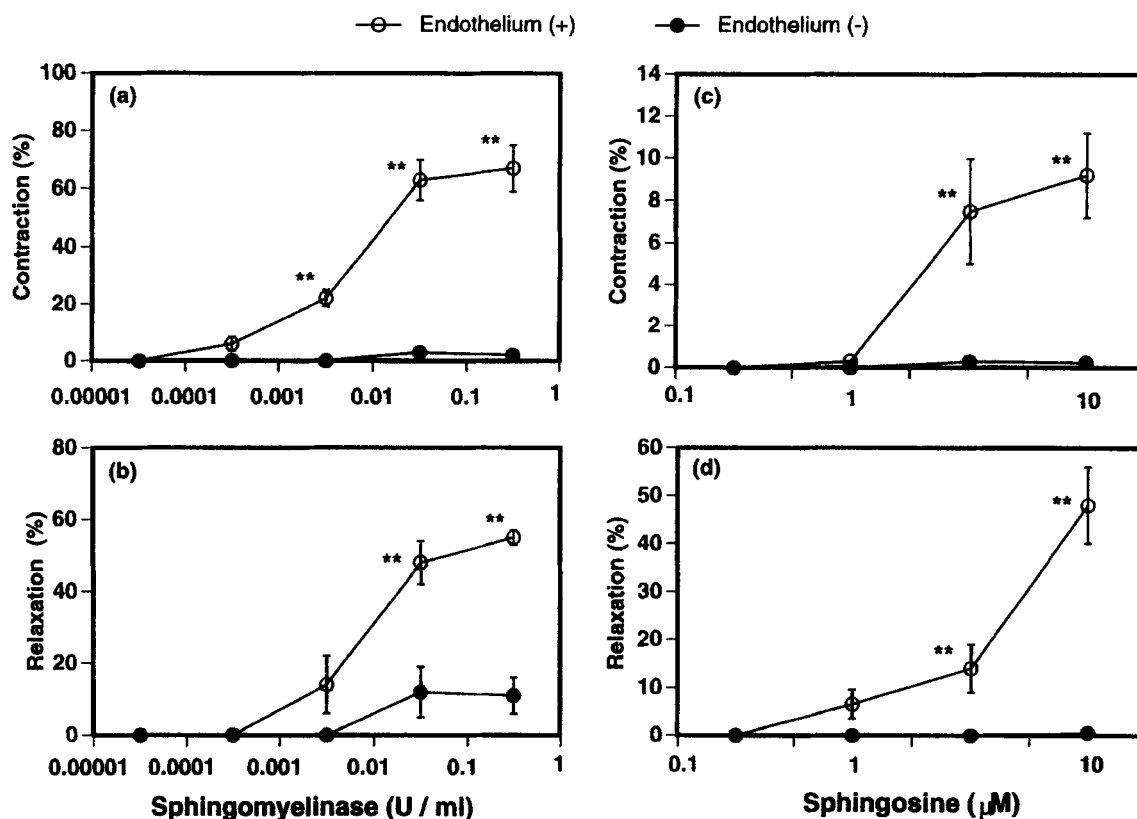


Fig. 2. Left panels: concentration–response curves of sphingomyelinase (SPMase)-mediated initial contraction (a) and subsequent relaxation (b) in rings with ($n = 6$) or without ($n = 7$) endothelium. Right panels: concentration–response curves of sphingosine-mediated initial contraction (c) and subsequent relaxation (d) in rings with ($n = 12$) or without ($n = 7$) endothelium; $**P < 0.01$ vs. precontraction induced by $\text{PGF}_{2\alpha}$. Data are means \pm SE.

arteries using various inhibitors. Sphingosine ($10 \mu\text{M}$) induced $10.2 \pm 3\%$ of initial contraction in control rings with intact endothelium (Fig. 3a). In endothelium-denuded rings, the vasoconstriction by sphingosine was virtually attenuated to $0.3 \pm 0.1\%$ ($P < 0.001$ vs. control rings). As normal endothelial cells release vasoactive substances such as NO and prostaglandins, we also examined the effects of the NO synthase inhibitor, L-NMMA ($300 \mu\text{M}$) and the cyclooxygenase inhibitor indomethacin ($10 \mu\text{M}$) on the sphingosine-induced vasomotion. In the presence of L-NMMA, the vasoconstriction in response to sphingosine was moderately but not significantly suppressed to $6.2 \pm 2\%$. However, indomethacin ($10 \mu\text{M}$) more markedly attenuated the sphingosine-induced contraction to $0.2 \pm 0.1\%$ ($P < 0.001$ vs. control) (Fig. 3a).

As lysophosphatidylcholine, another lysophospholipid contained in atherogenic lipoproteins, affects PKC activity of endothelial cells and can regulate vascular tone, we further examined whether the PKC inhibitor affected sphingosine-induced vasomotor regulation. Incubation of rings with a potent PKC inhibitor staurosporine (100 nM) failed to inhibit the sphingosine-induced initial contraction (Fig. 3a).

We also tested effects of superoxide radical scavenger SOD on sphingosine-induced vasoconstriction. SOD (100 U/ml) did not significantly affect sphingosine-induced initial contraction (Fig. 3a).

These results indicate that sphingosine-induced initial contraction is mainly mediated by cyclooxygenase-sensitive vasoconstrictive prostanoid(s) released from endothelium. Magnitudes of precontraction induced by $\text{PGF}_{2\alpha}$ were not significantly different among control, endothelium-denuded, and each inhibitor-treated group. L-NMMA ($300 \mu\text{M}$) added during the resting state induced significant vasoconstriction ($1.5 \pm 0.5 \text{ g}$, $n = 6$) from the baseline. The value of precontraction in the presence of L-NMMA was total amount of contraction included L-NMMA-induced basal tone increase.

Effects of inhibitors on sphingosine-induced second-phase vasorelaxation

As sphingosine induced the second-phase slowly developing relaxation (i.e., $49 \pm 8\%$ of precontraction), we also evaluated the effects of endothelial denudation and of other inhibitors on this second-phase relaxation (Fig. 3b). The relaxation was also attenuated in endothelium-denuded rings. Prior inhibition of nitric oxide by L-

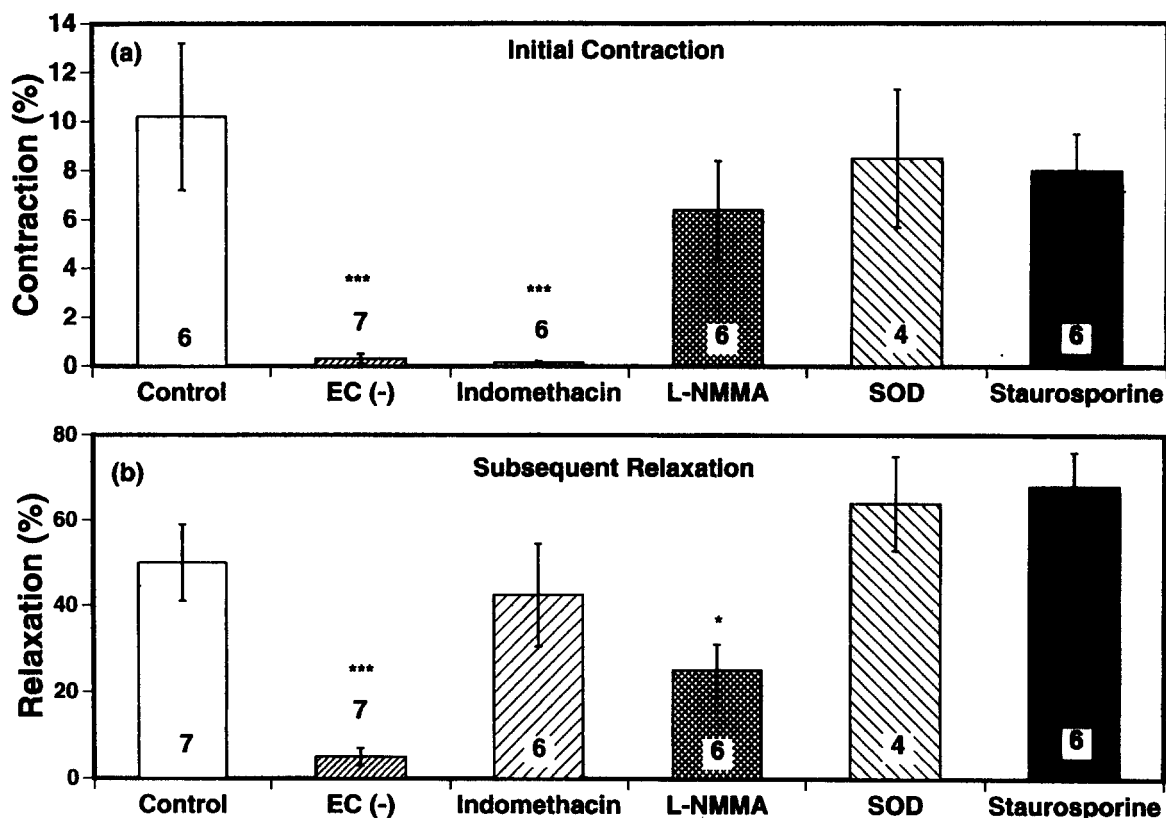


Fig. 3. Sphingosine-induced initial contraction (panel a) and second phase relaxation (panel b). Data are expressed as percent increase or decrease from precontraction induced by $\text{PGF}_{2\alpha}$. Values are means \pm SE; * $P < 0.01$, *** $P < 0.001$ vs. control rings.

NMMA (300 μM) treatment significantly attenuated sphingosine-mediated late relaxation by about 50%; however, indomethacin failed to inhibit the response. Staurosporine did not significantly affect the second-phase relaxation, but, had a tendency to augment the relaxation (Fig. 3b). Oxygen free radical scavenger SOD slightly augmented sphingosine-induced late relaxation (Fig. 3b). These results suggest that the second-phase endothelium-dependent relaxation to sphingosine may at least partially depend on L-NMMA-sensitive NO release from endothelium, and may not depend on cyclooxygenase-sensitive prostaglandins such as prostacyclin. Superoxide radical scavenger SOD significantly enhanced this NO-mediated relaxation in response to sphingosine.

Effects of 120-min incubation with sphingomyelin, SPMase, or sphingosine on endothelium-dependent and -independent relaxation

We further evaluated the effects of sphingosine on coronary endothelial nitric oxide release. After 120 min incubation of coronary rings with either SPMase (0.4

U/ml) or sphingosine (10 μM), endothelium-dependent relaxation in response to thrombin (0.001 to 0.3 U/ml) during precontraction in response to $\text{PGF}_{2\alpha}$ was significantly attenuated compared to control arteries (Fig. 4). Endothelium-dependent but receptor-independent relaxation to calcium ionophore A23187 (1 nM to 3 μM) was also significantly attenuated by incubation with either SPMase or sphingosine. In contrast, sphingomyelin (10 μM) did not influence the endothelium-dependent relaxation to these agonists (Fig. 4). Direct vascular smooth muscle relaxation in response to sodium nitroprusside (0.01–30 μM) was not altered after incubation with either sphingomyelin, SPMase, or sphingosine, suggesting no adverse effect of these three compounds on endothelium-independent relaxation of smooth muscle (Fig. 4). $\text{PGF}_{2\alpha}$ -induced precontraction was not significantly different comparing before and after incubation with these three compounds. The precontractions were not significantly different among the three compound groups after incubation either as we modified the concentration of $\text{PGF}_{2\alpha}$ to obtain a similar level of precontraction in these experiments.

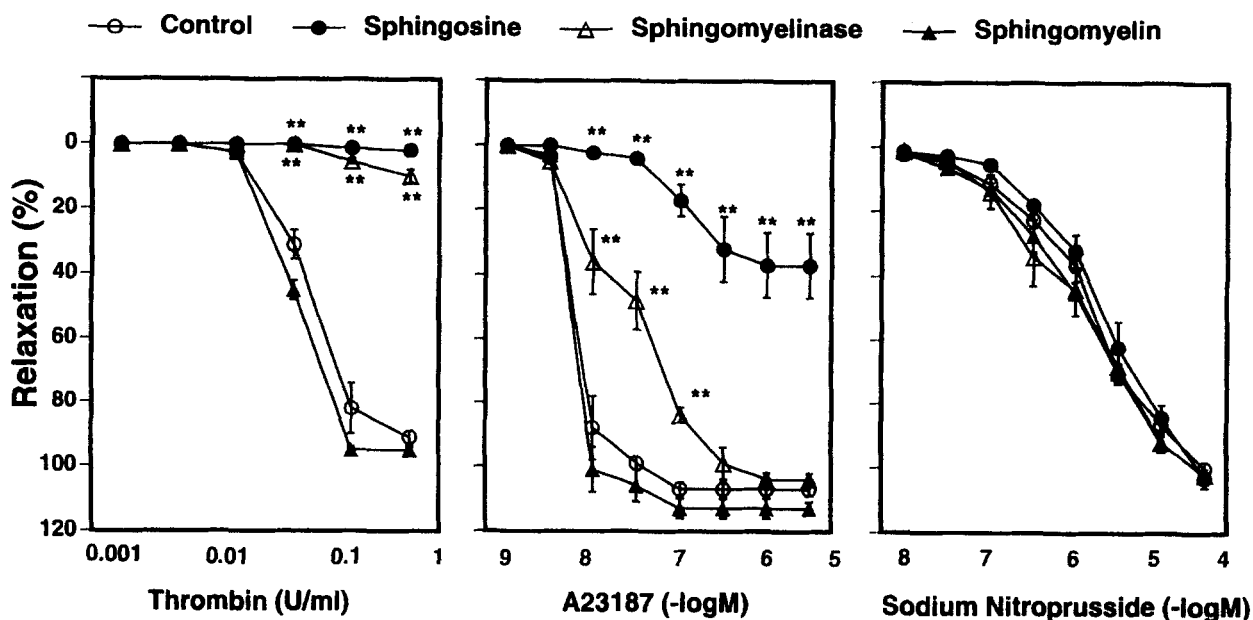


Fig. 4. Effects of 120-min incubation of coronary rings with either sphingomyelin (10 μ M, n = 8), sphingomyelinase (SPMase; 0.4 U/ml, n = 8) or sphingosine (10 μ M, n = 8) on agonist-induced endothelium-dependent (by thrombin and A23187) and -independent relaxation (by sodium nitroprusside). Control rings are rings incubated in control buffer (Krebs-Henseleit solution) for 120 min (n = 6). Relaxation data are expressed as percent decrease from precontraction. Values are means \pm SE; n = 6–8; ***P* < 0.01 vs. control rings.

DISCUSSION

The present study demonstrates the effects of sphingomyelin and sphingosine, backbone structures of naturally occurring sphingolipids, and SPMase, an enzyme that catalyzes sphingosine and initiates sphingomyelin pathway, on vascular tone in vitro. Both SPMase and sphingosine, but not sphingomyelin, elicited endothelium-dependent vascular responses, characterized by an initial contraction and subsequent gradual relaxation, in precontracted porcine coronary artery rings.

The initial contraction to sphingosine was endothelium-dependent and was significantly attenuated by a cyclooxygenase inhibitor indomethacin. These results suggest possibilities that *a*) sphingosine might stimulate endothelial cells to release cyclooxygenase-dependent vasoconstrictor prostanoids such as prostaglandin H_2 and thromboxane A_2 (PGH_2 - TXA_2) (27), or *b*) sphingosine might inhibit release of prostacyclin, leading to contraction. However, the latter possibility is less likely as indomethacin itself did not induce contraction during precontraction in our study. This is further supported by evidence that basal release of prostacyclin may be minimal in the isolated epicardial coronary arteries (28). The former possibility is more likely by evidence that sphingosine enhances cytokine (TNF α and IL-1 β)-induced synthesis of prostaglandin E_2 , another metabolite of arachidonic acid pathway via cyclooxygenase action

(14, 29), and that sphingosine may also directly stimulate cyclooxygenase in intact cells (13, 14).

The initial contraction in response to sphingosine was moderately but not significantly attenuated by inhibition of NO release by L-NMMA. This finding raises the question whether sphingosine would modulate the endothelial NO release. We therefore further examined effects of 120 min incubation with sphingomyelin, SPMase, and sphingosine on endothelium-dependent and -independent relaxation. Interestingly, incubation with sphingosine and SPMase significantly attenuated endothelium-dependent relaxation in response to either thrombin or A23187 without affecting vasorelaxation in response to endothelium-independent vasodilator sodium nitroprusside. In this connection, Higashi, Omori, and Yamagata (30) recently reported that sphingolipids can directly bind to calmodulin, which may inhibit the activity of calmodulin-dependent enzymes such as endothelial NO synthase (31). Thus, it is conceivable that sphingosine may inhibit endothelial NO synthase activity by calmodulin-dependent mechanism. Another possibility is that lysolipids in general can be readily incorporated into the lipid bilayer of the cell membranes, altering membrane fluidity and receptor/G protein coupling, thus leading to impaired agonist-induced release of NO (3, 8, 10, 12). For example, Flavahan (8) demonstrated that lysophosphatidylcholine, another form of lysolipid, can modify G_i protein-dependent signaling in porcine endothelial cells.

Sphingosine also acts as a regulator of membrane signal transduction system. Sphingosine is an endogenous inhibitor of PKC (13, 15–18); recent studies have shown that sphingosine, presumably via phosphorylation to sphingosine-1-phosphate, exerts a rapid Ca^{2+} release from intracellular stores (32, 33). Because increased $[\text{Ca}^{2+}]_i$ could activate PKC (34), it is conceivable that the initial contraction in response to sphingosine might be related to the increased $[\text{Ca}^{2+}]_i$ itself in VSMC or to endothelial PKC activation. Stimulation of endothelial PKC is known to attenuate NO release (35) and to promote vasoconstrictor prostanoid production such as $\text{PGH}_2\text{-TXA}_2$ (27, 36). However, sphingosine-induced initial contraction was endothelium-dependent and also was not inhibited by a PKC inhibitor staurosporine, thus, these possibilities are less likely in the present study.

Sphingosine elicited the second-phase gradual relaxation. This relaxation was endothelium-dependent and was not attenuated by indomethacin but was significantly attenuated by L-NMMA (Fig. 4). These results suggest that sphingosine may stimulate endothelial cells to produce NO from L-arginine and induce relaxation at late phase after exogenous addition. One possible mechanism of this late vasodilatory effect of sphingosine might be mediated through PKC inhibition (13, 16) as inhibition of PKC may stimulate NO synthesis (37). Indeed, the PKC inhibitor staurosporine had a tendency to augment the second-phase relaxation in response to sphingosine in the present study. However, L-NMMA could not totally abolish the sphingosine-induced second phase vasorelaxation as shown in Fig. 3 (about 50%). Thus, there may exist other possible vasodilator(s) released from endothelium in the sphingosine-induced second-phase relaxation. Because endothelial cells may also release a variety of vasorelaxation factors other than NO (e.g., endothelium-dependent hyperpolarizing factor, epoxyeicosatrienoic acids), effects of sphingosine metabolites on these substances remains to be further elucidated.

Sphingosine did not contract quiescent rings, but contracted $\text{PGF}_2\alpha$ -prestimulated rings. Thus, there may exist “contractile synergism” between sphingosine and $\text{PGF}_2\alpha$. In this regard, there has been the idea that sphingolipids interact with receptors to alter the responsiveness to growth factors and potentially other agonists (18). A similar phenomenon was observed between $\text{PGF}_2\alpha$ and ox-LDL or other lysolipids in previous studies (10, 11, 38). Ox-LDL-mediated vasoconstriction was minimal in resting state, but was enhanced after precontraction with agonists (11, 38). The contractile synergism between vasoconstrictors and lysolipids or ox-LDL may explain evidence that agonist-induced vasospasm is frequently observed at the atherosclerotic site (25). Precise

mechanism of the synergistic effect of sphingosine and $\text{PGF}_2\alpha$ is unknown. However, such synergism is often observed between K^+ channel inhibitors and agonists (39). Interestingly, Petrou et al. (40) recently demonstrated that high concentration of sphingosine ($50\ \mu\text{M}$) inhibits K^+ channel activity in gastric smooth muscle cells. Although it is unknown whether $10\ \mu\text{M}$ of sphingosine, the concentration used in the present study, has inhibiting effects on K^+ channel in endothelial cells, direct effects of sphingolipid on the K^+ channel activity should be further elucidated.

In conclusion, sphingosine and SPMase, two major substances involved in the sphingomyelin metabolism pathway, significantly influenced the endothelial regulation of isolated pig coronary arterial tone. Sphingomyelin signaling pathway possibly plays an important regulatory role in the arterial wall, and further studies are needed to elucidate its role in the atherosclerotic arteries. ■

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