EUROPEAN SOCIETY OF CARDIOLOGY®

Cardiovascular Research (2009) **82**, 212–220
doi:10.1093/cvr/cvp064

Review

Sphingosine-1-phosphate and modulation of vascular tone

Junsuke Igarashi[1]* and Thomas Michel[2]*

[1]*Department of Cardiovascular Physiology, Kagawa University, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan; and* [2]*Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Thorn Building, Room 1210A, 75 Francis Street, Boston, MA 02115, USA*

Received 26 August 2008; revised 30 January 2009; accepted 3 February 2009; online publish-ahead-of-print 20 February 2009

Time for primary review: 36 days

KEYWORDS
Vasoactive agents;
Lipid signalling;
Receptors;
G-proteins;
eNOS

Sphingosine-1-phosphate (S1P) is a phosphorylated product of sphingosine, the core structure of the class of lipids termed sphingolipids. S1P is a naturally occurring lipid metabolite, and usually is present at a concentration of a few 100 nanomolar in human sera. S1P has been found to exert a diverse set of physiological and pathophysiological responses in mammalian tissues through the activation of heterotrimeric G-proteins that in turn modulate the activity of various downstream effecter molecules. In blood vessels, vascular endothelial cells and smooth muscle cells express specific receptors for S1P that modulate vascular tone. This article will provide a brief overview of S1P metabolism in the vasculature and will discuss some of the pathways whereby S1P regulates intracellular signal transduction pathways in endothelial and smooth muscle cells, leading to the activation of both vasorelaxation and vasoconstriction responses.

1. Introduction

Many bioactive substances regulate vascular tone either by directly modulating smooth muscle layer or by stimulating endothelial cells (ECs) to release bioactive molecules that diffuse to and regulate vascular smooth muscle cell (VSMC) responses. Sphingosine-1-phosphate (S1P), a member of a large family of lipid metabolites termed sphingolipids, is capable of regulating a wide array of biological processes such as proliferation, migration, survival, differentiation, among others.[1] Many of these cellular responses are initiated by S1P binding to and activating a family of G-protein coupled S1P receptors. Five independent S1P receptor subtypes have been identified in mammals termed $S1P_{1-5}$ (previously known as endothelial differentiation gene receptors[2]). S1P and S1P receptors have critical effects on morphogenesis and embryonic development of the vasculature as well as the heart.[3,4] It should be also noted that at least some of the S1P actions appear to occur by way of its intracellular molecular targets, independently of S1P receptors.[5] A calcium channel termed sphingolipid calcium release-mediating protein of the endoplasmic reticulum (SCaMPER) may represent an intracellular target of S1P actions,[6] although the identity and characteristics of intracellular target molecules of S1P remain less well understood within endothelial and VSMCs. S1P also represents one of the key latest additions to the list of 'vasoactive' substances that modulate vascular tone. Regulation of vascular tone by S1P displays both common and distinct features with other vasoactive compounds, and differential modulation of S1P responses may have physiological and therapeutic implications for many cardiovascular disease states.

2. Biosynthesis and metabolism of sphingosine-1-phosphate

2.1 Metabolic pathways of sphingosine-1-phosphate

Sphingolipids are widely distributed in virtually every class of mammalian cells. For many years, sphingolipids were considered to serve principally as structural components of cell membranes, without discernable roles in signal transduction. We now know that many sphingolipids are also capable of functioning as signal mediators both as receptor ligands and as intracellular second messengers. Sphingosine is an amino alcohol containing an unsaturated hydrocarbon chain and undergoes a broad array of metabolic transformations in mammalian cells. The lipid-containing products of sphingosine are termed sphingolipids. For an extensive review of biosynthesis and metabolism of sphingolipids in general, see Futerman and Hannun.[7] In brief, sphingosine kinases (SphK) catalyse the ATP-dependent phosphorylation of sphingosine to produce S1P. Thus far, two different SphK

* Corresponding authors. Tel: +81 87 891 2099 (J.I.)/+1 617 732 7376 (T.M.); fax: +81 87 891 2101 (J.I.)/+1 617 732 5132 (T.M.).
E-mail addresses: igarashi@med.kagawa-u.ac.jp (J.I.)/thomas_michel@harvard.edu (T.M.)

Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2009.
For permissions please email: journals.permissions@oxfordjournals.org.

Downloaded from https://academic.oup.com/cardiovascres/article/82/2/212/279374 by guest on 16 August 2022

isoforms have been found in mammals named SphK-1 and SphK-2, each having distinct patterns of tissue distribution and enzymatic properties (see review[8]). Degradation of S1P is catalysed both by S1P lyase, an enzyme that cleaves S1P into hexadecanal and phosphoethanolamine, and by S1P phosphatase, which dephosphorylates S1P back into sphingosine.[9] S1P concentrations are typically in the range of several 100 nanomolar in normal adult human blood, and the serum S1P level is usually higher than that in plasma (see review[9] and references therein). It is interesting that S1P in blood is enriched in specific lipoprotein fractions, notably in high-density lipoproteins (HDLs),[10] while some other related lipid metabolites appear to exhibit differential pattern of plasma lipoprotein distribution. It is tempting to speculate that distinct distribution patterns of S1P and other related lysophospholipid mediators within specific plasma lipoprotein fractions are associated with differential responses of cardiovascular cells to a given plasma lipoprotein fraction (see review[10]). The cellular source of blood S1P is a topic of active investigation, and local S1P concentrations in vascular beds may vary considerably (discussed below). In general, S1P concentrations within most cells and organs (except for blood platelets) appear to be lower than those in blood; however, it is possible that intracellular S1P concentrations may be differentially modulated in various subcellular locales.

2.2 Cellular sources of blood sphingosine-1-phosphate that modulates vascular sphingosine-1-phosphate receptors

The cellular origins of plasma S1P that binds to and activates specific S1P receptors in the vascular cells remain incompletely understood. The work by Yatomi *et al.*[11] first postulated that platelets serve as a key source of S1P in blood. This was based on analyses of the specific enzymatic profiles of platelets, which are enriched in the activity of SphK, while being essentially devoid of S1P lyase activity. As a result, platelets are distinctly enriched in S1P. Furthermore, stimulation of platelets with thrombin leads these cells to release large amounts of S1P.[11] Recent studies have broadened our understanding of the S1P-producing[12] and -releasing[13] pathways in platelets.

Mammals express two distinct SphK isoforms, SphK-1 and SphK-2. It had been difficult to ascertain the differential cellular contributions to blood S1P in greater detail, in part because the complete deletion of all four SphK alleles is embryonic lethal in mice.[14] Recent studies, however, have reported major advances in our understanding of the cellular origins of blood S1P. For example, Pappu *et al.*[15] used a conditional gene ablation approach to obtain mice that are deficient in both SphK isoforms but are still able to survive until adulthood. Exploiting an elegant transfusion approach using cells derived from these 'SphK-deficient' mice, they demonstrated that red blood cells rather than platelets play a pivotal role to maintain blood S1P abundance in this model.[15] Another cellular source of blood S1P which activates cardiovascular cells may include ECs themselves. Vascular ECs express significant levels of SphK activity and contain detectable S1P. Several extracellular stimuli, including the cytokine tumour necrosis factor alpha, may activate endothelial SphK, and thereby lead to augmented S1P production.[16] Another feature of endothelial SphK enzyme is that SphK-1 isoform undergoes export to the outer leaflet of plasma membrane and releases S1P into the extracellular space.[17] Using multiple complementary experimental approaches, Venkatamaran *et al.*[18] recently showed that ECs indeed represent a key source of plasma S1P. Thus, SphK/S1P may modulate vascular S1P receptors through paracrine and/or autocrine pathways. VSMCs may also participate in local S1P metabolism that may influence vascular tone. A recent study by Peter *et al.*[19] showed that endogenously expressed S1P phosphatase isozyme SPP1 in Hamster gracilis muscle resistance arteries antagonizes the pro-constrictive effects of S1P-producing enzyme SphK-1. These and other findings suggest that modulation of local S1P metabolism by smooth muscle cells can participate in the regulation of vascular tone.

3. Sphingosine-1-phosphate as a novel activator of endothelial nitric oxide synthase

3.1 Endothelial nitric oxide as a key determinant of vascular tone

Promotion of NO production represents a key feature of vascular S1P system that modulates vascular tone. NO is synthesized in mammalian tissues by three distinct isoforms of NO synthase (NOS) and participates in a wide array of signalling processes in nearly all types of mammalian cells. NO was first identified as endothelium-derived relaxing factor, a naturally occurring signal mediator that is a key determinant of vascular tone. NO produced in the vascular endothelium by the endothelial isoform of NO synthase (eNOS) diffuses to the subjacent VSMCs, where NO activates the soluble isoform of guanylate cyclase, thereby increasing intracellular cyclic guanosine monophosphate (cGMP) and promotes vascular smooth muscle relaxation. The eNOS, encoded by the NOS3 gene, is the principal source of NO within cardiovascular cells. Vascular NO derived from eNOS modulates numerous essential vascular functions, including regulation of blood pressure, inhibition of platelet aggregation, and inhibition of leukocyte adhesion, among others (see review[20]). While we focus on eNOS regulation by S1P receptor systems in this article, readers are invited to another review paper in which we discussed more general aspects of the eNOS/NO system.[21]

3.2 Endothelial sphingosine-1-phosphate receptors that activate endothelial nitric oxide synthase

In vascular ECs, several S1P receptor subtypes serve to activate eNOS and promote NO production. Early studies of S1P regulation of eNOS exploited a heterologous expression system in which COS-7 cells were transiently transfected with plasmids encoding eNOS and $S1P_1$ (EDG-1) receptors.[22] Addition of S1P to these co-transfected cells led to robust NO production. S1P did not activate eNOS in the absence of co-transfected $S1P_1$ receptors, indicating that this action of S1P is mediated by $S1P_1$ receptors rather than by its intracellular actions. S1P was found to stimulate NO production in cultured native bovine aortic ECs (BAEC). S1P induced marked increases in NO production in a dose-dependent manner with an approximate EC_{50} value of 10 nM,[23] which is in good agreement with many other receptor-dependent endothelial responses to this lipid. Like many other lipid mediators, S1P in plasma is principally

Downloaded from https://academic.oup.com/cardiovascres/article/82/2/212/279374 by guest on 16 August 2022

protein-bound. This may explain why the total blood S1P concentration is so much higher than that sufficient to activate eNOS. The principal S1P receptor subtype in these cultured vascular ECs was found to be the $S1P_1$ subtype, as determined by quantitative northern blot assays.[24,25] 'Knock down' of $S1P_1$ receptor expression by means of transient transfection of small interfering RNA (siRNA) showed that the $S1P_1$ receptor subtype is indispensable in eNOS responses to S1P in cultured ECs,[26] indicating that the $S1P_1$ subtype plays a key role in mediating eNOS activation by S1P. It is important to note that the degree of eNOS activation by S1P is comparable to those attained by other classical eNOS agonists, including bradykinin or vascular endothelial growth factor (VEGF), suggesting quantitative importance of eNOS activation by S1P.[23,27] S1P has since been found to activate eNOS in many other cultured EC types, including human umbilical vein endothelial cells[28] (HUVEC) as well as bovine lung microvascular ECs.[25] In blood vessels isolated from rodent mesenteric arterioles and thoracic aorta, S1P activates eNOS via pertussis toxin-sensitive G-protein-coupled receptor (GPCR) pathways.[29] Taken together, these studies have now added the S1P/$S1P_1$ receptor system to the list of GPCR pathways of vascular ECs that lead to quantitatively important NO production by means of eNOS activation.

Although $S1P_1$ receptors appear to play a major role in mediating S1P activation of eNOS, other S1P receptor subtype(s) in vascular ECs may also play significant roles in distinct regulatory processes influencing vascular tone. For example, genetic as well as pharmacological experiments demonstrated that $S1P_3$ subtype activates eNOS in mouse arteries in response to S1P present in HDL.[30,31] Therefore, other S1P receptor subtype(s) than $S1P_1$ may also participate in the regulation of vascular tone in a manner that may depend on the receptor subtype expression profile in a given experimental model of blood vessels.

3.3 $S1P_1$ receptor expression levels as a determinant of sphingosine-1-phosphate activation of endothelial nitric oxide synthase

Another important feature of S1P/S1P receptors as an eNOS-activating system is that the expression levels of $S1P_1$ receptor subtype are subjected to dynamic regulation by cell stimulation, consequently determining the degrees of NO production in response to a given amount of S1P. In BAEC, VEGF up-regulates expression of $S1P_1$ receptors at both levels of mRNA and protein, in a manner sensitive to pharmacological inhibition of protein kinase C (PKC) pathways.[32] Increases in $S1P_1$ expression levels are associated with enhanced eNOS phosphorylation/activation of cultured ECs as well as those of isolated blood vessels in response to subsequent stimulation with S1P, suggesting that at least some of the newly synthesized $S1P_1$ receptor molecules are functional. Thus, VEGF may dynamically regulate the expression levels of $S1P_1$ receptors and the magnitudes of eNOS responses to S1P by way of PKC pathways. Indeed, the acute up-regulation of $S1P_1$ transcripts following the treatment of cultured ECs with phorbol esters was found at first during the initial discovery process of these receptors (which were originally termed EDG-1 receptors) by Hla and Maciag. By treating HUVEC with phorbol-12-myristate-13-acetate (PMA), these investigators found an immediate early gene whose expression levels are drastically up-regulated following PMA.[33] Since treatment with PMA induces ECs to differentiate to 'angiogenic' phenotypes, this gene was named endothelial differentiation gene-1 (EDG-1), now renamed as $S1P_1$ after the discovery of its ligand. Thus, PKC activation may play a pivotal role in modulating $S1P_1$ transcript/protein expression to determine the amounts of S1P-dependent NO production via eNOS. Endothelial stimulation with VEGF also leads to activation of SphK in HUVEC,[34] making it possible that endothelial S1P release evoked by VEGF may activate eNOS via up-regulated $S1P_1$ receptors in an autocrine/paracrine fashion to promote further NO production. Some groups have reported that treatment of cultured ECs with S1P leads to transactivation of the VEGF receptor kinase insert domain receptor (KDR) (also termed VEGR-2).[35,36] However, it is not clear whether this mechanism is so quantitatively important for endothelial S1P signalling, because siRNA-mediated down-regulation of KDR abundance in cultured ECs fails to attenuate eNOS responses to S1P.[37]

In addition to VEGF, many other extracellular stimuli are also capable of modulating expression of $S1P_1$ receptors in ECs and thereby affecting the eNOS responses to S1P. Blood vessels are continuously exposed to oxidative stress, which may be defined as excess production of reactive oxygen species (ROS), which in turn may be affected by cellular metabolism, by local inflammation, or even by turbulent blood flow.[38] Hydrogen peroxide (H_2O_2), an abundant ROS, was found to augment $S1P_1$ receptor expression and S1P-dependent eNOS activation.[39] Clinically relevant pharmacological agents may also regulate $S1P_1$ receptor expression. One example is the class of drugs termed 'statins', which are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a rate-limiting enzyme of cholesterol synthesis in liver, and widely used for cholesterol lowering therapy in patients. Statins exert pleiotropic actions outside the liver as well, notably in vascular endothelium (see review[40]). Some of these statins are able to augment expression of $S1P_1$ receptor mRNA and protein, associated with higher levels of NO production not only in response to S1P but also to HDL *in vitro*.[26] Thus, a wide spectrum of endothelial stimuli may modulate the abundance of $S1P_1$ receptors and the magnitude of S1P-elicited eNOS activation. These stimuli include polypeptide growth factors (e.g. VEGF), ROS such as H_2O_2, and therapeutic agents including statins. The therapeutic and pathophysiological implications of these findings remain to be explored in greater detail.

The promoter region of $S1P_1$ gene has not yet been extensively characterized in ECs, and mechanistic insights into the factors modulating $S1P_1$ transcriptional regulation remain to be elucidated in detail. The degrees of $S1P_1$ receptor expression in T-lymphocytes appear to represent a key determinant of egress of these cells from thymus into peripheral blood,[41] and the transcription factor Kruppel-like factor 2 has been observed to activate lymphocyte $S1P_1$ expression.[42]

3.4 Endothelial signalling machinery that connects sphingosine-1-phosphate receptors to endothelial nitric oxide synthase activation

In vascular ECs, signalling pathways that connect S1P receptor activation to eNOS activation have been extensively characterized. Compartmentalization of signalling proteins is an important cellular tool to afford efficacy and specificity of

Downloaded from https://academic.oup.com/cardiovascres/article/82/2/212/279374 by guest on 16 August 2022

inter-molecular communication processes. eNOS is targeted to the invaginated domains of the cell membrane called plasmalemmal caveolae,[43] and this distinctive subcellular localization plays a vital role in eNOS regulation. Targeting of eNOS to caveolae is determined by a unique feature of this NOS isoform, which is dually acylated at its N terminus by the saturated fatty acids myristate and palmitate (see review[44]). eNOS myristoylation occurs co-translationally at its glycine 2 residue[45] and is irreversible, whereas eNOS palmitoylation occurs post-translationally at cysteine residues 15 and 26 and is reversible.[46] A large body of biochemical as well as cell biological evidence has accumulated to establish that a major fraction of eNOS protein is specifically enriched in caveolae microdomains.[47] Compared with the surrounding plasma membrane, caveolae are relatively enriched in cholesterol and sphingolipids, which together decrease the fluidity of these discrete membrane regions. The distinct 'ordered fluid' phase characteristic of caveolae seems to influence targeting of proteins involved in a variety of signalling pathways, and thus may function to facilitate a broad range of protein–protein and protein–lipid interactions necessary for cellular signal transduction. Importantly, $S1P_1$ receptors are targeted to caveolae, thereby establishing the physical proximity of receptor/effecter proteins required for S1P-induced eNOS activation.[22]

eNOS was found to interact with caveolin, which is the transmembrane scaffolding protein that characterizes caveolae. There are three isoforms of caveolin, of which the ubiquitous caveolin-1 isoform and the muscle-restricted caveolin-3 isoform interact with and regulate eNOS within vascular ECs and within cardiac myocytes, respectively.[48] The direct interaction of caveolin with eNOS leads to inhibition of eNOS enzyme activity; this inhibitory interaction of caveolin with eNOS takes place through the caveolin mid-molecule 'scaffolding domain'.[49] Importantly, when ECs are stimulated to elevate $[Ca^{2+}]_i$, calmodulin replaces caveolin to increase NO production. With more prolonged agonist stimulation, eNOS translocates from caveolae to intracellular membranes, but this reversible targeting probably is not significantly influenced by eNOS–caveolin interactions, but rather is a consequence of reversible acylation of eNOS. eNOS palmitoylation is dynamically regulated, and this reversible post-translational modification appears to play a key role in the receptor-modulated subcellular translocation of eNOS in native ECs, as seen in bradykinin B2 receptor-elicited eNOS regulation.[50] Because eNOS mutants that are deficient in palmitoylation or myristoylation exhibit aberrant phosphorylation patterns in response to S1P in cultured BAEC,[51] it is plausible that caveolae targeting of eNOS afforded by enzyme's acylation plays pivotal roles in S1P receptor-elicited activation processes as well. Thus, the reversible fatty acid modification of the enzyme seems to provide an additional level of control in eNOS subcellular localization. Together, these regulatory processes involving plasmalemmal caveolae, termed the eNOS-caveolin regulatory cycle,[52] play key roles to determine eNOS activity, which is reciprocally controlled by caveolin vs. calcium/calmodulin. S1P elevates $[Ca^{2+}]_i$ concomitant with eNOS activation in cultured ECs,[53,54] and conversely, chelation of intracellular calcium abolishes S1P-mediated NO production.[23,27] Overexpression of caveolin in heterologous cells co-expressing eNOS and $S1P_1$ receptors attenuates the degree of eNOS activation induced by S1P.[22] Thus, eNOS regulation by S1P is fundamentally dependent on intracellular calcium, and appears likely to be reciprocally modulated by caveolin/calmodulin in a fashion similar to that of the other calcium-mobilizing eNOS agonists.

eNOS regulation by S1P involves another set of signalling pathways that modulate enzyme's phosphorylation at the serine 1177 residue; this phosphorylation of eNOS at its C terminus 'sensitizes' the enzyme to activation at lower levels of calcium *in vitro*.[55] Full activation of eNOS by S1P requires a pathway comprising G-protein-coupled S1P receptors; pertussis toxin-sensitive G-proteins, especially G-protein β-γ subunits that modulate the β-isoform of phosphoinositide 3-kinase (PI3-K); and protein kinase Akt, which phosphorylates eNOS at the serine 1177 residue.[23,27] Subsequent studies, using siRNA-mediated knock down of selected signalling proteins in ECs, established that the AMP-activated protein kinase and the small G-protein Rac1 represent a key upstream regulatory pathway that couples S1P receptor activation and stimulation of PI3-Kβ/Akt.[37,56] It is important to mention that both AMP kinase and Rac1 are negatively regulated by caveolin.[37] Collectively, these observations support a model in which at least two inter-related pathways of eNOS regulatory processes are involved in S1P-dependent activation of eNOS: the eNOS activation pathway modulated by calcium/calmodulin vs. caveolin is importantly influenced by phosphorylation pathways, which in turn involve multiple phosphorylation/dephosphorylation events that alter phosphorylation of eNOS at multiple sites on the enzyme. Thus, there are multiple regulatory loci that could provide opportunities for molecular cross-talk in the pathways for S1P-dependent eNOS activation (much remains to be learnt). These proximal signals evoked by S1P receptor activation exhibit significant similarities and differences in comparison with other eNOS activators,[57] identifying potentially important points of control in receptor-regulated eNOS activation pathways. eNOS phosphorylation pathways also involve other protein kinases and phosphatases at additional serine/threonine and tyrosine residues.[21,58]

4. Sphingosine-1-phosphate as a constricting agent of vascular smooth muscle cells

4.1 Sphingosine-1-phosphate receptors in vascular smooth muscle cells

As also observed in ECs, VSMCs express several S1P receptor subtypes, some of which mediate constriction responses of these cells to S1P. Expression of the S1P receptor subtypes $S1P_1$, $S1P_2$, as well as $S1P_3$ has been detected in immunoblots of smooth muscle cell membrane preparations derived from rat cerebral artery as well as thoracic aorta.[59] Salomone *et al*. recently showed that S1P loses its vasoconstricting activity in knockout animals lacking $S1P_3$ receptors and, conversely, compound VPC23019, an antagonist of $S1P_1$ and $S1P_3$ receptors, blunts S1P-elicited constriction. S1P itself induces robust constriction responses in basilar arteries isolated from either wild-type or homozygous knockout mice of $S1P_2$. These authors, therefore, conclude that $S1P_3$ subtype plays a major role to mediate constriction responses under these conditions.[60] However, Lorenz *et al*.[61] demonstrated that $S1P_2$ receptor knockout mice exhibit markedly decreased mesenteric as well as renal vascular resistance, suggesting that this receptor

Downloaded from https://academic.oup.com/cardiovascres/article/82/2/212/279374 by guest on 16 August 2022

subtype plays a key role in maintaining basal vascular tone in these organs. In addition, in isolated Hamster gracilis muscle resistance arteries, either genetic inactivation of $S1P_2$ subtype by transient transfection using antisense oligonucleotide or the use of a selective $S1P_2$ antagonist JTE-013 blunts the enhanced myogenic constriction responses elicited by S1P.[19] Thus, S1P may differentially evoke vasoconstriction or vasorelaxation responses depending on the expression of distinct receptor subtypes ($S1P_2$ and/or $S1P_3$) in different organs and in different animal species.

The abundance of $S1P_1$ receptors is dynamically regulated in smooth muscle cells[62] as well as in ECs (discussed above). Cultured VSMCs derived from the blood vessels of rat pups appear to express much higher levels of transcripts encoding $S1P_1$ than do smooth muscle cells from adult rat aorta. Conversely, overexpression of $S1P_1$ receptors in adult aortic smooth muscle cells leads to higher degrees of cell migration as well as proliferation responses of these cells to S1P,[62] suggesting that perhaps the $S1P_1$ receptor subtype participates in developmental regulation of VSMCs and blood vessel formation. Interestingly, a recent study showed that $S1P_1$/$S1P_3$ receptors promote proliferative responses of smooth muscle cells in balloon injury models *in vivo* and in cultured cells *in vitro*, whereas $S1P_2$ receptors antagonize these responses.[63] However, the roles of $S1P_1$ in constriction responses of VSMCs remain less completely characterized. In addition, the activation of S1P receptors induces constriction responses in smooth muscle cell of non-vascular origin. For example, in rabbit gastric smooth muscle cells, S1P induces biphasic constriction responses via $S1P_1$ and $S1P_2$ receptor subtypes.[64] In human airway smooth muscle cells, S1P elicits constriction responses mediated by $S1P_2$ receptors.[65]

4.2 Vasoconstriction pathways elicited by sphingosine-1-phosphate in smooth muscle cells

Signalling pathways that mediate smooth muscle constriction have been extensively studied, and the activation of vascular smooth muscle S1P receptors modulates at least some of these molecules. The major trigger for smooth muscle contraction is a rise in $[Ca^{2+}]_i$. Increased $[Ca^{2+}]_i$ promotes binding of calcium to calmodulin and this complex activates a protein kinase that phosphorylates myosin light chain (MLC) termed MLC kinase (MLCK).[66] Classical agonists of smooth muscle G-protein-coupled receptors such as serotonin and histamine activate the phosphoinositide cascade, which in turn stimulates calcium release from the sarcoplasmic reticulum.[67] These pathways also promote calcium influx from extracellular sources by the activation of various calcium channels.[68] S1P was found to induce calcium release from intracellular stores in rat cerebral arteries.[59] S1P also induces pertussis toxin-sensitive transient calcium mobilization in cultured smooth muscle cells derived from rat aorta.[69] Thus, S1P appears to modulate calcium-mobilizing pathways in smooth muscle cells in a manner similar to other classical G-protein-coupled receptor agonists.

Phosphorylation of the MLC by MLCK in VSMCs enhances the interaction of myosin II with actin, thereby results in enhanced cross-bridge cycling, ultimately leading to smooth muscle contraction.[66] MLC phosphatase (MLCP) is an enzyme that dephosphorylates MLC. Agonist stimulation of G-protein-coupled receptors leads to activation of the small G-protein RhoA, which in turn regulates a protein kinase termed Rho-associated kinase (ROK) as well as specific isozymes of PKC. ROK and PKC attenuate the activity of MLCP either independently or synergistically, and thereby promote smooth muscle cell contraction.[66] Vasoconstriction responses evoked by S1P receptor activation in smooth muscle cells have been attributed to RhoA/ROK pathways based on pharmacological experiments. For example, in human coronary artery smooth muscle cells, S1P-elicited constriction responses are blunted by C3 botulinum toxin, which inhibits RhoA, and are also inhibited by compound Y-27632, which inhibits ROK.[70] In cultured smooth muscle cells derived from rat cerebral arteries, S1P activates RhoA.[59] The ROK inhibitor Y-27632 also counteracts S1P-elicited constriction responses in canine basilar arteries[71] as well as in hamster gracilis muscle small resistance arteries.[72] The roles of RhoA/ROK pathway have been documented in several smooth muscle models, but roles of PKC in S1P-dependent contraction remain less completely characterized. When smooth muscle cells are exposed to eNOS-derived NO, cGMP-dependent protein kinase (PKG) gets activated. PKG promotes activation of MLCP, thereby leading to relaxation of smooth muscle cells.[66] Although smooth muscle cells express caveolae microdomains and caveolin (see review[73]), the roles of caveolae and caveolins in S1P signalling remain less well characterized in VSMCs. Of note, in hamster gracilis muscle small resistance arteries, S1P induces acute translocation of the MLCP from cytosol to membrane fractions.[72]

5. Sphingosine-1-phosphate overall vessel tone reactions

S1P is capable of activating both vasorelaxation responses mediated by eNOS/NO in ECs and vasoconstriction responses mediated by RhoA/ROK pathways in smooth muscle cells. What then are the overall consequences of S1P receptor activation for vascular tone? The answer appears to lie in the fact that S1P modulation of vascular tone takes place in a highly context-dependent manner, depending on the specific vascular bed being studied, by specific experimental conditions, including important differences between animal species. In any experimental system, the net effect of S1P on vasorelaxation vs. vasoconstriction responses is also importantly influenced by the S1P concentrations being studied and by the use of other vasoactive drugs, possibly with additional influences being reflective of vascular disease states.

S1P induces eNOS-dependent vasorelaxation in epinephrine-preconstricted mesenteric arterioles derived from either rats or mice.[29] It is interesting to note that S1P-induced vasodilatation appears to be solely dependent on eNOS-derived NO, whereas bradykinin-induced response appears to depend both on eNOS/NO and endothelium-derived hyperpolarizing factor pathway.[29] In addition to S1P itself, several related molecules are also capable of modulating eNOS to induce vasodilatation responses. HDL, which is enriched in S1P (discussed below), is able to promote the dilation of phenylephrine-preconstricted rat thoracic aorta preparations by way of $S1P_3$ receptors and eNOS.[30] FTY-720 is a novel immunosuppressant, structurally related to sphingosine; this agent, when phosphorylated by the action of sphingosine kinase, binds to and activates S1P receptors.[74] Interestingly, *in vivo* administration of FTY-720 inhibits egress of lymphocytes from lymph

Downloaded from https://academic.oup.com/cardiovascres/article/82/2/212/279374 by guest on 16 August 2022

nodes and Peyer's patches, leading to lymphocytopenia in peripheral blood.[74] A recent study reported that FTY-720 mediates eNOS-dependent vasorelaxation in phenylephrine-preconstricted mouse thoracic aorta preparations by way of $S1P_3$ receptor activation.[75] Collectively these studies help establish that treatment with S1P leads to physiologically relevant amounts of NO production in intact blood vessels.

It is important to note that in some experimental systems, S1P has been found to induce vasoconstriction responses instead of vasodilatation. For example, S1P-induced vasoconstriction was observed in canine basilar arteries,[71] in rodent cerebral arteries,[59,60] and in mesenteric resistance arteries from aged rats.[76] In general, higher concentrations of S1P are required to elicit vasoconstriction (several 100 nanomolar to micromolar) than required for S1P-induced vasorelaxation, which typically shows an EC_{50} in the low nanomolar range. Thus, different receptor subtypes and different cell types in the vascular wall may subserve S1P-mediated vasoconstriction responses. In support of this hypothesis, pharmacological as well as genetic experiments have shown that $S1P_2$ and/or $S1P_3$ receptor subtypes coupled with ROK may mediate S1P-provoked vasoconstriction in VSMCs.[60,70,77,78] It has also been shown that pharmacological inhibition of NOS activity leads to an enhancement of S1P-elicited vasoconstriction,[76,79] and conversely, S1P fails to induce vasorelaxation in eNOS*null* animals.[29] Further support for differential receptor-mediated vasorelaxation and vasoconstriction responses comes from studies in which $S1P_1$ antagonists were found to potentiate S1P-induced vasoconstriction responses in rodent cerebral arteries; however, effects of $S1P_1$ receptor antagonism are lost when the endothelium is removed from the blood vessel preparation.[60] Taken all together, these studies are consistent with a model in which S1P-dependent activation of endothelial $S1P_1$ receptors promotes vasorelaxation responses and antagonizes vasoconstriction by the activation of eNOS and production of NO—even in blood vessels where the response to high doses of S1P may lead to vasoconstriction. Thus, whether a given blood vessel preparation responds to S1P stimulation with vasodilatation or vasoconstriction may depend on multiple experimental variables: the animal species being studied, the vascular bed being analysed, the S1P concentrations used, S1P receptor subtype expression profile, as well as other factors (*Figure 1*). Further studies are required for a more detailed understanding of the mechanisms whereby S1P and related lipids regulate vascular tone. It is notable that other classical GPCR pathways also show differential responses in vasoregulation: like S1P, the vasoactive neurotransmitter acetylcholine evokes eNOS-dependent vasorelaxation in ECs at lower ligand concentrations, yet promotes vasoconstriction at higher acetylcholine concentrations (or under conditions of endothelial dysfunction) by the activation of a distinct muscarinic receptor subtype located on VSMCs.[80]

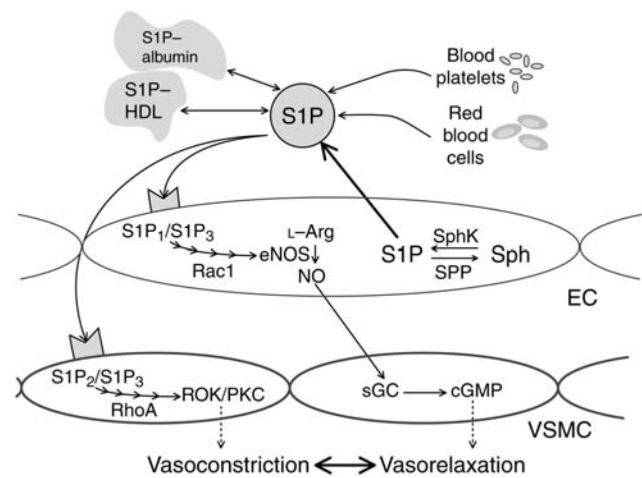

Figure 1 Sphingosine-1-phosphate (S1P)-mediated regulation of vascular tone. The figure shows a schematic representation of how stimulation of the vascular wall with S1P may lead to modulation of vascular tone. Vascular endothelial cells (ECs) express sphingosine kinases (SphK) that catalyse ATP-dependent phosphorylation of sphingosine (Sph) to produce S1P. S1P phosphatases dephosphorylate S1P back into sphingosine. Endothelial cells actively produce and release S1P into blood stream and appear to represent a major source of S1P *in vivo*, with red blood cells, and to a lesser extent, blood platelets also producing significant quantities of S1P. S1P metabolized by vascular smooth muscle cells (VSMCs) may modulate vascular tone as well. In blood, S1P is highly protein-bound and can be found in lipid–protein complexes with high-density lipoproteins (HDLs) or serum albumin. S1P binds to and activates S1P receptors expressed on endothelial cell surface, including $S1P_1$ as well as $S1P_3$ receptor subtypes. In other cell types, S1P has been found also to have intracellular sites of action, but cell surface receptors appear to modulate the principal responses to S1P in vascular cells. Ligand binding of endothelial cell S1P receptors modulates numerous downstream effect or molecules as summarized in the main text, under the control of the small GTP-binding cytoskeleton signalling protein Rac1. These S1P receptor-activated signalling events can lead to the activation of the endothelial isoform of nitric oxide synthase (eNOS), leading to the synthesis of the important vascular messenger molecule nitric oxide (NO). Endothelial cell-derived nitric oxide diffuses to vascular smooth muscle cells, where it binds with and activates the soluble isoform of guanylate cyclase (sGC), which in turn produces the messenger molecule cGMP. cGMP is the key determinant of the vasorelaxation responses evoked by S1P actions in the endothelium. In contrast, S1P is also able to bind with and activate S1P receptors expressed on the surface of vascular smooth muscle cells, including the $S1P_2$ as well as $S1P_3$ receptor subtypes. In vascular smooth muscle cells, ligand binding of S1P receptors leads to the activation of the small G-protein RhoA and its effector molecules, including Rho-associated protein kinase (ROK), as well as some isoforms of protein kinase C (PKC). Activation of the RhoA/ROK/PKC pathway by vascular smooth muscle cells S1P receptors ultimately leads to vasoconstriction responses by mechanisms summarized in the main text. Thus, the overall effects of S1P of blood vessels in regulation of vascular tone take place in a highly context-dependent manner based on the balance responses modulated by endothelial cells vs. vascular smooth muscle cells, and the differential roles of Rac1/NO/cGMP vs. RhoA/ROK/PKC pathways. The effects of S1P on vascular tone can vary significantly between different vascular beds and different animal species; key experimental variables can influence S1P-mediated vascular responses, as vasoconstriction and vasorelaxation responses can be importantly affected by the S1P concentrations being studied, the specific S1P receptor subtype expression profiles present under different conditions, as well as other factors. See text for more details.

6. Pathophysiological implications of sphingosine-1-phosphate-induced control of vascular tone

The central role of S1P in modulation of vascular tone may have important pathophysiological implications. For example, local S1P concentration may acutely increase when platelets get activated. It therefore seems plausible that platelet-derived S1P, for which concentrations may be locally regulated in spatially constrained regions of platelet activation, plays a key role in the activation of S1P receptors on vascular cells. Because eNOS-derived NO limits the degrees of platelet activation, adhesion, and

Downloaded from https://academic.oup.com/cardiovascres/article/82/2/212/279374 by guest on 16 August 2022

aggregation,[81] it is tempting to speculate that S1P activation of eNOS plays a significant role during pathophysiological vascular thrombosis. Since platelet-related molecules other than S1P, including thrombin,[20] are also able to activate eNOS, it remains to be determined whether or not S1P activates eNOS in the face of vascular coagulation *in vivo*. Yet in blood vessels in which S1P exerts overall vasoconstriction responses, the local production of S1P by platelets might help reduce bleeding by decreasing local blood flow. In this context, it is noteworthy that S1P modulates functions of the molecules residing in focal adhesion sites and adherence junctions, as well as molecules localized in the cytoskeleton.[24,82] S1P thereby augments vascular integrity and decreases permeability, largely attributed to effects on vascular ECs (see review[82]). It is also plausible that smooth muscle S1P receptors may elicit pathological spastic responses in cerebral arteries in the face of brain stroke associated with cerebral haemorrhage.[71]

S1P is enriched in HDL fractions of human serum (see review[10]) and in serum albumin. HDL removes excess cholesterol from the vessel wall and delivers this cholesterol to liver; clinically, higher concentrations of HDL are associated with more favourable outcomes in the patients with cardiovascular diseases. In contrast, other lysophospholipids, such as lysophosphatidylcholine and lysophosphatidic acid, are present in higher concentrations in oxidized forms of low-density lipoproteins, which in turn are associated with increased cardiovascular risk.[83,84] These observations raise the possibility that S1P and other lysophospholipids within various lipoprotein fractions may exert differential actions on vascular cells.

7. Concluding remarks

S1P is a key determinant of eNOS activity, and importantly influences NO-dependent signalling pathways in the vascular wall. S1P receptor-elicited molecular responses share both common and distinct features with other agonist-mediated eNOS activation pathways in vascular ECs. In contrast to the vasorelaxation elicited by S1P through $S1P_1$ receptors in vascular endothelium mediated by Rac1, S1P receptors in smooth muscle cells can elicit vasoconstriction responses through the activation of RhoA/ROK pathways, particularly at higher concentrations of S1P. Activation of vasorelaxing Rac1/eNOS pathways in ECs and of vasoconstricting RhoA/ROK pathways in VSMCs are seen in response to diverse agonists in both cell types, but subtle differences in proximal receptor-specific molecular pathways translate into strikingly different physiological responses. Understanding the cross-talk and co-ordinated regulation of these S1P-modulated pathways in the vascular wall is a critical ongoing area of investigation. A major unanswered question relates to the cellular synthesis, transport, and ultimate delivery of S1P to vascular S1P receptors, a topic with important implications for the vascular disease states that are influenced by dyslipidemias and/or ameliorated by statins, in which S1P-modulated signalling pathways play a clear role in vascular pathophysiology.

Conflict of interest: none declared.

Funding

The research programme of T.M. is supported in part by National Institutes of Health grants HL46457, HL48743, and GM36259.

References

1. Hla T. Physiological and pathological actions of sphingosine 1-phosphate. *Semin Cell Dev Biol* 2004;**15**:513–520.
2. Hla T, Lee MJ, Ancellin N, Paik JH, Kluk MJ. Lysophospholipids–receptor revelations. *Science* 2001;**294**:1875–1878.
3. Liu Y, Wada R, Yamashita T, Mi Y, Deng CX, Hobson JP *et al*. Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. *J Clin Invest* 2000;**106**:951–961.
4. Kupperman E, An S, Osborne N, Waldron S, Stainier DY. A sphingosine-1-phosphate receptor regulates cell migration during vertebrate heart development. *Nature* 2000;**406**:192–195.
5. Payne SG, Milstien S, Spiegel S. Sphingosine-1-phosphate: dual messenger functions. *FEBS Lett* 2002;**531**:54–57.
6. Cavalli AL, O'Brien NW, Barlow SB, Betto R, Glembotski CC, Palade PT *et al*. Expression and functional characterization of SCaMPER: a sphingolipid-modulated calcium channel of cardiomyocytes. *Am J Physiol Cell Physiol* 2003;**284**:C780–C790.
7. Futerman AH, Hannun YA. The complex life of simple sphingolipids. *EMBO Rep* 2004;**5**:777–782.
8. Spiegel S, Milstien S. Functions of the multifaceted family of sphingosine kinases and some close relatives. *J Biol Chem* 2007;**282**:2125–2129.
9. Yatomi Y. Plasma sphingosine 1-phosphate metabolism and analysis. *Biochim Biophys Acta* 2008;**1780**:606–611.
10. Okajima F. Plasma lipoproteins behave as carriers of extracellular sphingosine 1-phosphate: is this an atherogenic mediator or an anti-atherogenic mediator? *Biochim Biophys Acta* 2002;**1582**:132–137.
11. Yatomi Y, Yamamura S, Ruan F, Igarashi Y. Sphingosine 1-phosphate induces platelet activation through an extracellular action and shares a platelet surface receptor with lysophosphatidic acid. *J Biol Chem* 1997; **272**:5291–5297.
12. Tani M, Sano T, Ito M, Igarashi Y. Mechanisms of sphingosine and sphingosine 1-phosphate generation in human platelets. *J Lipid Res* 2005;**46**: 2458–2467.
13. Kobayashi N, Nishi T, Hirata T, Kihara A, Sano T, Igarashi Y *et al*. Sphingosine 1-phosphate is released from the cytosol of rat platelets in a carrier-mediated manner. *J Lipid Res* 2006;**47**:614–621.
14. Mizugishi K, Yamashita T, Olivera A, Miller GF, Spiegel S, Proia RL. Essential role for sphingosine kinases in neural and vascular development. *Mol Cell Biol* 2005;**25**:11113–11121.
15. Pappu R, Schwab SR, Cornelissen I, Pereira JP, Regard JB, Xu Y *et al*. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science* 2007;**316**:295–298.
16. Xia P, Gamble JR, Rye KA, Wang L, Hii CS, Cockerill P *et al*. Tumor necrosis factor-alpha induces adhesion molecule expression through the sphingosine kinase pathway. *Proc Natl Acad Sci USA* 1998;**95**:14196–14201.
17. Ancellin N, Colmont C, Su J, Li Q, Mittereder N, Chae SS *et al*. Extracellular export of sphingosine kinase-1 enzyme. Sphingosine 1-phosphate generation and the induction of angiogenic vascular maturation. *J Biol Chem* 2002;**277**:6667–6675.
18. Venkataraman K, Lee YM, Michaud J, Thangada S, Ai Y, Bonkovsky HL *et al*. Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. *Circ Res* 2008;**102**:669–676.
19. Peter BF, Lidington D, Harada A, Bolz HJ, Vogel L, Heximer S *et al*. Role of sphingosine-1-phosphate phosphohydrolase 1 in the regulation of resistance artery tone. *Circ Res* 2008;**103**:315–324.
20. Loscalzo J, Welch G. Nitric oxide and its role in the cardiovascular system. *Prog Cardiovasc Dis* 1995;**38**:87–104.
21. Dudzinski DM, Igarashi J, Greif D, Michel T. The regulation and pharmacology of endothelial nitric oxide synthase. *Annu Rev Pharmacol Toxicol* 2006;**46**:235–276.
22. Igarashi J, Michel T. Agonist-modulated targeting of the EDG-1 receptor to plasmalemmal caveolae: eNOS activation by sphingosine 1-phosphate and the role of caveolin-1 in sphingolipid signal transduction. *J Biol Chem* 2000;**275**:32363–32370.
23. Igarashi J, Bernier SG, Michel T. Sphingosine 1-phosphate and activation of endothelial nitric oxide synthase: differential regulation of Akt and MAP kinase pathways by EDG and bradykinin receptors in vascular endothelial cells. *J Biol Chem* 2001;**276**:12420–12426.

Downloaded from https://academic.oup.com/cardiovascres/article/82/2/212/279374 by guest on 16 August 2022

24. Lee MJ, Thangada S, Claffey KP, Ancellin N, Liu CH, Kluk M *et al*. Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. *Cell* 1999;**99**:301–312.
25. Morales-Ruiz M, Lee MJ, Zoellner S, Gratton JP, Scotland R, Shiojima I *et al*. Sphingosine-1-phosphate activates Akt, nitric oxide production and chemotaxis through a Gi-protein/ phosphoinositide 3-kinase pathway in endothelial cells. *J Biol Chem* 2001;**276**:19672–19677.
26. Igarashi J, Miyoshi M, Hashimoto T, Kubota Y, Kosaka H. Statins induce S1P(1) receptors and enhance endothelial nitric oxide production in response to high-density lipoproteins. *Br J Pharmacol* 2007;**150**:470–479.
27. Igarashi J, Michel T. Sphingosine 1-phosphate and isoform-specific activation of phosphoinositide 3-kinase beta. Evidence for divergence and convergence of receptor-regulated endothelial nitric-oxide synthase signaling pathways. *J Biol Chem* 2001;**276**:36281–36288.
28. Kimura T, Sato K, Kuwabara A, Tomura H, Ishiwara M, Kobayashi I *et al*. Sphingosine 1-phosphate may be a major component of plasma lipoproteins responsible for the cytoprotective actions in human umbilical vein endothelial cells. *J Biol Chem* 2001;**276**:31780–31785.
29. Dantas AP, Igarashi J, Michel T. Sphingosine 1-phosphate and control of vascular tone. *Am J Physiol Heart Circ Physiol* 2003;**284**:H2045–H2052.
30. Nofer JR, Van Der Giet M, Tolle M, Wolinska I, Von Wnuck Lipinski K, Baba HA *et al*. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P(3). *J Clin Invest* 2004;**113**:569–581.
31. Theilmeier G, Schmidt C, Herrmann J, Keul P, Schafers M, Herrgott I *et al*. High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury *in vivo* via the $S1P_3$ lysophospholipid receptor. *Circulation* 2006; **114**:1403–1409.
32. Igarashi J, Erwin PA, Dantas AP, Chen H, Michel T. VEGF induces $S1P_1$ receptors in endothelial cells: implications for cross-talk between sphingolipid and growth factor receptors. *Proc Natl Acad Sci USA* 2003;**100**: 10664–10669.
33. Hla T, Maciag T. An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to G-protein-coupled receptors. *J Biol Chem* 1990;**265**:9308–9313.
34. Shu X, Wu W, Mosteller RD, Broek D. Sphingosine kinase mediates vascular endothelial growth factor-induced activation of ras and mitogen-activated protein kinases. *Mol Cell Biol* 2002;**22**:7758–7768.
35. Endo A, Nagashima KI, Kurose H, Mochizuki S, Matsuda M, Mochizuki N. Sphingosine 1-phosphate induces membrane ruffling and increases motility of human umbilical vein endothelial cells via vascular endothelial growth factor receptor and CrkII. *J Biol Chem* 2002;**277**:23747–23754.
36. Tanimoto T, Jin ZG, Berk BC. Transactivation of vascular endothelial growth factor (VEGF) receptor Flk-1/KDR is involved in sphingosine 1-phosphate-stimulated phosphorylation of Akt and endothelial nitric-oxide synthase (eNOS). *J Biol Chem* 2002;**277**:42997–43001.
37. Levine YC, Li GK, Michel T. Agonist-modulated regulation of AMP-activated protein kinase (AMPK) in endothelial cells: evidence for an AMPK → Rac1 → Akt → endothelial nitric-oxide synthase pathway. *J Biol Chem* 2007;**282**:20351–20364.
38. Cai H. Hydrogen peroxide regulation of endothelial function: origins, mechanisms, and consequences. *Cardiovasc Res* 2005;**68**:26–36.
39. Igarashi J, Miyoshi M, Hashimoto T, Kubota Y, Kosaka H. Hydrogen peroxide induces $S1P_1$ receptors and sensitizes vascular endothelial cells to sphingosine 1-phosphate, a platelet-derived lipid mediator. *Am J Physiol Cell Physiol* 2007;**292**:C740–C748.
40. Mason RP, Walter MF, Jacob RF. Effects of HMG-CoA reductase inhibitors on endothelial function: role of microdomains and oxidative stress. *Circulation* 2004;**109**:II34–II41.
41. Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V *et al*. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 2004;**427**:355–360.
42. Kabashima K, Haynes NM, Xu Y, Nutt SL, Allende ML, Proia RL *et al*. Plasma cell $S1P_1$ expression determines secondary lymphoid organ retention versus bone marrow tropism. *J Exp Med* 2006;**203**:2683–2690.
43. Shaul PW, Anderson RGW. Role of plasmalemmal caveolae in signal transduction. *Am J Physiol* 1998;**275**:L843–L851.
44. Sase K, Michel T. Expression and regulation of endothelial nitric oxide synthase. *Trends Cardiovasc Med* 1997;**7**:28–37.
45. Busconi L, Michel T. Endothelial nitric oxide synthase. N-terminal myristoylation determines subcellular localization. *J Biol Chem* 1993;**268**: 8410–8413.
46. Yeh CD, Duncan JA, Yamashita S, Michel T. Depalmitoylation of endothelial nitric-oxide synthase by acyl-protein thioesterase 1 is potentiated by Ca^{2+}-calmodulin. *J Biol Chem* 1999;**274**:33148–33154.
47. Shaul PW. Regulation of endothelial nitric oxide synthase: location, location, location. *Annu Rev Physiol* 2002;**64**:749–774.
48. Feron O, Belhassen L, Kobzik L, Smith TW, Kelly RA, Michel T. Endothelial nitric oxide synthase targeting to caveolae. Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells. *J Biol Chem* 1996;**271**:22810–22814.
49. Michel JB, Feron O, Sase K, Prabhakar P, Michel T. Caveolin versus calmodulin. Counterbalancing allosteric modulators of endothelial nitric oxide synthase. *J Biol Chem* 1997;**272**:25907–25912.
50. Robinson LJ, Busconi L, Michel T. Agonist-modulated palmitoylation of endothelial nitric oxide synthase. *J Biol Chem* 1995;**270**:995–998.
51. Gonzalez E, Kou R, Lin AJ, Golan DE, Michel T. Subcellular targeting and agonist-induced site-specific phosphorylation of endothelial nitric-oxide synthase. *J Biol Chem* 2002;**277**:39554–39560.
52. Michel T, Feron O. Nitric oxide synthases: which, where, how, and why? *J Clin Invest* 1997;**100**:2146–2152.
53. Lee H, Goetzl EJ, An S. Lysophosphatidic acid and sphingosine 1-phosphate stimulate endothelial cell wound healing. *Am J Physiol* 2000;**278**:C612–C618.
54. Muraki K, Imaizumi Y. A novel function of sphingosine 1-phosphate to activate a non-selective cation channel in human endothelial cells. *J Physiol* 2001;**537**:431–441.
55. McCabe TJ, Fulton D, Roman LJ, Sessa WC. Enhanced electron flux and reduced calmodulin dissociation may explain 'calcium-independent' eNOS activation by phosphorylation. *J Biol Chem* 2000;**275**:6123–6128.
56. Gonzalez E, Kou R, Michel T. Rac1 modulates sphingosine 1-phosphate-mediated activation of phosphoinositide 3-kinase/Akt signaling pathways in vascular endothelial cells. *J Biol Chem* 2006;**281**:3210–3216.
57. Igarashi J, Michel T. More sweetness than light? A search for the causes of diabetic vasculopathy. *J Clin Invest* 2001;**108**:1425–1427.
58. Fulton D, Ruan L, Sood SG, Li C, Zhang Q, Venema RC. Agonist-stimulated endothelial nitric oxide synthase activation and vascular relaxation. Role of eNOS phosphorylation at Tyr83. *Circ Res* 2008;**102**:497–504.
59. Coussin F, Scott RH, Wise A, Nixon GF. Comparison of sphingosine 1-phosphate-induced intracellular signaling pathways in vascular smooth muscles: differential role in vasoconstriction. *Circ Res* 2002;**91**:151–157.
60. Salomone S, Potts EM, Tyndall S, Ip PC, Chun J, Brinkmann V *et al*. Analysis of sphingosine 1-phosphate receptors involved in constriction of isolated cerebral arteries with receptor null mice and pharmacological tools. *Br J Pharmacol* 2008;**153**:140–147.
61. Lorenz JN, Arend LJ, Robitz R, Paul RJ, MacLennan AJ. Vascular dysfunction in $S1P_2$ sphingosine 1-phosphate receptor knockout mice. *Am J Physiol Regul Integr Comp Physiol* 2007;**292**:R440–R446.
62. Kluk MJ, Hla T. Role of the sphingosine 1-phosphate receptor EDG-1 in vascular smooth muscle cell proliferation and migration. *Circ Res* 2001; **89**:496–502.
63. Wamhoff BR, Lynch KR, Macdonald TL, Owens GK. Sphingosine-1-phosphate receptor subtypes differentially regulate smooth muscle cell phenotype. *Arterioscler Thromb Vasc Biol* 2008;**28**:1454–1461.
64. Zhou H, Murthy KS. Distinctive G protein-dependent signaling in smooth muscle by sphingosine 1-phosphate receptors $S1P_1$ and $S1P_2$. *Am J Physiol Cell Physiol* 2004;**286**:C1130–C1138.
65. Rosenfeldt HM, Amrani Y, Watterson KR, Murthy KS, Panettieri RA Jr, Spiegel S. Sphingosine-1-phosphate stimulates contraction of human airway smooth muscle cells. *FASEB J* 2003;**17**:1789–1799.
66. Somlyo AP, Somlyo AV. Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev* 2003;**83**:1325–1358.
67. Sanders KM. Invited review: mechanisms of calcium handling in smooth muscles. *J Appl Physiol* 2001;**91**:1438–1449.
68. McFadzean I, Gibson A. The developing relationship between receptor-operated and store-operated calcium channels in smooth muscle. *Br J Pharmacol* 2002;**135**:1–13.
69. Bischoff A, Czyborra P, Fetscher C, Meyer Zu Heringdorf D, Jakobs KH *et al*. Sphingosine-1-phosphate and sphingosylphosphorylcholine constrict renal and mesenteric microvessels *in vitro*. *Br J Pharmacol* 2000; **130**:1871–1877.
70. Ohmori T, Yatomi Y, Osada M, Kazama F, Takafuta T, Ikeda H *et al*. Sphingosine 1-phosphate induces contraction of coronary artery smooth muscle cells via $S1P_2$. *Cardiovasc Res* 2003;**58**:170–177.
71. Tosaka M, Okajima F, Hashiba Y, Saito N, Nagano T, Watanabe T *et al*. Sphingosine 1-phosphate contracts canine basilar arteries *in vitro* and *in vivo*: possible role in pathogenesis of cerebral vasospasm. *Stroke* 2001;**32**:2913–2919.
72. Bolz SS, Vogel L, Sollinger D, Derwand R, de Wit C, Loirand G *et al*. Nitric oxide-induced decrease in calcium sensitivity of resistance arteries is

Downloaded from https://academic.oup.com/cardiovascres/article/82/2/212/279374 by guest on 16 August 2022

attributable to activation of the myosin light chain phosphatase and antagonized by the RhoA/Rho kinase pathway. *Circulation* 2003;**107**:3081–3087.
73. Hardin CD, Vallejo J. Caveolins in vascular smooth muscle: form organizing function. *Cardiovasc Res* 2006;**69**:808–815.
74. Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J *et al*. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* 2002;**296**:346–349.
75. Tolle M, Levkau B, Keul P, Brinkmann V, Giebing G, Schonfelder G *et al*. Immunomodulator FTY720 induces eNOS-dependent arterial vasodilatation via the lysophospholipid receptor $S1P_3$. *Circ Res* 2005;**96**:913–920.
76. Hemmings DG, Xu Y, Davidge ST. Sphingosine 1-phosphate-induced vasoconstriction is elevated in mesenteric resistance arteries from aged female rats. *Br J Pharmacol* 2004;**143**:276–284.
77. Alewijnse AE, Peters SL, Michel MC. Cardiovascular effects of sphingosine-1-phosphate and other sphingomyelin metabolites. *Br J Pharmacol* 2004;**143**:666–684.
78. Salomone S, Yoshimura S, Reuter U, Foley M, Thomas SS, Moskowitz MA *et al*. $S1P_3$ receptors mediate the potent constriction of cerebral arteries by sphingosine-1-phosphate. *Eur J Pharmacol* 2003;**469**:125–134.
79. Hemmings DG, Hudson NK, Halliday D, O'Hara M, Baker PN, Davidge ST *et al*. Sphingosine-1-phosphate acts via rho-associated kinase and nitric oxide to regulate human placental vascular tone. *Biol Reprod* 2006;**74**:88–94.
80. Russo G, Leopold JA, Loscalzo J. Vasoactive substances: nitric oxide and endothelial dysfunction in atherosclerosis. *Vascul Pharmacol* 2002;**38**:259–269.
81. Loscalzo J. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. *Circ Res* 2001;**88**:756–762.
82. McVerry BJ, Garcia JG. *In vitro* and *in vivo* modulation of vascular barrier integrity by sphingosine 1-phosphate: mechanistic insights. *Cell Signal* 2005;**17**:131–139.
83. Kume N, Gimbrone MAJ. Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human endothelial cells. *J Clin Invest* 1994;**93**:907–911.
84. Maschberger P, Bauer M, Baumann-Siemons J, Zangl KJ, Negrescu EV, Reininger AJ *et al*. Mildly oxidized low density lipoprotein rapidly stimulates via activation of the lysophosphatidic acid receptor Src family and Syk tyrosine kinases and Ca^{2+} influx in human platelets. *J Biol Chem* 2000;**275**:19159–19166.

Downloaded from https://academic.oup.com/cardiovascres/article/82/2/212/279374 by guest on 16 August 2022