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Minireviews

S1P Signaling and De Novo Biosynthesis in Blood Pressure Homeostasis

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ABSTRACT

Initially discovered as abundant components of eukaryotic cell membranes, sphingolipids are now recognized as important bioactive signaling molecules that modulate a variety of cellular functions, including those relevant to cancer and immunologic, inflammatory, and cardiovascular disorders. In this review, we discuss recent advances in our understanding of the role of sphingosine-1-phosphate (S1P) receptors in the regulation of vascular function, and focus on how de novo biosynthesized sphingolipids play a role in blood pressure homeostasis. The therapeutic potential of new drugs that target S1P signaling is also discussed.

Introduction

Sphingolipids (SLs) are pleiotropic lipids with important structural and functional roles in mammalian physiology (Hannun and Obeid, 2008; Blaho and Hla, 2011). De novo SL biosynthesis begins in the membrane of the endoplasmic reticulum (ER), where serine palmitoyltransferase (SPT) converts serine and palmitoyl-CoA to 3-dehydro-D-sphinganine (Fig. 1). This constitutes the first and rate-limiting step of the de novo pathway, which through various steps forms ceramide, a central product of this pathway. Ceramide can be phosphorylated, transformed into higher-order SL such as sphingomyelins and glycosphingolipids, and converted into sphingosine, which can be phosphorylated by sphingosine kinase (SPHK)-1 and SPHK2 to form sphingosine-1-phosphate (S1P), a highly bioactive lipid. The sphingolipid biosynthetic pathways have been extensively reviewed elsewhere (Hannun and Obeid, 2008; Gault et al., 2010; Merrill, 2011). The biologic functions of S1P are mainly mediated by a family of five G-protein-coupled receptors called S1P receptors (S1PRs) 1–5 (Sanchez and Hla, 2004). S1P is known to be an important regulator of vascular functions, including angiogenesis, endothelial barrier integrity, and vascular tone in isolated vessels.

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In this review, we focus on studies that contribute to our understanding of the roles of S1P signaling in vascular tone and blood pressure (BP) regulation. We outline new developments, such as the recently described role of locally produced S1P in the vascular response to flow and pressure and the discovery of Nogo-B, an ER membrane protein, as a negative regulator of de novo SL biosynthesis within the vascular wall.

S1P: Sources and Carriers

S1P is present at high concentrations in plasma (0.1–1 μM) and its half-life is only ~15 minutes (Venkataraman et al., 2008), suggesting that in vivo there exists a significant biosynthetic capacity for S1P to replenish the one rapidly degraded in the plasma (Peest et al., 2008). In physiologic conditions, erythrocytes (Hänel et al., 2007) and endothelial cells (ECs) (Venkataraman et al., 2008; Xiong et al., 2014) are the major source of plasma S1P, whereas platelets (Yatomi et al., 1997a; Pappu et al., 2007) become an important source of S1P in pathologic conditions, following their activation. Platelets produce and store large amounts of S1P because they express highly active SPHK enzymes and virtually no S1P lyase (Yatomi et al., 1997b). S1P is released from platelets following stimulation (i.e., thrombin) through a membrane transporter, which remains to be identified (Kobayashi et al., 2006).

Mice with low numbers of platelets (Shivdasani and Orkin, 1995) still have normal S1P levels, which is likely due to S1P

ABBREVIATIONS: AngII, angiotensin II; ApoM, apolipoprotein M; BP, blood pressure; CV, cardiovascular; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; FTY720, fingolimod; GFP, green fluorescent protein; HDL, high-density lipoprotein; HR, heart rate; MABP, mean arterial blood pressure; NO, nitric oxide; SL, sphingolipid; SPHK, sphingosine kinase; SPT, serine palmitoyltransferase; S1P, sphingosine-1-phosphate; S1PR, sphingosine-1-phosphate receptor; VSMC, vascular smooth muscle cell; WT, wild type.

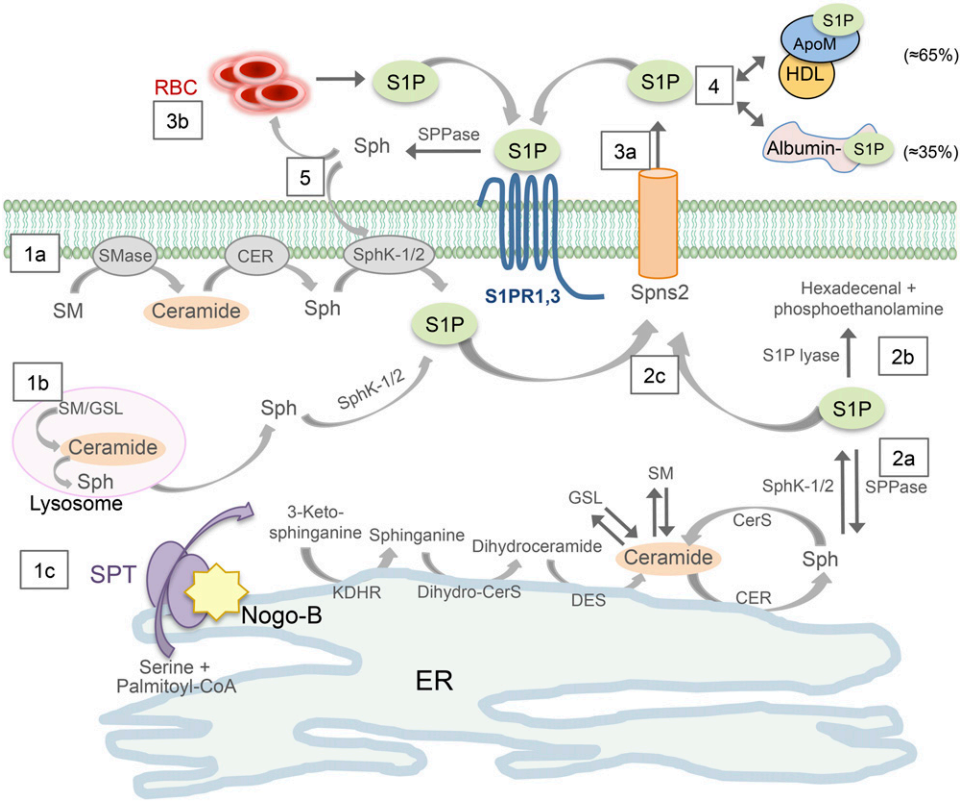

Fig. 1. De novo SL biosynthesis and S1P metabolism. S1P is the product of ceramide metabolism, which can be originated by three pathways: 1) sphingomyelin catabolism in the plasma membrane; 2) salvage pathway in the lysosome; and 3) de novo biosynthesis in the ER membrane. (1a) Sphingomyelinase (SMase) catabolizes sphingomyelin (SM) into ceramide, which is transformed into sphingosine (Sph) by ceramidase and finally phosphorylated by SPHK-1/2 to form S1P; (1b) the salvage pathway involves first the breakdown of SM and complex SLs into ceramide and then sphingosine in the endolysosomal compartment; and (1c) de novo biosynthesis initiates with the condensation of serine and palmitoyl-CoA into 3-ketosphinaganine by serine-palmitoyltransferase (SPT), the rate-limiting enzyme of this pathway. Nogo-B binds to and inhibits the activity of SPT. In the next step, 3-ketosphinganine is rapidly converted into sphinganine by ketodihydrosphingosine reductase (KDHR). Dihydroceramide synthase (dihydro-CerS) converts sphinganine into dihydroceramide, which is further catabolized into ceramide by dihydroceramide desaturase (DES). Ceramide can be transformed into higher-order SLs, such as glycosphingolipids (GSL) and SM, or metabolized into Sph and ultimately S1P. Once produced, S1P can follow three different paths: (2a) it can be dephosphorylated into Sph by S1P phosphatase (SPP) to form ceramide; (2b) it can be irreversibly degraded by the S1P lyase; or (2c) it can be transported out of the cell, through transporters, such as spinster 2 (Spns2). Once released by (3a) ECs or (3b) red blood cells (RBCs), (4) S1P binds to circulating protein carriers, ApoM on the HDL (≈65%) or albumin (≈35%), which transport and deliver S1P to S1PRs. Endothelial-derived S1P can also directly activate the S1PR1/3 in an autocrine manner to regulate vascular tone. (5) S1P can be recycled and converted into Sph, which is uptaken by RBC or EC and converted to S1P.

produced by the erythrocytes (Pappu et al., 2007) and ECs (Venkataraman et al., 2008; Fukuhara et al., 2012). Similar to platelets, erythrocytes have high SPHK and minimal S1P lyase activity. Interestingly, pharmacological inhibition of the enzymes involved in S1P production, including SPT, ceramidase, and SPHK, did not affect the amount of S1P released by erythrocytes, suggesting that these cells can efficiently uptake extracellular sphingosine and accumulate S1P (Hänel et al., 2007). Therefore, these studies suggest that erythrocytes, rather than platelets, play an important role in maintaining physiologic levels of S1P. A year after Hänel et al. (2007) published their study Venkataraman et al. (2008) demonstrated that ECs actively contribute to the maintenance of the plasma S1P pool. Interestingly, mechanical shear stress can stimulate the production of S1P from ECs and downregulate S1P lyase, suggesting a link between hemodynamic changes and SL metabolic pathways.

Once produced, S1P is transported out of ECs through the spinster-2 transporter (Fukuhara et al., 2012), where it activates S1PR1 on the cell surface to induce barrier protective functions and control vascular tone in an autocrine manner (Cantalupo et al., 2015); additionally, it binds to protein carriers such as albumin (≈35%) and high-density lipoprotein (HDL) (≈60%), and to a lesser extent to low-density and very low-density lipoproteins (Murata et al., 2000). Christoffersen et al. (2011) showed that in mice lacking apolipoprotein M (ApoM), HDL has no S1P content, indicating that S1P binds to HDL through ApoM. This finding was also confirmed for human HDL. The S1P-ApoM-HDL complex was able to activate downstream signaling through the S1PR1, suggesting that ApoM can carry and deliver S1P to the receptor. Indeed, plasma S1P levels were lower (~46%) and the basal endothelial barrier was decreased in *Apom* knockout mice (Christoffersen et al., 2011), suggesting a role of HDL/ApoM-bound S1P in maintaining vascular barrier integrity. It is now well accepted that many of the beneficial effects attributed to HDL are due to S1P and receptor-mediated S1P activation (Nofer et al., 2004). Furthermore, Galvani et al. (2015) demonstrated that endothelial S1PR1 exerts an important atheroprotective role and proposed that HDL-bound S1P acts as a biased agonist on S1PR1 to inhibit vascular inflammation in atheroprone areas of the aorta. This suggests

that S1P chaperoned by HDL imparts a specific signaling mechanism, which may in part explain the cardioprotective functions of HDL; nevertheless, whether ApoM exerts additional vascular effects, including the regulation of vascular tone and BP, remains to be determined.

S1P in Endothelial and Flow-Mediated Vasodilation

Exogenous S1P: Vasodilation and Atheroprotection through S1PR1 and S1PR3 Receptors. The endothelium dynamically regulates BP through real-time integration of chemical and rheological stimuli (Cowley, 2006). Vasoactive factors released by ECs include nitric oxide (NO), prostacyclin, endothelin, and thromboxane, among others; S1P, which is released by the endothelium (Venkataraman et al., 2008), has been identified as a potent activator of endothelial NO synthase (eNOS) through S1PR1 (Igarashi and Michel, 2000; Igarashi et al., 2001; Kimura et al., 2001). Igarashi et al. (2001) elegantly dissected the signaling pathways downstream of S1P/S1PR1 activation using a pharmacological approach in bovine aortic ECs which included Ca^{++} mobilization and activation of MAPK and protein kinase B pathways, with the latter leading to eNOS phosphorylation at the Ser1179 in a time- and concentration-dependent manner. The molecular mechanisms activated by the S1P/S1PR1 signaling axis have been extensively reviewed elsewhere (Igarashi and Michel, 2009).

In isolated murine arteries, exogenous S1P (0.1–100 nM) induced endothelial-dependent relaxation of preconstricted arteries, mostly mediated by eNOS-derived NO (Sugiyama et al., 2000; Dantas et al., 2003). However, the importance of S1PR1 signaling in vascular tone and BP regulation remains poorly described, in part because S1PR1-null mice are embryonic lethal due to defective vascular maturation (Liu et al., 2000b; Allende et al., 2003). A recent study by Jung et al. (2012), in which a mouse model with inducible deletion of S1PR1 in ECs was used, showed that eNOS phosphorylation in the aorta was reduced in the absence of S1PR1, providing in vivo evidence of the importance of S1PR1-mediated eNOS activation.

Although S1PR1 is estimated to be 16-fold more abundant than S1PR3 (Lee et al., 1999), HDL and S1P-induced vasorelaxation is absent in aortas from *S1pr3*$^{-/-}$ mice (Nofer et al., 2004), suggesting that S1PR3 actively contributes to S1P-mediated vascular tone regulation. However, the role of S1PR1 in ECs cannot be excluded because in this study neither a genetic *S1pr1* knockout model nor pharmacological inhibitors of S1PR1 were used. Interestingly, both W146 (a S1PR1 inhibitor) and VPC23019 (2-Amino-*N*-(3-octylphenyl)-3-(phosphonooxy)-propanamaide) a S1PR1/3 inhibitor enhanced S1P-mediated vasoconstriction in rodent cerebral arteries (Salomone et al., 2008). Collectively, these studies suggest that S1PR1/3 activation in ECs counteracts S1PR2/3-mediated vasoconstriction in vascular smooth muscle cells (VSMCs), likely through eNOS activation. However, the relative role of these receptors in vascular tone and BP regulation in pathophysiological conditions remains unknown. Further studies in mice knocked out for S1PR1 and/or S1PR3 specifically in the ECs or VSMCs will help to fill this knowledge gap.

Fingolimod (FTY720), phosphorylated in vivo by SPHK2 to the active form FTY720 phosphate (Brinkmann et al., 2002; Billich et al., 2003; Paugh et al., 2003; Sanchez et al., 2003; Zemann et al., 2006), is a potent agonist of four S1P receptors (S1PR1, 3, 4, and 5) (Brinkmann et al., 2002; Mandala et al., 2002; Zemann et al., 2006). Similar to S1P, FTY720 and FTY720 phosphate (1 nM–10 μM) induced eNOS-dependent vasodilation in murine aortas (Tölle et al., 2005). Consistent with a dual S1P effect on the vasculature, FTY720 phosphate induced vasoconstriction of rat basilar arteries (Salomone et al., 2008). Notably, the internalization and down regulation of S1PR1 is a lasting functional effect of FTY720, and therefore FTY720 is considered a functional antagonist (Oo et al., 2007; Brinkmann, 2009; Sykes et al., 2014). Despite its effects on vascular tone regulation and BP in vivo, subsequently discussed in this review, whether long-term FTY720 treatment has any effect in hypertensive conditions has not yet been investigated. Using a S1PR1-GFP (green fluorescent protein) reporter mouse model that expresses intracellular GFP following S1PR1 activation (Kono and Proia, 2015), Galvani et al. (2015) demonstrated that S1PR1 signaling increased in the vasculature at points of turbulent but not laminar flow, such as the lesser curvature, thoracic branching point, and aortic valves, suggesting an important role of S1PR1 in proinflammatory and atherogenic areas of the vessels exposed to turbulent flow.

Endothelial-Derived S1P: Role in Flow-Mediated Vasodilation. The endothelium is not only a target but also an important source of plasma S1P. Recent studies reveal a novel role of endothelial-derived S1P and autocrine S1P-S1PR1 signaling in flow-mediated vasodilation (Jung et al., 2012; Cantalupo et al., 2015). Jung et al. (2012) reported that flow-induced EC alignment and eNOS activation were suppressed in the absence of S1PR1, both in vitro and in vivo. By using the pressure myograph system, Cantalupo et al. (2015) demonstrated that the endothelial S1P-S1PR1-eNOS autocrine loop is physiologically active in regulating vascular tone in response to flow. In control mice, W146 markedly reduced flow-induced vasodilation of mesenteric arteries in a concentration-dependent manner [Fig. 2, adapted from Cantalupo et al. (2015)] as well as eNOS activation, with a subsequent increase in myogenic tone (Cantalupo et al., 2015).

Recently, Cantalupo et al. (2015) discovered a novel regulatory mechanism of endothelial de novo sphingolipid biosynthesis by Nogo-B (Fig. 3), an ER membrane protein that is highly expressed in blood vessels. Nogo-B binds to and inhibits

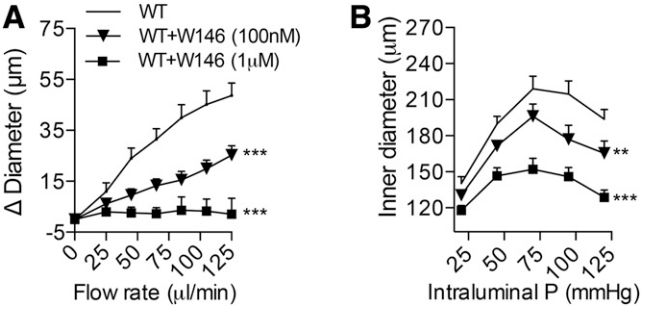

Fig. 2. S1P-S1PR1 regulates and controls vascular responses to flow and pressure murine mesenteric arteries (MAs). MAs isolated from C57Bl6 mice and mounted in the pressure myograph system (DMT, Danish Myotechnology) were incubated with W146 (100 nM and 1 μM), a S1PR1 receptor inhibitor, and response to the flow and transmural pressure was assessed. (A) W146 induced a marked decrease in flow-induced vasodilation and (B) a significant increase in myogenic response in a concentration-dependent manner.

the activity of SPT, thereby controlling local endothelial S1P production and its autocrine G-protein-coupled receptor-dependent signaling actions. In the absence of endothelial Nogo-B, SPT activity and S1P production increase, and S1P-S1PR1 autocrine signaling is upregulated, leading to enhanced vasodilation of resistance arteries. Interestingly, SPT inhibition with myriocin or S1PR1 inhibition with W146 restored the vasodilation to flow in Nogo-A/B-deficient mice to wild-type (WT) levels.

Together, these findings suggest that S1PR1 is tonically activated by endothelial-derived S1P to preserve vascular tone through the eNOS-NO pathway and reveal a key role of the endothelial S1P-S1PR1 autocrine axis in mechanotransduction signaling in response to flow. Although further studies are needed to elucidate the specific molecular interactions leading to this phenomenon, one could speculate that S1P transporters on ECs facilitate the autocrine activation of S1P receptors in the absence of circulating albumin and HDL, as in ex vivo vascular studies.

Recent pharmacological studies suggest that endothelial-derived S1P might also play a role in vascular responses elicited by other vasoactive mediators, such as muscarinic M3 receptor agonists (Roviezzo et al., 2006; Mulders et al., 2009). Interestingly, following carbachol stimulation of ECs, YFP (yellow fluorescent protein)-tagged SPHK1 rapidly translocated to the plasma membrane, suggesting that endothelial S1P may be involved in carbachol-induced NO production. However, DL-threo-dihydrosphingosine and *N*,*N*-dimethylsphingosine used in these studies are competitive inhibitors of both isoforms, SPHK1 and SPHK2 (Liu et al., 2000a), with *N*,*N*-dimethylsphingosine having poor selectivity for SPHKs since it also binds other kinases (Igarashi et al., 1989; McDonald et al., 1991; Megidish et al., 1995; King et al., 2000; Sugiura et al., 2002). Considering that SPHK1 and SPHK2 have distinct and sometimes opposing roles (Pyne and Pyne, 2010), studies to dissect the role of SPHK1- and SPHK2-driven S1P signaling in vascular tone regulation should be re-evaluated based on the availability of more selective inhibitors. Finally, the use of mice deficient in *Sphk1* or *Sphk2* specifically in the ECs or VSMCs may provide great insights into dissecting the role of endothelial autocrine S1P-S1PR1 signaling in other vasoactive signaling pathways known to regulate vascular tone (Table 1).

S1P in Vascular and Myogenic Tone Regulation

S1P-Induced Vasoconstriction: Role of S1PR2 and S1PR3. Multiple studies have shown that exogenous S1P induces vasoconstriction in several vascular beds, particularly in resistance arteries, at concentrations greater than those required to elicit vasodilation. These findings are summarized in Table 2.

An initial study by Bischoff et al. (2000a,b) demonstrated that S1P constricts mesenteric and intrarenal rat arteries ex vivo and reduces renal and mesenteric blood flow in vivo (Table 3). The latter effect was inhibited by pertussis toxin, confirming the involvement of $G_{i/o}$ proteins. S1P (10^{-7} up to 10^{-5} M) was also shown to constrict canine basilar arteries, with and without the endothelium, and this effect was abolished by the Rho-kinase inhibitor Y27632 (+)-(R)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl)cyclohexanecarboxamide dihydrochloride (Tosaka et al., 2001). Interestingly, injection of S1P (50 nmol/Kg) into the canine cisterna magna led to vasoconstriction of the basilar arteries up to 48 hours post-administration, despite a decrease in S1P concentration in cerebrospinal fluid to basal levels 5 hours postinjection, suggesting that additional mechanisms are involved in long-lasting vasoconstriction initiated by S1P (Tosaka et al., 2001). Other groups subsequently confirmed the involvement of the Rho pathway in S1P-mediated vasoconstriction in mice

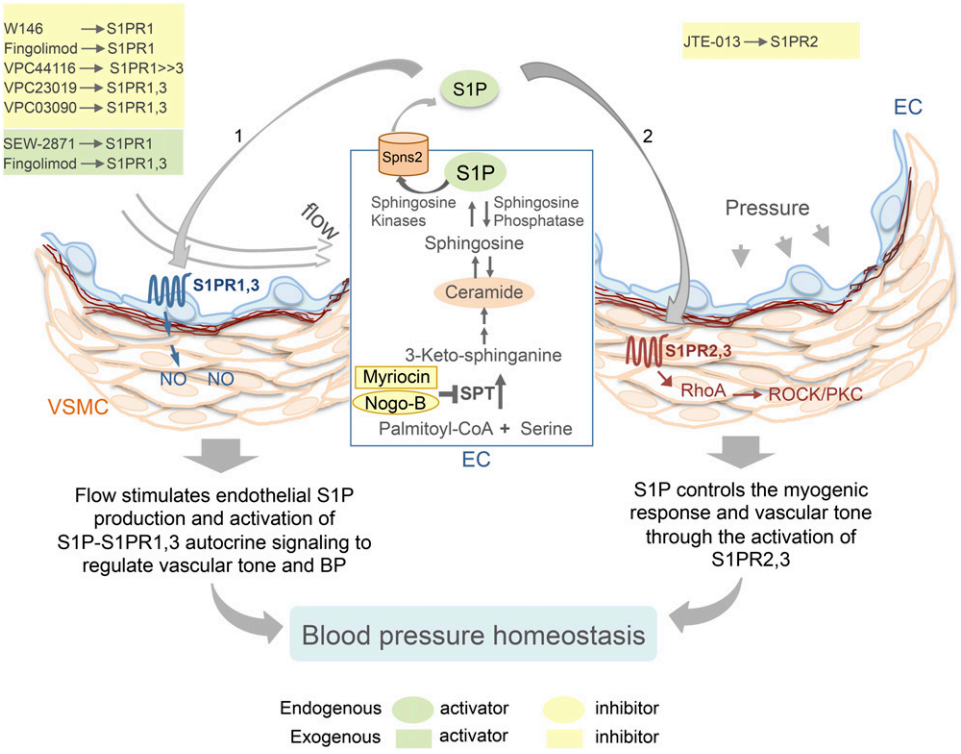

Fig. 3. Nogo-B regulates de novo SL biosynthesis through the inhibition of SPT activity to control vascular tone and BP. Nogo-B, a membrane protein of the ER, is a negative regulator of SPT activity, the enzyme controlling the first and rate-limiting step of the de novo biosynthesis of SLs. A highly bioactive SL, S1P has been identified as a key player in vascular homeostasis: 1) S1P induces vasorelaxation through the activation of S1PR1,3 expressed on EC leading to eNOS-NO-mediated vasodilation. S1P plays an important role in flow-mediated vasodilation. Changes in flow stimulate endothelial S1P production and activation of S1P-S1PR1,3 autocrine signaling to regulate vascular tone. 2) S1P induces vasoconstriction by activating S1PR2,3 on VSMCs through the RhoA/Rho kinase pathway. Moreover, S1P signaling regulates the myogenic response to pressure.

TABLE 1
Vasodilation mediated by S1P signaling in different vascular beds

Vascular Bed	Species	Vasorelaxant agent/Stimulus	Concentration or Flow	Endothelium	S1PR	Signaling	Reference
Mesenteric artery	SD rat Mouse	S1P	10 nM–30 μM	+ − (no effect)	N.R.	$G_{i/o}$ (PTX) eNOS (*eNOS*$^{-/-}$) Ca^{++} (BAPTA) PI3-kinase (wortmannin)	Dantas et al. (2003)
Thoracic aorta	Mouse	S1P	100 nM–10 μM	+	S1PR3 (S1pr3$^{-/-}$)	eNOS (*eNOS*$^{-/-}$)	Nofer et al. (2004)
	Wistar rat	HDL	0.5 mg/ml (single dose) 0.1–0.5 mg/ml (dose-response)	+		Akt (LY294002)	
		SPC	100 nM–10 μM	+			
Thoracic aorta	Mouse	FTY720	1 nM–10 μM	+	S1PR3 (S1pr3$^{-/-}$)	eNOS	Tölle et al. (2005)
		FTY720-P	1 nM–10 μM	+		Akt	
Mesenteric artery	Mouse	S1P	1 pM–10 nM	+	S1PR1 (W146)	eNOS (L-NAME)	Cantalupo et al. (2015)
		Flow	0–125 μl/min	+			
Thoracic aorta	Mouse	S1P	10 nM–30 μM	+		eNOS (L-NAME)	Roviezzo et al. (2006)

Akt, protein kinase B; BAPTA, 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′*N*′-tetraacetic acid; FTY720-P, FTY20 phosphate; L-NAME, L-N^{G}-nitroarginine methyl ester; N.R., not reported; PTX, pertussis toxin; SD, Sprague Dawley; −, absence of endothelium; +, presence of endothelium.

(Coussin et al., 2002; Bolz and Pohl, 2003; Bolz et al., 2003; Salomone et al., 2003; Scherer et al., 2006), as well as in human resistance arteries, including omental and myometrial arteries from pregnant women (Hudson et al., 2007), placenta and stem villous arteries (Hemmings et al., 2006), and porcine retinal arterioles (Kamiya et al., 2014).

TABLE 2
S1P signaling in vasoconstriction and myogenic response in different vascular districts

Vascular Bed	Species	Contracturant agent/stimulus	Concentration or Pressure	Endothelium	S1PR	Signaling	Reference
Cerebral artery	SD rat	S1P	1 and 5 μM	—	N.R.	Rho kinase	Coussin et al. (2002)
Cerebral artery	SD rat	S1P	10 nM–10 μM	—	N.R.	Rho kinase	Salomone et al. (2003)
Cerebral artery	Mouse Rat	S1P	10 nM–30 μM	—	S1PR3 (*S1pr3*$^{-/-}$)	N.R.	Salomone et al. (2008)
Retinal artery	Pig	S1P	1 nM–10 μM	—	S1PR2 (JTE-013)	Rho kinase PLC PKC L-VOCCS MLCK	Kamiya et al. (2014)
Pulmonary artery	Mouse	S1P	1 μM–100 μM	+	S1PR2 (JTE-013/*S1pr2*$^{-/-}$)	Rho kinase	Szczepaniak et al. (2010)
Mesenteric artery Renal artery	Wistar rat	S1P	100 nM–300 μM	+	N.R.	$G_{i/o}$ (PTX)	Bischoff et al. (2000a)
Cerebral artery	Dog	S1P	10 nM–100 μM	+ and −	N.R.	Rho kinase	Tosaka et al. (2001)
Spiral modiolar artery	Gerbil	S1P	0.1 nM–30 μM	N.R.	S1PR2 (JTE-013)	N.R.	Kono et al. (2007)
Mesenteric artery	Wistar rat	S1P	0–100 μg/Kg i.v.	+	N.R.	$G_{i/o}$ (PTX)	Bischoff et al. (2000b)
Pulmonary artery	Mouse	S1P	10 μM	+	S1PR2/4	Rho kinase (Y27632)	Tabeling et al. (2015)
Mesenteric artery	Mouse	S1P Pressure	10 nM–3 μM 20–120 mmHg	+	N.R.	N.R.	Cantalupo et al. (2015)
Gracilis muscle resistance artery	Hamster	Pressure	45 mmHg	N.R.	N.R.	RhoA/Rho kinase	Bolz et al. (2003)
Gracilis muscle resistance artery	Hamster	Pressure S1P	45–110 mmHg 0.1 nM–10 μM	N.R. N.R.	S1PR2 (JTE-013; antisense oligonucleotide)	SPP1	Peter et al. (2008)
Femoral artery Mesenteric artery	SD rat	S1P	10 nM–30 μM	+	N.R.	SphK (DMS)	Salomone et al. (2010)
Posterior cerebral artery	Rabbit	S1P Pressure	1 μM 20–100 mmHg	—	N.R.	Ca^{++} Rho kinase MLC_{20}	Lim et al. (2012)
Mesenteric artery Cremaster muscle artery	Mouse	Pressure	20–120 mmHg	N.R.	S1PR2 (JTE-013)	N.R.	Hoefer et al. (2010)
Omental artery Myometrial artery	Human	S1P	0.01 μM–100 μM	+	N.R.	N.R.	Hudson et al. (2007)
Spiral modiolar artery	Gerbil	S1P	3 nM–30 μM	+	N.R.	Rho kinase (Y27632)	Scherer et al. (2006)
Thoracic aorta	Mouse	KCl PE	10–50 mM 1 nM–10 μM	+ and −	S1PR2 (*S1pr2*$^{-/-}$)	N.R.	Lorenz et al. (2007)

DMS, *N*,*N*-dimethylsphingosine; L-VOCC, L-type voltage-operated calcium channel; MLCK, myosin light-chain kinase; N.R., not reported; PKC, protein kinase C; PLC, phospholipase C; PTX, pertussis toxin; SD, Sprague Dawley, SPP1, S1P-phosphohydrolase 1; Y27632, 4-[(1R)-1-aminoethyl]N-pyridin-4-ylcyclohexane-1-carboxamide dihydrochloride; −, absence of endothelium; +, presence of endothelium.

TABLE 3
Effects of S1P administration on BP and HR

Administration Route	Dose	Species	Anesthesia/BP Measurement Method	BP	HR	Reference
S1P i.v. bolus	1–100 μg/kg	Wistar rat	Thiobutabarbitone/pressure transducer	N.C. (MABP)	N.C.	Bischoff et al. (2000b)
S1P i.v. bolus	1 μg/kg	SD rat	Pentobarbital/Pressure transducer	N.C. (MABP)	N.C.	Sugiyama et al. (2000)
	10 μg/kg			N.C. (MABP)	N.C.	
	100 μg/kg			TR ↓ (MABP)	TR ↓	
S1P intra-aortic (post-endothelin i.v. stimulation)	0.2 μmol	WKY rat	N.R.	TR ↓ (SBP)		Nofer et al. (2004)
HDL intra-aortic (post-endothelin i.v. stimulation)	1mg	WKY rat	N.R.	TR ↓ (MABP)		
S1P i.v. perfusion	0.05 μM	SD rat	Pentobarbital/pressure transducer	N.C. (SBP)		Ikeda et al. (2004)
	0.5 μM	Mouse		↑ (SBP)		
	5.0 μM			↑ (SBP)		
S1P i.v. bolus	0.2 mg/kg	SD rat	Nembutal/pressure transducer	TR ↓ followed by ↑ (MABP)	TR ↓	Forrest et al. (2004)
S1P i.v. bolus	0.1 mg/kg	Mouse	Conscious/pressure transducer	↑ (MABP)	TR ↓	
S1P i.v. bolus	38 μg/kg	Mouse	Isoflurane/tail cuff	N.C. (SBP)	N.C.	Levkau et al. (2004)
HDL i.v. bolus	2.0 mg/kg	Mouse	Isoflurane/tail cuff	N.C. (SBP)	N.C.	
S1P intra-carotid	1.9–380 μg/kg	SD rat	Pentobarbital/pressure transducer	N.C. (MABP)	N.C.	Lee et al. (2009)

N.C., no change; N.R., not reported; SBP, systolic blood pressure; SD, Sprague Dawley; TR, transient WKY, Wistar Kyoto.

Initially, it was suggested that S1P exerts selective constrictive actions on cerebral arteries; however, it is now clear that S1P constricts both peripheral (Salomone et al., 2010; Cantalupo et al., 2015) and cerebral arteries (Coussin et al., 2002; Salomone et al., 2003). However, sensitivity to S1P vasoconstriction is higher in cerebral arteries compared with peripheral arteries, most likely through variable expression levels of S1PR1-3 (Coussin et al., 2002; Salomone et al., 2003). Interestingly, gerbil spiral modiolar arteries, which control inner ear blood flow and protect the capillary bed from high perfusion pressure, are markedly sensitive to S1P-induced contraction compared with other types of vascular beds, responding to S1P concentrations as low as 0.3 nM (Scherer et al., 2006). This correlates well with a subsequent study showing that *S1pr2*$^{-/-}$ mice were completely deaf and display defects in the stria vascularis. S1P-mediated vasoconstriction of gerbil spiral modiolar arteries was reduced by JTE-013 (1-[1,3-dimethyl-4-(2-methylethyl)-1-*H*-pyrazolo[3,4-*b*]pyridine-6-yl]-4-(3,5-dichloro-4-pyridinyl)-semicarbazide), an inhibitor of S1PR2, suggesting a possible role of S1PR2-mediated vascular tone in the deafness reported in *S1pr2*$^{-/-}$ mice (Kono et al., 2007). However, JTE-013 interferes with KCl-, ET-1-, and U-46619-induced vasoconstriction in WT and *S1pr2*$^{-/-}$ mice (Salomone et al., 2008), strongly suggesting that JTE-013 can bind to multiple targets, and thus has poor selectivity; therefore, the assessment of S1P-induced vasoconstriction in *S1pr2*$^{-/-}$ gerbil spiral modiolar arteries will confirm or refute these observations.

Interestingly, studies using *S1pr2*$^{-/-}$ mice to assess the role of S1PR2 in vascular tone regulation have yielded controversial results. Lorenz et al. (2007) demonstrated that phenylephrine failed to raise mean arterial BP (MABP) in *S1pr2* mice, whereas Salomone et al. (2008) demonstrated that S1PR2 did not play a role in mediating S1P-induced vasoconstriction in basilar arteries. Moreover, in their study Lorenz et al. (2007) showed that the vascular responses elicited by KCl and phenylephrine in *S1pr2* aortic rings were also reduced, suggesting that locally produced S1P within the vessel wall may contribute to tonic S1PR2 activation in an autocrine manner to maintain vascular tone homeostasis. Alternative approaches, such as the use of antisense oligonucleotides, have also shown that S1PR2 mediates, at least in part, the S1P-induced vasoconstriction of hamster gracilis muscle resistance arteries (Peter et al., 2008). Additionally, S1PR2 mice are resistant to S1P-increased pulmonary vascular resistance of isolated perfused lungs (Szczepaniak et al., 2010). On the other hand, S1PR2 did not play any role in hypoxia-induced vasoconstriction in isolated and perfused lungs, despite the inhibition of hypoxia-induced pulmonary vasoconstriction by JTE-013 (Tabeling et al., 2015).

The work by Salomone et al. (2003) supports the role of S1PR3, but not S1PR2, in the S1P-mediated vasoconstriction of basilar arteries. These experiments were initially conducted using adenovirus-delivered S1PR2 and S1PR3 antisense oligonucleotides and by pharmacological S1PR3 inhibition using suramin. The same group subsequently confirmed the importance of S1PR3-mediated S1P vasoconstriction in *S1pr3*$^{-/-}$ cerebral arteries (Salomone et al., 2008). Importantly, the authors demonstrated also that S1P-induced vasoconstriction of cerebral arteries was enhanced in the absence of the endothelium as well as when the vessels were pretreated with VPC23019 (a nonselective S1PR1/3 antagonist), indicating that S1PR1 and S1PR3 in the endothelium are together accountable for the overall effect of S1P-mediated vascular tone regulation. The role of S1PR3 in mediating S1P-induced vasoconstriction was further corroborated using a S1PR3/4 antagonist TY-5215) (*N*-(4-chlorophenyl)-3,3-dimethyl-2-oxobutamidic 2-(4-chlorophenyl) hydrazide) in canine cerebral arteries ex vivo (Murakami et al., 2010).

Collectively, these studies suggest that S1PR3 plays a predominant role over S1PR2 in S1P-mediated vasoconstriction, at least in cerebral arteries from rats and mice, and that the S1PR1,3-signaling in the endothelium might counteract S1PR2,3-mediated vasoconstriction of the smooth muscle

layer of the vessels. However, the roles of S1PR2 and S1PR3 in vascular tone regulation during pathologic conditions remain unknown. The lack of specificity of JTE-013 for the S1PR2 (Salomone et al., 2008) suggests that experimental findings based on this inhibitor need to be carefully interpreted. Selective S1P receptor agonists and specific S1P receptor inhibitors would be very useful tools not only to aid our understanding of the roles of S1P receptors in pathophysiological conditions, but also to therapeutically modulate S1P signaling in disease states involving the alteration of this pathway.

S1P and the Myogenic Response. Bayliss (1902), was the first to observe that the myogenic response is an intrinsic property of vascular smooth muscle consisting of the contraction of blood vessels in response to stretch induced by increases in transmural pressure. This response is independent of neural or hormonal influences, is inversely correlated with vessel size (Davis, 1993), and ensures constant blood flow to the downstream capillary network during changes in systemic BP (Davis and Hill, 1999). How vascular cells convert mechanical stimuli into vasoconstriction is still unclear.

Recent studies suggest that locally produced S1P in the vascular wall contributes to the myogenic response (Bolz et al., 2003). SPHK1 overexpression in resistance arteries from hamster gracilis muscle increased resting and pressure-induced tone compared with GFP-transfected control arteries (Bolz et al., 2003). In line with this finding, a follow-up study from the same group demonstrated that S1P-phosphohydrolase degrading enzyme overexpression significantly reduced the myogenic responses of hamster gracilis muscle resistance arteries, whereas S1P-phosphohydrolase degrading enzyme knockdown enhanced myogenic tone in the same vessels (Peter et al., 2008), implicating a locally produced S1P in the myogenic response to pressure.

Pharmacological inhibition of SPHK1 also suggested a role of SPHK1 in the myogenic response (Lim et al., 2012). In the Lim et al. (2012) study, vessels were also incubated with exogenous S1P (1 μM), which as expected increased the basal tone at 20 mmHg, most likely through the activation of S1PR2,3, without affecting the magnitude of the myogenic response in response to pressure. This suggests that endogenous, and not exogenous, S1P modulates myogenic tone. However, a genetic approach validating these pharmacological findings is warranted, considering the poor specificity of these inhibitors.

An additional study by Hoefer et al. (2010) described an important role of S1PR2 in myogenic tone control through locally produced S1P. The authors showed that heart failure triggered an increase in myogenic response in mesenteric arteries from WT but not $Sphk1^{-/-}$, $Sphk1^{-/-}/Sphk2^{+/-}$, or $S1pr1^{-/-}$ mice, suggesting a clear role of S1P-S1PR2 autocrine signaling in the pathologic increase of myogenic tone and total peripheral resistance.

Recently, using SMC (smooth muscle cells)-Nogo-A/B-deficient mice, our group showed that upregulation of de novo sphingolipid (SL) biosynthesis within VSMCs led to a specific decrease in the myogenic response of mesenteric arteries and protected mice from the onset of hypertension (although to a lesser extent than in endothelial-specific Nogo-A/B-deficient mice). C18- and C22-ceramides increased in VSMCs in culture in the absence of Nogo-B, while S1P levels remained the same. This is not surprising since S1P is rapidly secreted from cells as it is being formed. These data suggest that SLs produced in the VSMCs exert a vasculoprotective function by resetting vascular tone and BP to a lower-than-normal value in physiologic and pathologic states (Cantalupo et al., 2015). Notably, pressure also increased S1P production in rabbit posterior cerebral arteries (Lim et al., 2012), suggesting the involvement of locally produced S1P in mechano-transduction signaling.

Overall, de novo sphingolipid production and downstream S1P-S1PR2 signaling appear to play an important role in cardiovascular (CV) homeostasis. These studies present new questions. For example, what is the relative contribution of different sphingolipid species (e.g., ceramides, sphingosine, and S1P) to vascular homeostasis and how is SL production regulated in pathologic conditions such as hypertension? While Spijkers et al. (2011b) showed that ceramide but not S1P is increased in the plasma of spontaneously hypertensive rats (SHR) and humans, and it seems to play a role in the pathogenesis of hypertension, the specific molecular mechanisms of ceramide, S1P, and other SLs in ECs versus VSMCs in vascular tone regulation are poorly understood. Additionally, it would be fruitful to determine whether the effects of SLs on vascular tone are associated with the magnitude of de novo biosynthesis (and thus sphingolipid levels) as well as to determine whether S1P-S1PR2 autocrine signaling changes in hypertension, and if so, what its precise role is in the process. Finally, the relative contributions of S1P synthesizing/degrading enzymes in pathophysiological conditions also deserve further consideration.

S1P: New Player in BP Regulation?

Based on the major vascular effects of S1P signaling, recent research has focused on the potential role of S1P signaling in BP regulation (Table 3). Here, we review preclinical and clinical studies implicating S1P signaling as an emerging novel pathway in BP regulation and hypertension.

Administration of S1P and S1P Receptor Agonists In Vivo. Although many currently available pharmacological S1P receptor agonists and antagonists serve as great tools to decipher the role of S1P receptors in different biologic systems (Blaho and Hla, 2014), very few studies using in vivo models have investigated the role of S1PR1, S1PR2, or S1PR3 signaling in BP regulation. An early study showed that S1P (0.1 mg/Kg, i.v.) induced a rapid and transient decrease in heart rate (HR) and MABP in rats (Sugiyama et al., 2000). However, these findings were disputed by others (Bischoff et al., 2000b; Levkau et al., 2004). In a subsequent study, Nofer et al. (2004b) confirmed that S1P induced transient hypotension in mice pretreated with ET-1 to raise BP. They additionally demonstrated that S1P bound to HDL is responsible, at least in part, for a similar transient decrease in MABP following intra-arterial administration of HDL in rats. However, it is unclear whether the observed hypotension was due exclusively to an effect on the vasculature or whether it was secondary to transient bradycardia. The authors also showed that HDL and S1P fail to induce vasodilation in arteries isolated from $S1pr3^{-/-}$ mice, although no effect associated with S1PR3 in HDL- or S1P-induced hypotension in vivo was reported. Forrest et al. (2004) additionally demonstrated in rats a transient decrease in HR and MABP in response to a single dose of S1P, while continuous infusion

of S1P analog, a derivate from FTY720, was predominantly associated with hypertension. Interestingly, in *S1pr3*$^{-/-}$ mice, acute administration of S1P did not affect MABP and HR, confirming the effect of S1PR3 in hemodynamic changes induced by S1P.

Considering that S1P induces only a moderate and transient decrease in BP in vivo, different S1P doses and pretreatment with endothelin to increase BP may explain controversial findings. Furthermore, the net effect of S1P on BP is the result of changes in both vascular and heart functions. Both S1PR1 and S1PR3 are expressed in the heart (Mazurais et al., 2002). Some studies suggested that S1PR3, and not S1PR1, mediates transient bradycardia in response to S1P (Forrest et al., 2004) and nonselective S1PR agonists (Forrest et al., 2004; Sanna et al., 2004); however, recent studies demonstrated that S1PR1 can also contribute to the bradycardia upon treatment with FTY720 (Murakami et al., 2010), with BAF312, a S1PR1,5 selective agonist (Fryer et al., 2012), and other S1PR1 agonists (Hamada et al., 2010; Bolli et al., 2013).

The lack of S1PR2 in vivo does not alter systemic MABP in physiologic conditions (Lorenz et al., 2007; Olivera et al., 2010). S1PR2 is upregulated in the bile duct following ligation in mice and appears to regulate hepatic blood flow and portal pressure in pathologic conditions in both mice and rats (Kageyama et al., 2012), as well as portal pressure in isolated rat liver (Ikeda et al., 2004; Kageyama et al., 2012). However, the specific role of S1PR2 in hypertension and in other hypertensive-based diseases remains to be elucidated.

Recently, Obinata et al. (2014) identified several single nucleotide polymorphisms in the S1PR1 gene that influence receptor function. One single nucleotide polymorphism in S1PR1 correlated with a differential coronary artery disease risk, suggesting that genetic variations of S1PR1 may be involved in the pathogenesis of CV diseases.

Recently, Cantalupo et al. (2015) showed that the S1PR1-signaling pathway exerts important antihypertensive functions. The S1PR1 agonist, SEW2871 (5-[4-phenyl-5-(trifluoromethyl)-2-thienyl]-3-[3-(trifluoromethyl)phenyl]-1,2,4-oxadiazole) (3mg/Kg, i.p.), induced a marked decrease in systolic BP in hypertensive mice (~25 mmHg) but did not affect HR. Notably, SEW2871 did not have any effect in Nogo-A/B-deficient mice, resistant to hypertension (Cantalupo et al., 2015), and in normotensive mice (unpublished data). In the absence of Nogo-B, endothelial-derived S1P and the autocrine S1P-S1PR1-eNOS signaling are upregulated and the mice are protected from angiotensin II (AngII)–induced hypertension. These findings suggest that during the onset of hypertension, the endogenous S1P/S1PR1 signaling axis could be impaired, while remaining responsive to the exogenous modulation of this pathway.

Although the role of S1P receptors in BP regulation in vivo is still unclear, transient hypotension induced by S1P has been consistently observed by several groups. Notably, the hypotensive effects of S1P signaling are more pronounced in animals with acute or chronic hypertension (Nofer et al., 2004; Cantalupo et al., 2015), suggesting that S1P signaling may represent a potential therapeutic target for treating hypertension.

Role of Endothelial-Derived and Circulating S1P in BP Regulation. In addition to red blood cells, ECs represent an important source of plasma S1P (Pappu et al., 2007; Venkataraman et al., 2008; Fukuhara et al., 2012; Xiong et al., 2014). Levels of circulating S1P, bound to ApoM of the HDL fraction (~65%) and albumin (~35%), are relatively high (0.1–1 μM) compared with the potency of S1P on S1PR1 (Cyster and Schwab, 2012). However, it is still unclear how endothelial S1P receptors remain responsive to S1P despite the high concentrations of plasma S1P. Whether and how plasma chaperones control the availability and/or the binding of S1P to its cognate receptors remains unknown.

Studies from our laboratory revealed a novel important role of endothelial-derived S1P in blood flow and pressure homeostasis. In mice lacking Nogo-B, endothelial S1P-S1PR1 autocrine signaling is upregulated and exerts an antihypertensive function through the eNOS-NO pathway. Interestingly, a single dose of the SPT inhibitor myriocin reinstated AngII-induced hypertension in Nogo-A/B-deficient mice, suggesting that locally produced SLs within the vasculature are key regulators of vascular tone and BP (Cantalupo et al., 2015). On the other hand, increasing levels in circulating and local SLs, specifically ceramides and S1P, have recently been correlated with hypertension in both animal models and humans (Spijkers et al., 2011a,b). Still, specific molecular mechanisms are poorly understood. It is reasonable to hypothesize that while the upregulation of this pathway within a physiologic range (such as in the absence of Nogo-B) protects from hypertension, exaggerated levels of SLs may be harmful.

A double knockout of Sphk1 and Sphk2, generating S1P, is lethal, while mice knocked out for either Sphk1 or 2 are viable (Mizugishi et al., 2005). One caveat to be considered is that while circulating levels of S1P are reduced in *Sphk2*$^{-/-}$/*Sphk1*$^{+/-}$ mice (Pappu et al., 2007; Camerer et al., 2009) and in *Sphk1*$^{-/-}$ mice (Allende et al., 2004), circulating levels of S1P are actually increased in *Sphk2*$^{-/-}$ mice (Olivera et al., 2007; Kharel et al., 2012), possibly due to overcompensation by SPHK1 (Olivera et al., 2007).

In physiologic conditions, MABP was not altered by the loss of Sphk1$^{-/-}$ or Sphk2$^{-/-}$ (Furuya et al., 2013; Gorshkova et al., 2013), or the overexpression of SPHK1 (Takuwa et al., 2010). In a model of histamine-induced hypotension, Olivera et al. (2010) showed that *Sphk1*$^{-/-}$ mice, but not *Sphk2*$^{-/-}$ mice, were significantly more hypotensive than WT mice. Interestingly, S1P administration reverted the hypotension induced by histamine in *Sphk1*$^{-/-}$ mice, suggesting SPHK1 as a positive regulator of MABP. In line with this evidence, the lack of SPHK1 attenuated the elevation of BP in response to the acute (Furuya et al., 2013; Wilson et al., 2015) and chronic (Wilson et al., 2015) administration of AngII. A positive role of SPHK1 in BP homeostasis has also been shown in an animal model of potassium-induced cardiac arrest with resuscitation (Gorshkova et al., 2013). Interestingly, Furuya et al. (2013) also demonstrated that the absence of *Sphk2* prolonged the acute hypertension induced by AngII, suggesting that SPHK2 protects from high BP.

Collectively, these findings suggest that SPHK1 exerts positive effects and SPHK2 exerts negative effects on BP. Considering the ubiquitous expression of SPHK1, whether SPHK1-mediated effects on BP are due to circulating or local S1P levels, and the cell-type/tissues involved in this regulation remain unknown. Further studies deleting *Sphk1* and *Sphk2* in specific cell types (e.g., ECs or VSMCs) will provide insights into the respective roles of these enzymes in BP regulation. Measurements of circulating and local (within the vessel wall) S1P levels in normotensive and hypertensive states are also warranted to clarify the role of S1P in hypertension.

Effects of FTY720 on Systemic BP. Recently, the U.S. Food and Drug Administration approved FTY720 for the oral treatment of relapsing multiple sclerosis (Brinkmann et al., 2002; Kappos et al., 2006; Foster et al., 2007). Preclinical studies on the acute effects of FTY720 on MABP and HR are more controversial and depend on the doses, routes of administration, and pathophysiological state (i.e., normotensive versus hypertensive). An initial study on normotensive rats demonstrated that FTY720 induced a transient increase in MABP at 1 mg/Kg (i.v.) and a decrease in MABP at supratherapeutic doses of 5.0 mg/Kg (i.v.) (Tawadrous et al., 2002). A subsequent study by Fryer et al. (2012) elegantly showed that the infusion of FTY720 in anesthetized normotensive rats (0.1, 0.3, and 0.5 mg/Kg) elicited a dose-dependent decrease in HR and MABP; on the contrary, 24 hours following the oral administration of FTY720, MABP increased only at high doses (3 and 10 mg/Kg), while HR decreased at 10 mg/Kg, suggesting that the acute effects of FTY720 on BP are also dependent on the route of administration. Of note, a lower dose of FTY720 (0.3 mg/kg; p.o., 24 hours) induced robust elevation in MABP in spontaneously hypertensive rats (Spijkers et al., 2012).

Studies on the long-term effects of FTY720 treatment on BP provided more consistent findings. Orally administered FTY720 elicited a dose-dependent increase in BP in rats (Tawadrous et al., 2002; Fryer et al., 2012) at doses below those necessary to induce bradycardia (Fryer et al., 2012) and consistent with the rise in BP observed clinically (Cohen et al., 2010). It is possible that the acute decrease in MABP is due to direct activation of the S1PR1-eNOS pathway and/or bradycardia, while the chronic increase in MABP is due to functional antagonism of the S1PR1 pathway, thereby favoring the vasoconstriction of VSMCs. It is also possible that FTY720 could act on the kidneys, thus influencing BP, although 7-day FTY720 treatment at supratherapeutic doses did not alter renal functions and structure (Tawadrous et al., 2002). Corroborating these results, clinical studies assessing the therapeutic effects of FTY720 in healthy volunteers and multiple sclerosis patients reported transient bradycardia within 6 hours after the first dose of FTY720, and delay in atrioventricular conduction (Kappos et al., 2006, 2010; Schmouder et al., 2006; Cohen et al., 2010; Calabresi et al., 2014; Gold et al., 2014). These acute effects were paralleled by a small and transient decrease in systolic and diastolic BP (Kovarik et al., 2008; DiMarco et al., 2014).

Notably, multiple studies have reported a slight increase in BP (≈2–5 mmHg) following chronic treatment with FTY720 (Kappos et al., 2006, 2010; Comi et al., 2010). A 2.3% increase in BP was demonstrated in a recent postmarketing study of a cohort of 212 patients receiving 0.5 mg/d FTY720 (Paolicelli et al., 2015). A case of peripheral vascular adverse effects associated with FTY720 treatment in a multiple sclerosis patient was recently reported (Russo et al., 2015). Altogether, these studies strongly emphasize the importance of S1P signaling in the maintenance of CV homeostasis, and suggest that further studies are needed to elucidate the effects of FTY720 on BP regulation at different doses and treatment durations, and under different pathologic conditions, especially within the context of hypertension where S1PR1 signaling was shown to exert protective functions on the vasculature (Cantalupo et al., 2015).

Nogo-B/SPT: Novel Regulatory Pathway of Endothelial-Derived S1P to Control BP

SPT catalyzes the first rate-limiting step in the pathway to de novo sphingolipid synthesis in the ER. Two major subunits required for SPT activity are Sptlc1 and Sptlc2. A third subunit, Sptlc3, has also been identified (Hornemann et al., 2009); however, the stoichiometry of the complex is still controversial. Knockout of either Sptlc1 or Sptlc2 is embryonically lethal in mice (Hojjati et al., 2005a), suggesting that endogenously synthetized SLs have an important role during development.

The regulation of SPT is an active area of investigation. Breslow et al. (2010) identified ormdl proteins, involved in childhood asthma, as regulators of the SL biosynthesis by interacting and inhibiting SPT. The role and the regulation of SPT in vivo are poorly understood, particularly within the context of CV diseases. Recently, we identified Nogo-B as a negative regulator of SPT within blood vessels. Mechanistically, the absence of Nogo-B in ECs increased SL content, particularly S1P, which regulates vascular tone and blood flow through the S1P-S1PR1-eNOS signaling axis. It is noteworthy that pharmacological inhibition of SL biosynthesis with myriocin increased BP in AngII-treated mice lacking Nogo-B, suggesting a protective role of de novo SL biosynthesis in the pathogenesis of hypertension (Cantalupo et al., 2015).

Notably, while the upregulation of SPT activity in VSMCs decreased myogenic tone (most likely through ceramides), the upregulation of SPT activity in ECs enhanced the vasodilation in response to flow through the autocrine S1P-S1PR1-eNOS signaling axis. Interestingly, pharmacological inhibition of SPT or S1PR1 with myriocin and W146, respectively, reduced flow-mediated vasodilation in the absence of Nogo-B to WT levels (Cantalupo et al., 2015). Altogether, these results demonstrated that de novo SL biosynthesis within the vascular wall and regulation of this synthesis by Nogo-B play a key role in BP regulation in physiologic and pathologic conditions, mainly through the endothelial S1P/S1PR1/eNOS autocrine loop.

Given these results, the S1P/S1PR1/eNOS signaling pathway may represent a new therapeutic target for the treatment of hypertension. Although our data suggest that endothelial-derived S1P is accountable for the Nogo-B/SPT regulation of vascular tone and BP, further studies are needed to dissect the role of S1P from that of ceramides, and perhaps the biologic function of specific ceramide species in vascular tone regulation. Due the variety of downstream products, the effects of SPT regulation in different pathophysiological conditions are very complex, and they are only just beginning to be understood.

It has been shown that in other pathologic conditions, such as atherosclerosis, SPT has a deleterious role (Jiang and Liu, 2013). Inhibition of SPT with myriocin decreased atherosclerotic lesions, plasma and aortic ceramide levels, and sphinganine and sphingomyelin in $ApoE^{-/-}$ mice challenged with a high fat diet (Park et al., 2004, 2008). S1P and phosphatidylcholine plasma levels also decreased following myriocin treatment, with no change in cholesterol levels (Hojjati et al., 2005b), suggesting a pro-atherogenic role of SPT apart from cholesterol.

The accumulation of ceramides in the vasculature has been correlated with endothelial dysfunction in obesity. Zhang et al.

(2012) showed that mice treated with myriocin and challenged with a high fat diet or genetically deficient for dihydroceramide desaturase had decreased levels of ceramides in the aorta and liver and experienced increased body weight and triglyceride levels. Interestingly, myriocin improved vascular function and endothelial-derived NO. Mechanistically, ceramide promotes the protein phosphatase 2A/eNOS interaction, leading to impairment of NO production; therefore, it causes endothelial dysfunction, suggesting that the accumulation of SLs within the vascular wall is ultimately deleterious for normal endothelial function.

Therefore, two main conclusions can be stated. First, pathologic conditions may affect the activity of SPT in vascular cells, thereby modifying their endogenous biosynthesized SLs. Second, modulation of SPT activity can also affect vascular tone and BP in mice.

Conclusions

In this review, we have attempted to summarize recent advances in the role of SLs, particularly S1P in vascular tone and BP regulation (Fig. 3). Although there have been significant advances in our knowledge on the role of S1PRs in vascular tone regulation in physiologic conditions, much information is still lacking. What is the role of S1PRs in BP regulation in vivo, particularly in the pathogenesis of hypertension? This is an important question, especially since FTY720 (which targets S1P receptors) has been approved for the treatment of multiple sclerosis. S1PR1 is activated by changes in flow (Jung et al., 2012; Cantalupo et al., 2015), most likely by the increased production of endothelial S1P triggered by the flow (Venkataraman et al., 2008). What are the molecular mechanisms involved in the activation of this mechanotransduction autocrine loop, S1P-S1PR1, and what is its relevance during hypertension? What is the biologic function of local versus circulating S1P in BP homeostasis? Furthermore, the finding that Nogo-B controls local production of SLs to impact vascular tone and BP raises other questions. How do circulating and local (vascular wall) SL levels change in hypertension? What regulates the inhibitory action of Nogo-B on SPT in hypertension and how? These and other questions will most likely be the focus of further studies on the role of SL in the pathophysiology of the CV system.

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Wrote or contributed to the writing of the manuscript: Cantalupo, Di Lorenzo.

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