Vascular dysfunction in S1P2 sphingosine 1-phosphate receptor knockout mice

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Lorenz JN, Arend LJ, Robitz R, Paul RJ, MacLennan AJ. Vascular dysfunction in S1P2 sphingosine 1-phosphate receptor knockout mice. Am J Physiol Regul Integr Comp Physiol 292: R440–R446, 2007. First published September 21, 2006; doi:10.1152/ajpregu.00085.2006.—There is growing evidence that sphingosine 1-phosphate (S1P) plays an important role in regulating the development, morphology, and function of the cardiovascular system. There is little data, however, regarding the relative contribution of endogenous S1P and its cognate receptors (referred to as S1P1–5) to cardiovascular homeostasis. We used S1P2 receptor knockout mice (S1P2−/−) to evaluate the role of S1P2 in heart and vascular function. There were no significant differences in blood pressure between wild-type and S1P2−/− mice, measured awake. Cardiac function, evaluated in situ by using a Millar catheter, was also not different in S1P2−/− mice under baseline or stimulated conditions. In vivo analysis of vascular function by flowmetry revealed decreases in mesenteric and renal resistance in S1P2−/− mice, whereas the maximal isometric forces were not different. By contrast, in deno- thelialized rings the concentration-force relations were not different but the maximal force was significantly greater in S1P2−/− aorta. Histologically, there were no apparent differences in vascular morphology. These data suggest that the S1P1 receptor plays an important role in the function of the vasculature and is an important mediator of normal hemodynamics. This is mediated, at least in part, through an effect on the endothelium, but direct effects on vascular smooth muscle cannot be ruled out and require further investigation.

lyosphingolipids; endothelium; vascular smooth muscle; blood flow; myocardial contractility

IN VITRO RESEARCH INDICATES that the lyosphingolipid sphingosine 1-phosphate (S1P) can regulate the proliferation, morphology, migration, and survival of a wide variety of cell types, and most, if not all of these effects result from activation of one or more high-affinity G protein-coupled S1P receptors, referred to as S1P1–S1P5 (6, 10, 23, 28). S1P is synthesized from membrane phospholipids as part of the sphingomyelin metabolic cycle, which can be influenced acutely and chronically by a variety of physiological and pathophysiological stimuli (34). Although S1P circulates in the blood in high nanomolar concentrations (derived primarily from platelets), it is also produced avidly at the local level and therefore has the potential to strongly influence organ and tissue function in an autocrine and/or paracrine manner (1). A rapidly growing literature suggests that S1P signaling mediates several aspects of cardiovascular function. In vitro studies indicate that S1P administration can contract a variety of cultured and freshly isolated smooth muscle cell types (2, 5, 7, 12, 13, 25, 29, 33, 35).

Likewise, intravenous administration of S1P in vivo induces cerebral artery constriction and decreases cerebral, renal, and mesenteric blood flow (3, 29, 35). In contrast, it has been reported that S1P can dilate mesenteric arteries and aorta in vitro (8, 24) and that these effects appear to be dependent upon endothelial nitric oxide (NO) production. In the intact animal, it has been reported that infusion of S1P in vivo can increase, decrease, or have no effect on blood pressure (3, 9, 24). Finally, the reported effects of S1P on cardiac function include tachycardia in vitro (33), bradycardia or no effect in vivo (3, 9, 33), and in vivo decrease in ventricular contraction (33). Although there is little available data regarding the potential effects of endogenous S1P, these observations suggest that S1P, either circulating or local, may play a direct role in regulating cardiovascular function or, alternatively, that it may modify the cardiovascular responses to other stimuli.

Studies of specific S1P receptors in this field are relatively limited. The S1P-induced constriction of isolated cerebral arteries was found to be inhibited by an adenovirus containing a S1P3 antisense construct, thereby suggesting a S1P3 role in vasoconstriction (29). In contrast, targeted disruption of the S1P1 gene eliminated the S1P-induced dilation of preconstricted aorta in vitro (24). In a separate study, S1P3 gene disruption decreased the impact of S1P administration on heart rate and blood pressure (9). A role for S1P2 receptors in vasoconstriction is supported by work with the S1P2-specific antagonist JTE-013, which was found to decrease S1P-mediated contraction of both cultured coronary smooth muscle cells (25, 26) and isolated portal vessels (14). On the other hand, an adenovirus antisense S1P2 construct was without effect on the cerebral artery preparation noted above that responds to an antisense S1P3 construct (29). These data, although contradictory, raise the possibility that S1P receptors may serve as pharmacologically accessible targets for cardiovascular therapeutics. Clearly, a prerequisite to such therapeutic development is a better understanding of what physiological roles are played by the individual S1P receptor subtypes, and indeed, whether they actively participate in the maintenance of cardiovascular homeostasis. We therefore carried out the present series of experiments using S1P2 receptor knockout mice to evaluate the contribution of this receptor subtype to normal cardiovascular function using both in vivo and in vitro approaches. We report here that targeted disruption of the S1P2 gene leads to decreased vascular tone and blunted responsiveness to vasoconstrictor agents. These data describe an important in vivo role for the S1P2 receptor in the maintenance of normal cardiovascular function and will provide the necessary basis for future studies.

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After 30 min of recovery, dose-dependent responses to phenylephrine (PE: 10, 40, and 80 ng·min⁻¹·g body wt⁻¹ infusion) and sodium nitroprusside (SNP; 10 and 33 ng·min⁻¹·g body wt⁻¹ infusion) were tested. Individual doses were infused until a plateau was achieved (1–2 min) with a 5- to 10-min recovery period between doses. Data were recorded and analyzed using PowerLab hardware and software.

Vascular smooth muscle reactivity. Analyses of contractile properties of vascular smooth muscle were performed in both intact and endothelium-denuded thoracic aortas as previously described (19). Briefly, aortas were dissected and in some of them, the endothelia was removed mechanically by gently rolling between thumb and index finger. After the experiment, the aorta was gently blotted and weighed, and its dimensions were measured. Aortic wall thickness (tw) was estimated from the equation tw = blot weight/(1.05 × length × circumference), and the cross-sectional area (CSA) for force normalization was calculated as CSA = 2 (t × length). The bath solution contained (in mmol/l) 118 NaCl, 4.73 KCl, 1.2 MgCl₂, 0.026 EDTA, 1.2 KH₂PO₄, 2.5 CaCl₂, and 5.5 glucose, buffered with 25 NaHCO₃; pH when bubbled with 95%O₂/5% CO₂ was 7.4 at 37°C. For force measurement, rings were mounted on 100-μm stainless steel wires, and attached to a Harvard Apparatus differential capacitor force transducer (South Natick, MA). Resting tension on each aorta was set to 25 mN, the estimated in vivo tension calculated for a pressure of 100 mmHg and a CSA of 0.42 mm². Data were collected and analyzed with a BioPac MP100 and AcqKnowledge software (BioPac Systems, Goleta CA). Before the start of the experiment, each aortic segment was challenged once with 50 mM KCl and three times with 1 μM PE to ensure reproducible forces. Cumulative KCl- and PE-concentration-isometric force relationships were generated for each intact and endothelium-denuded aorta. EC₅₀ and maximal force of contraction was determined by using a logistic nonlinear curve-fitting routine (OriginLab, Northampton MA).

Histological and morphological evaluation. S1P2⁺/⁺ and S1P2⁻/⁻ mice were anesthetized with pentobarbital sodium (70 μg/g body wt) and prepared for whole body fixation by inserting catheters into the thoracic aorta via the left carotid artery for infusion and into the abdominal aorta below the kidneys via the femoral artery for measurement of arterial pressure. Mice were then given a priming dose of 2.5 μg/g body wt papaverine to maximally dilate vascular smooth muscle. The vena cava was cut 2–4 min later, and whole body perfusion through the carotid catheter was initiated with 20 ml PBS containing 75 μg/ml papaverine, followed by 20 ml of 10% buffered formalin. Input pressure was carefully adjusted to produce a perfusion pressure in the aorta of 40–50 mmHg as measured via the femoral artery, which is equivalent to the pressure achieved during initial perfusion of papaverine. The kidneys were bisected and postfixed in 10% buffered formalin. Mesenteric vascular arcades containing first-, second-, and third-order branches of the mesenteric artery and a small section of intestine were carefully dissected and postfixed in 10% buffered formalin. Following dehydration through graded alcohols and xylene, tissues were embedded in paraffin, sectioned at 3 μm onto glass slides, and stained with hematoxylin and cosin. Serial sections were examined by light microscopy under ×400 magnification. For mesenteric sections, second-order arteries near the mesenteric-intestinal junction were evaluated for wall thickness and composition, and for renal cortical sections, afferent arterioles adjacent to glomeruli were evaluated. Electronic images were captured on a Spot Insight digital camera (Diagnostic Instruments, Sterling Heights, MI), and wall thickness was measured along the short axis of each vessel using Image-Pro (Media Cybernetics, Silver Spring, MD). The observer was blinded as to genotype.

Statistics. Statistical analysis was performed by ANOVA, using a single factor, two-factor, or mixed factorial design with repeated measures as appropriate. Where necessary, individual contrasts were used to compare individual group means. For PE concentration-response relationships, data analysis was performed on log data. Data

MATERIALS AND METHODS

Animals. Mice lacking the S1P2 receptor, as described by MacLennan et al. (22) were obtained from an established colony at the University of Cincinnati College of Medicine. Wild-type (S1P2⁺/⁺) and knockout (S1P2⁻/⁻) mice were generated from heterozygote (S1P2⁺/⁻ × S1P2⁻/⁻) crosses. Genotyping was performed by Southern blot analysis of tail biopsy DNA as previously described (22), or through two rounds of genomic PCR employing nested primers: 5'-CTGTCACAGCTCTGC-3', and 5'-GACACCCCCCCTGCTG-3' followed by 5'-GCAATGCATATGCTCATAC-3', and 5'-AGCAGTCTTGTAGATCATGTTG-3' to detect the intact allele and 5'-GTGGGGGAGCCTATAC-3', and 5'-GACACCCCCCCTGCTG-3' followed by 5'-GCAATGCATATGCTCATAC-3', and 5'-AGCAGTCTTGTAGATCATGTTG-3' to detect the mutated allele. Prior to experiments, mice were maintained on normal rodent chow and water ad libitum, and on a 12:12-h light-dark cycle (7:00 AM to 7:00 PM). We previously reported that mice on 14 g/g body wt ip) and 10, 40, and 80 ng·min⁻¹·g body wt⁻¹ infusion) were measured in S1P2⁺/⁺ and S1P2⁻/⁻ mice, aged 4–6 mo, on 14 consecutive days using a Visitech Systems computerized tail cuff apparatus (model BP-2000; Apex, NC) as described previously (20). Each day, 10 preliminary cuff-inflation cycles were performed to acclimate the mice to the operation of the apparatus, followed by 10 measurement cycles. Pulse tracings were carefully monitored and reviewed, and any recordings showing evidence of end point detection artifacts were removed from the analysis, and a minimum of five (out of 10) valid measurements were acquired for each mouse on each day. Left ventricular function. S1P2⁺/⁺ and S1P2⁻/⁻ mice (n = 5 per group), aged 22–24 mo, were anesthetized with ketamine (50 μg/g body wt ip) and thiothreabat (Inactin; 100 μg/g body wt ip) and instrumented as previously described (21). Following tracheostomy and cannulation of the femoral artery and vein with polyethylene catheters, a high-fidelity micromanometer-tipped catheter (model SPR-671; Millar Instruments, Houston TX) was inserted into the left ventricle (LV) via the right carotid artery. Signals for LV pressure and its first derivative (dP/dt) were recorded at 1,000 Hz and analyzed by using a PowerLab system (AD Instruments, Colorado Springs, CO). Pressure waveforms were also recorded from the femoral artery catheter. Hemodynamic measurements were taken at the baseline state and after the administration of graded doses of dobutamine (0.01, 0.02, 0.04, 0.08, 0.16, and 0.32 ng·min⁻¹·g body wt⁻¹) to evaluate contractile reserve and β-adrenergic responsiveness. Each dose was administered at an infusion rate of 0.1 μl·min⁻¹·g body wt⁻¹ for 3 min, and measurements were taken from the final 30 s of each dosage period. Animals were allowed to recover to baseline for 5–10 min between doses. Maximum dP/dt (dP/dtmax) and dP/dt at 40 mmHg of developed pressure (dP/dt0.4) were calculated from the first derivative of the LV pressure waveforms.

Regional blood flow and vascular resistance. S1P2⁺/⁺ (n = 8) and S1P2⁻/⁻ mice (n = 6) aged 4–6 mo were anesthetized and prepared for measurement of femoral blood pressure as described above. Animals were mechanically ventilated (120 breaths/min, 200 tidal volume) to prevent hypoxia during subsequent procedures and were provided a maintenance infusion of 2.5% BSA in PBS at 0.1 μl·min⁻¹·g body wt⁻¹ to maintain euoxygen. The left renal and mesenteric arteries were exposed through a flank incision, and perivascular flow probes were positioned around each artery (models 0.5 PSB and 0.5 V, respectively; Transonic Systems, Ithaca NY).
are presented as means ± SE, and statistical significance was regarded as \( P < 0.05 \).

**RESULTS**

Baseline conscious systolic blood pressure, measured by tail cuff plethysmography, was not different between S1P\(_2^{+/+}\) and S1P\(_2^{-/-}\) mice: \( 113 \pm 5 \) vs. \( 117 \pm 3 \) mmHg, respectively (means ± SE). Likewise, in anesthetized mice prepared for in vivo measurement of LV pressure development, control mean arterial blood pressure (femoral) and LV systolic pressure were also not different between S1P\(_2^{+/+}\) and S1P\(_2^{-/-}\) mice (Fig. 1, A and B). However, in response to increasing doses of the \( \beta \)-adrenergic receptor agonist dobutamine, LV systolic pressure increased in S1P\(_2^{+/+}\) mice, but did not change in S1P\(_2^{-/-}\) mice, such that at the higher dose range, LV systolic pressure was significantly lower in the S1P\(_2\)-deficient mice (Fig. 1B). Importantly, cardiac contractile function, as measured by either \( \mathrm{dP}/d\tau\max \) or \( \mathrm{dP}/d\tau_{40} \) (an index of contractility that attempts to correct for differences in afterload), was not different between the two genotypes over the entire dose range of dobutamine (Fig. 1, C and D). There were also no differences observed in parameters of myocardial relaxation, such as \( \mathrm{dP}/d\tau_{\min} \) and tau (the time-constant of pressure decline), nor in left ventricular end-diastolic pressure (not shown). These data suggest that differences in blood pressure response between wild-type and S1P\(_2\)-deficient mice are primarily related to differences in vascular responsiveness, rather than to inherent differences in cardiac contractile performance. Finally, heart rate was not different between the genotypes throughout the dose-response range (Fig. 1E). It is noteworthy that these experiments were carried out in aged mice (20–24 mo) to allow for the possibility that any cardiac deficiencies resulting from S1P\(_2\) deficiency might develop with advancing age. This appears to not be the case.

Since a vascular phenotype was suggested by these experiments, we performed a series of studies to evaluate regional blood flow in S1P\(_2^{+/+}\) and S1P\(_2^{-/-}\) mice. Mean arterial pressure and blood flow to the renal and mesenteric vascular beds was determined by transit-time flowmetry in anesthetized mice, and results are presented in Fig. 2. As before, arterial pressure was not different between the genotypes under baseline conditions. However, the increases in pressure in response to graded doses of PE were significantly less in S1P\(_2^{-/-}\) mice compared with S1P\(_2^{+/+}\). The blood pressure responses to the NO donor SNP were not different. In contrast to pressure measurements, measurements from both renal and mesenteric artery demonstrated that blood flow was significantly elevated in S1P\(_2^{-/-}\) mice under basal conditions, and during vasoconstriction with PE or vasodilation with SNP. Analysis of regional vascular resistance (Fig. 3; calculated as mean arterial pressure/regional blood flow) showed that baseline renal vascular resistance was indeed reduced, and mesenteric vascular resistance tended to be lower (although not significantly) in the S1P\(_2^{-/-}\) mice compared with S1P\(_2^{+/+}\). In addition, the vasoconstrictor responses to PE infusion in both vascular beds were dramatically blunted in the S1P\(_2^{-/-}\) mice. The vascular re-

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**Fig. 1.** Mean arterial blood pressure (MAP) and left ventricular (LV) performance in anesthetized, closed-chest wild-type (S1P\(_2^{+/+}\)) and knockout mice (S1P\(_2^{-/-}\)) mice under baseline conditions and increasing doses of the \( \beta \)-adrenergic agonist dobutamine. \( \mathrm{dP}/d\tau_{\max} \): maximum LV pressure and change in pressure over time. \( \mathrm{dP}/d\tau_{40} \): LV \( \mathrm{dP}/d\tau \) at 40 mmHg of developed pressure. Values are means ± SE. *\( P < 0.05 \) compared with corresponding value in the S1P\(_2^{+/+}\) group.
responses to SNP appear somewhat puzzling at first glance. Based on calculated resistance measurements, renal vascular resistance increased in both groups of mice at higher doses of SNP. On the other hand, blood pressure and mesenteric blood flow measurements indicate a generalized vasodilation in response to administration of NO. Thus, rather than suggesting that SNP caused a paradoxical increase in renal vascular resistance, we consider it more likely that the systemic vasodilation and subsequent profound decrease in blood pressure resulted in a decrease in renal perfusion pressure below the critical closing pressure in intrarenal vascular beds, and therefore a disproportionate fall in renal blood flow. In any case, these data demonstrate that S1P2-deficient mice have reduced levels of resting vascular tone, and impaired vasoconstrictor responses to α-adrenergic stimulation.

To further explore functional responses of vascular smooth muscle, we performed muscle bath experiments in aortic rings isolated from S1P2+/+ and S1P2−/− mice. In intact vessels, the concentration-response relationships for both KCl and PE were significantly blunted (increased EC50) in S1P2−/− compared with S1P2+/+ vessels (Fig. 4 and Table 1). Maximal force generation in intact vessels was largely comparable between the two genotypes, but maximal force generation by both KCl and PE was markedly elevated in the S1P2−/− aortas. Although the data in Fig. 4 and Table 1 are normalized to CSA of the rings, it should be noted that the thickness of the aortic rings was not different between S1P2+/+ and S1P2−/− (77 ± 3 vs. 71 ± 2 μm). Relaxation responses to SNP following preconstriction with PE were not different between the two genotypes (data not shown).

We also evaluated whether the differences in vascular function observed in the S1P2−/− mice were associated with gross changes in microvascular morphology. The composition of the medial layer of second-order mesenteric arteries and renal afferent arterioles did not appear different between the S1P2−/− and S1P2+/+ mice, and no differences in wall thickness could be detected. Second-order mesenteric arteries from S1P2−/− mice had a wall thickness of 3.6 ± 0.1 vs. 3.9 ± 0.1 μm for S1P2+/+ mice. Likewise, the wall thickness of renal afferent arterioles was 2.1 ± 0.1 vs. 2.0 ± 0.1 μm in S1P2−/− and S1P2+/+ mice, respectively. The intima and adventitia from both sets of mice were normal. Finally, inspection of the renal cortical parenchyma showed no remarkable differences in glomeruli, tubules, and interstitium between S1P2−/− and S1P2+/+ mice.
Since no differences were observed in cardiac contractile performance or in vascular morphology, these data clearly indicate that the S1P2 receptors play an important physiological role in modulating vascular tone in the adult mouse, particularly under stimulated conditions.

The data presented here provide critical evidence, previously lacking, that the activation of S1P2 receptors can importantly modify the vascular responsiveness to known physiological stimuli, such as α-adrenergic stimulation. In this regard, there are several potential paradigms suggested in the literature by which the S1P2 receptor could contribute to the maintenance of vascular contractile tone. One readily apparent possibility is that S1P2 receptors expressed in vascular smooth muscle cells (VSMC) could act directly to promote contraction, as suggested by the apparent preferential expression of S1P2 in VSMC (1) and the ability of the S1P2-specific antagonist, JTE-013, to block S1P-induced contraction in cultured coronary VSMC (25, 26). Our in vivo blood flow data showing decreased vascular resistance in S1P2-deficient mice support this interpretation. On the other hand, our observation that endothelium-denuded, S1P2-deficient aortas exhibit increased maximal force generation compared with wild type conflicts with this view (Table 1). An alternative possibility is that S1P2 activation might antagonize vasodilatory influences from the endothelium. Although S1P2 receptors are highly expressed in vascular smooth muscle, there is evidence that they are also expressed in endothelial cells (27). Furthermore, Nofer et al. (24) recently reported that administration of S1P resulted in vasodilation of precontracted aortas from rats or mice and that these effects could be blunted by NO synthase blockade, and were eliminated in both endothelial NO synthase and S1P3 receptor knockout mice. It is possible, therefore, that S1P3 receptor activation may stimulate endothelium-dependent vasodilation, whereas S1P2 receptors may oppose it. Accordingly, our present finding that intact aortas show a decreased sensitivity to PE (EC50), despite the increased maximal force generation in denuded vessels, is consistent with an elevated production of endothelium-derived relaxing factors in S1P2-deficient mice. In this case, the elevated maximal force of contraction in denuded vessels might be regarded as a compensation for a life-long overproduction of vasorelaxing factors by the endothelium. In any event, it is apparent that S1P can strongly influence vascular function through a complex interaction of S1P receptor subtypes in both vascular smooth

**Table 1. Contractile parameters for aortic ring preparation**

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<th>Intact</th>
<th>Endothelium-denuded</th>
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<tr>
<td></td>
<td>S1P2+/+</td>
<td>S1P2−/−</td>
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<tr>
<td><strong>KCl Contraction</strong></td>
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<tr>
<td>EC50, mM</td>
<td>23.7±0.3</td>
<td>27.5±0.2*</td>
</tr>
<tr>
<td>Max force/area, mN/mm²</td>
<td>31.2±0.4</td>
<td>29.7±0.4*</td>
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<tr>
<td><strong>PE Contraction</strong></td>
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<tr>
<td>EC50, μM</td>
<td>0.20±0.03</td>
<td>0.82±0.25*</td>
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<tr>
<td>Max force/area, mN/mm²</td>
<td>26.8±1.1</td>
<td>27.0±2.3</td>
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Values are means ± SE; n = 6 for each group. *p < 0.05 compared to S1P2+/+; †p < 0.05 compared to intact vessel
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S1P receptors are expressed in reasonable abundance on cardiac myocytes and a variety of studies have demonstrated direct effects of S1P and other sphingomyelin metabolites on ion channel activity, cytosolic Ca\(^{2+}\) concentration, cardiac automaticity, and cardiac inotropy (1). It has been previously reported that administration of S1P can induce either tachycardia or bradycardia in vitro (11, 32) and primarily bradycardia in vivo (9, 30, 33). S1P has also been shown to decrease myocardial contractility in vitro and in vivo (32, 33). In the present study, we found that S1P2-deficiency did not influence either basal or \(\beta\)-adrenergic-stimulated cardiac chronotropy or inotropy in the intact mouse. Interestingly, in the studies cited above, the tachycardia produced by S1P was eliminated in S1P2-deficient mice (9, 30), and our data further support the notion that the S1P2 receptor does not directly influence myocardial performance in vivo. Other studies have also implicated S1P and other lysophospholipids in the development of hyper- trophy (31) and in cardioprotective responses (16–18). The S1P2-deficient mouse may therefore represent an important model for future investigations regarding these clinically relevant phenomena. For the purposes of our study, the apparently normal myocardial function in the S1P2-deficient mice is noteworthy in that the observed differences in blood pressure and flow rate can therefore be attributed primarily to a vascular effect of the S1P2 receptor.

The regional vascular effects of S1P have previously been evaluated in a series of studies by Bischoff and coworkers (2–4). It was reported that S1P and sphingosyl 1-phosphorylcholine resulted in concentration-dependent vasconstriction in isolated vessels and decreases in renal and mesenteric blood flow in the intact animal (2). In another report, administration of S1P resulted in diuresis and natriuresis that had a different time course from the observed vascular effect, indicating a direct renal tubular influence of exogenous S1P (4). Although we did not evaluate renal function in the present experiments, our data indicate that endogenous S1P2 receptor signaling can have an important influence on both renal and mesenteric hemodynamics.

Bolz et al. (5) used a dominant negative approach to selectively inhibit the S1P-producing enzyme sphingosine kinase in the VSMC of isolated resistance vessels and reported that this smooth muscle-specific reduction in S1P synthesis led to a decrease in both resting tone and myogenic response. These results suggest that at least some of the S1P that presumably activates VSMC S1P2 receptors and regulates in vivo vascular tone is produced by the VSMC themselves and may therefore be acting in an autocrine fashion. Our data from the isolated aorta preparation are consistent with our in vivo data in that intact S1P2-deficient aortas displayed a blunted contractile response to KCl and PE challenge compared with wild-type aortas. This S1P2-dependent difference in the intact vessels is seen with no exogenously added S1P, thereby suggesting that S1P is generated in the vessels to activate the S1P2 receptors and that the regulation of vascular contractility by S1P2 does not require blood-borne S1P or other signals in the blood. As discussed above, the reduced vascular tone observed following sphingosine kinase inhibition in VSMC suggests that the S1P is generated by the VSMC themselves.

Taken together the data presented here indicate that endogenous S1P2 receptor signaling makes an essential contribution to physiological vascular function. It is possible, therefore, that appropriate targeting of this small molecule, cell surface receptor may yield therapeutic benefits in the treatment of vascular disorders.

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