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Review

Sphingosine-1-phosphate receptor signalling in the heart

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The five known members of the sphingosine-1-phosphate (S1P) receptor family exhibit diverse tissue expression profiles and couple to distinct G-protein-mediated signalling pathways. $S1P_1$, $S1P_2$, and $S1P_3$ receptors are all present in the heart, but the ratio of these subtypes differs for various cardiac cells. The goal of this review is to summarize data concerning which S1P receptor subtypes regulate cardiac physiology and pathophysiology, which G-proteins and signalling pathways they couple to, and in which cell types they are expressed. The available information is based on studies using a lamentably limited set of pharmacological agonists/antagonists, but is complemented by work with S1P receptor subtype-specific knockout mice and sphingosine kinase knockout mice. In cardiac myocytes, the $S1P_1$ receptor subtype is the predominant subtype expressed, and the activation of this receptor inhibits cAMP formation and antagonizes adrenergic receptor-mediated contractility. The $S1P_3$ receptor, while expressed at lower levels, mediates the bradycardic effect of S1P agonists. Studies using knockout mice indicate that $S1P_2$ and $S1P_3$ receptors play a major role in mediating cardioprotection from ischaemia/reperfusion injury *in vivo*. S1P receptors are also involved in remodelling, proliferation, and differentiation of cardiac fibroblasts, a cell type in which the $S1P_3$ receptor predominates. Receptors for S1P are also present in endothelial and smooth muscle cells where they mediate peripheral vascular tone and endothelial responses, but the role of this regulatory system in the cardiac vasculature is unknown. Further understanding of the contributions of each cell and receptor subtype to cardiac function and pathophysiology should expedite consideration of the endogenous S1P signalling pathway as a therapeutic target for cardiovascular disease.

1. Introduction

The lysophospholipid, sphingosine-1-phosphate (S1P), is a circulating bioactive lipid metabolite that has been known for many years to induce cellular responses, including proliferation, migration, contraction, and intracellular calcium mobilization.[1,2] There is evidence that S1P can function as an intracellular second messenger.[3,4] However, several early studies showed that S1P-mediated responses could be blocked by pertussis toxin, which prevents G-protein coupled receptors (GPCRs) from activating the heterotrimeric G-proteins G_i or G_o. These data suggested that S1P was functioning through a GPCR. Now that a family of GPCRs for which S1P is the high affinity ligand has been discovered, it is well accepted that the activation of these receptors is the mechanism by which S1P elicits most of its biological responses.[5–7]

There are currently five receptors for which S1P is the high affinity ligand. All of these are GPCRs. Binding of S1P to the receptor activates specific signalling pathways as a consequence of receptor coupling to selected G-proteins. Biochemical and molecular studies have revealed that there is specificity in the coupling of the different S1P receptor subtypes to various G-proteins. The $S1P_1$ receptor is unique in that it couples exclusively to the G_i protein.[8–10] In contrast, the $S1P_2$ and $S1P_3$ receptors couple promiscuously to G_i, G_q, and $G_{12/13}$ proteins,[11–13] whereas the $S1P_4$ and $S1P_5$ receptors couple to both G_i and $G_{12/13}$ proteins.[14–17] Upon activation, the alpha subunit of the heterotrimeric G-protein is released and able to interact with downstream effectors. The effector for the alpha subunit of G_i is adenylate cyclase (which it inhibits); release of beta/gamma subunits from the G_i protein can also affect cellular responses through the activation of ion channels and downstream kinases. The effector mediating the response to the alpha subunit of G_q is phospholipase C (PLC) while that for the alpha subunit of $G_{12/13}$ is a RhoA exchange factor. G-protein signalling can also be regulated by regulator of G-protein signalling proteins,[18,19] expression of which can be affected via S1P receptor activation.[20] Thus, coupling to particular G-proteins and downstream effectors dictates the nature of the cellular response and provides a level of selectivity to S1P signalling.

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While selective G-protein coupling can explain the divergent signalling pathways associated with the members of the S1P receptor family, differential expression patterns for the receptor subtypes are also important determinants of cellular responses.[6,21] The $S1P_1$, $S1P_2$, and $S1P_3$ receptors are widely expressed, whereas expression of $S1P_4$ and $S1P_5$ receptors is limited to the immune and nervous system.[6,16,22] Furthermore, while $S1P_1$, $S1P_2$, and $S1P_3$ receptors are all present in the cardiovascular system,[23–28] the predominant receptor subtypes in specific cardiac cell types differ, as described below.

In addition to S1P receptors, the myocardium has been shown to express both isozymes of sphingosine kinase, the enzyme that produces S1P.[29,30] This enzyme is particularly enriched in cardiac fibroblasts. Furthermore, the myocardium is known to express S1P phosphatase, an enzyme that degrades S1P.[31–33] Finally, circulating S1P is present at a high level in the blood within platelets and erythrocytes or bound to albumin and HDL.[34] As circulating S1P is present in abundant quantities, particularly under pathophysiological conditions, the tissue distribution, cellular localization, and subtype selective coupling of S1P receptors to G-proteins would be expected to determine which signalling pathways are activated.

The effects of S1P receptor activation on the different cell types present in the cardiovascular system (myocytes, fibroblasts, endothelial cells, and smooth muscle cells) have been characterized to varying extents. S1P receptors on cardiomyocytes were initially studied for their effect on ion channels and contractility, but are now known to also be involved in hypertrophy and cardioprotection. Activation of S1P receptors on fibroblasts can mediate migration and proliferation, responses that are necessary for fibrosis and critical to cardiac remodelling. Finally, S1P can modulate vascular permeability, angiogenesis, and vascular tone by activating certain S1P receptor subtypes. While many reviews have been written about S1P receptor signalling, the purpose of this review is to discuss S1P receptor-mediated signalling in the various cell types within the cardiovascular system. We will focus on S1P signalling in cardiomyocytes, but will also provide comparative relevant information on the roles of S1P receptors in cardiac fibroblasts and the vasculature. More in-depth analysis of the role of S1P in the vasculature will be provided by other contributors to this spotlight issue.

2. Role of S1P receptors on cardiomyocytes

Cardiomyocytes express $S1P_1$, $S1P_2$, and $S1P_3$ receptors. Quantitative PCR has shown the $S1P_1$ receptor to be the predominant S1P receptor subtype on cardiomyocytes, with $S1P_2$ and $S1P_3$ receptor mRNA present at much lower levels[23,24] (*Table 1*). Activation of cardiac S1P receptors has been reported to affect cardiac contractility and heart rate, induce hypertrophy, provide protection from ischaemia, and mobilize intracellular calcium. Determining which S1P receptor subtypes mediate specific responses has been difficult due to ubiquitous S1P receptor expression and a paucity of commercially available subtype-specific agonists/antagonists. We will, however, call attention to responses that can be specifically assigned to particular S1P receptor subtype(s) whenever possible.

Table 1 Relative expression of S1P receptor subtypes in various cell types

Tissue	Relative S1P receptor expression
Cardiac myocytes	$S1P_1 >> S1P_3 > S1P_2$
Cardiac fibroblasts	$S1P_3 >> S1P_1 > S1P_2$
Aortic smooth muscle cells	$S1P_2 > S1P_3 >> S1P_1$
Vascular endothelial cells	$S1P_1 > S1P_3 >> S1P_2$

Comparison of S1P receptor subtype expression in various cell types found in the heart. Data for myocytes and fibroblasts are derived from studies of cells isolated from heart; those for vascular endothelium and smooth muscle are based on information from non-cardiac sources. $>>$, much greater than; $>$, greater than; $=$, similar.

2.1 S1P and cardioprotection

S1P has been demonstrated to protect the heart, as well as isolated myocytes, from a variety of insults both *in vivo* and *ex vivo*. In an early study, exogenous S1P as well as the related lysophospholipid, LPA, were shown to protect neonatal rat ventricular myocytes from hypoxia.[35] In addition S1P generated intracellularly, via ganglioside GM-1 mediated activation of sphingosine kinase, appeared to protect myocytes from hypoxia to a similar extent as exogenously applied S1P.[35] More recent experiments have shown that S1P also protects adult mouse ventricular myocytes from hypoxia.[23] In order to determine which receptors mediate the observed cardioprotection, Karliner's laboratory developed an antibody that functions as an agonist only at $S1P_1$ receptors.[36] Subsequent studies showed that this $S1P_1$ receptor agonist antibody protected myocytes from hypoxia to the same extent as exogenously applied S1P. Furthermore, studies using pharmacological inhibitors indicated that this protection occurred through either $S1P_1$ or $S1P_3$ receptors, was mediated by a PI3 kinase pathway, and likely involved activation of Akt and inactivation of GSK-3β.[23] Additional studies showed that pertussis toxin and the $S1P_{1/3}$ antagonist/$S1P_4$ agonist, VPC23019, could block GM-1 mediated cardioprotection against hypoxia and glucose deprivation, suggesting that intracellularly produced S1P is exported from the cell and acts on cardiomyocyte $S1P_1$ and $S1P_3$ receptors coupled to G_i in an autocrine or paracrine manner. The additional finding that GM-1 is unable to reproduce this protective effect in sphingosine kinase knockout cardiomyocytes further substantiates that the effects of GM-1 are relatively specific for sphingosine kinase and lend support to the concept that intracellular S1P is exported from the cell and acts via cell surface S1P receptors.[37]

In the intact heart, S1P has also been shown to protect against global ischaemia reperfusion (I/R). Both exogenous S1P as well as intracellularly generated S1P (produced via GM-1 stimulation of sphingosine kinase) have been reported to improve recovery of cardiac function as measured by LVDP or creatine kinase release.[38–40] Whereas the protection afforded by exogenous S1P administration was demonstrated to occur through a PKCε independent pathway (based on studies with PKCε knockout mice), protection afforded by intracellularly generated S1P required PKCε.[40] Furthermore, deletion of the sphingosine kinase 1 gene and the subsequent decrease in the ability to produce endogenous S1P could be rescued by exogenous S1P treatment.[41] The role of PKCε in mediating cardioprotection is unclear although it is

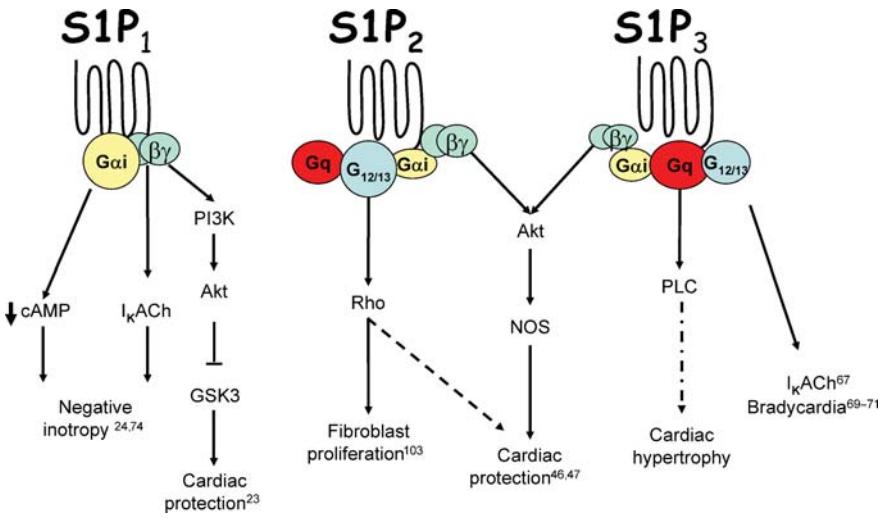

Figure 1 S1P receptor-mediated signalling in the heart. Activation of the $S1P_1$ receptor induces negative inotropy through effects of $G_{\alpha i}$ to decrease cAMP concentration and effects of $G_{\beta\gamma}$ on ion channels. The $S1P_2$ receptor is the primary receptor for mediating Rho activation and this receptor mediated pathway may participate in cardioprotection. The $S1P_3$ receptor is the primary receptor coupling to PLC and the activation of this receptor also results in bradycardia. While the $S1P_2$ and $S1P_3$ receptors appear to collaborate in providing cardioprotection from ischaemia reperfusion injury *in vivo*, the $S1P_1$ receptor has been suggested to be cardioprotective in the isolated cardiomyocytes.

noteworthy that while S1P does not cause translocation of PKCε, GM-1 stimulation results in PKC translocation and this may be a necessary step in GM-1 mediated sphingosine kinase activation. Further work from the Karliner laboratory suggests that it is the $S1P_1$ receptor that mediates cardioprotection from global I/R. On the other hand, perfusion of the $S1P_1$ receptor agonist, SEW2871, did not protect adult rat hearts from global I/R to the same extent as S1P, indicating that additional S1P receptor subtypes are involved in the cardioprotective response.[42]

Notably, several studies have examined the cardioprotective effects of S1P on the heart *in vivo*. Administration of exogenous S1P was shown by Levkau's group to protect the heart against damage from 30 min ischaemia/24 h reperfusion and a comparable effect was seen following administration of HDL, a known carrier of S1P.[43–45] The protective effects of HDL and S1P were abolished when this response was examined in hearts from $S1P_3$ receptor knockout mice[46] and interestingly, inhibition of nitric oxide synthase (NOS) completely abolished HDL- or S1P-mediated cardioprotection, implicating NOS as an important mediator in this pathway.[46] Studies from our laboratory utilized $S1P_2$, $S1P_3$, and $S1P_{2,3}$ receptor double knockout mice and showed a significant increase in infarct size after 30 min ischaemia/2 h reperfusion when both $S1P_2$ and $S1P_3$ receptors were deleted.[47] These data implicate endogenously supplied S1P in cardioprotection through effects on $S1P_{2,3}$ receptors. A significant reduction in *in vivo* Akt phosphorylation was also observed in $S1P_{2,3}$ receptor double knockout hearts following I/R, suggesting that S1P acting on $S1P_2$ and $S1P_3$ receptors mediates cardioprotection via Akt.[47] The aforementioned findings on NOS and Akt as *in vivo* mediators of cardioprotection may be related since the loss of Akt activation in the $S1P_{2,3}$ receptor double knockout mice could result in a decrease in eNOS activation; eNOS is a known substrate of Akt which has itself been shown to protect against cardiac damage from I/R injury[48–50] (*Figure 1*).

Recent unpublished studies from the Levkau group have yielded the first data on the cardiac-specific $S1P_1$ receptor knockout mouse (B. Levkau, personal communication). Generation of this alpha-MHC-Cre driven knockout line was necessary as the conventional $S1P_1$ receptor knockout mouse shows embryonic lethality.[51] Initial findings indicate that the $S1P_1$ receptor knockout mouse displays a progressive heart failure phenotype, consistent with a basal protective effect of S1P. Interestingly, when subjected to 30 min ischaemia/24 h reperfusion, the $S1P_1$ receptor knockout heart showed the same amount of IR-induced damage as the WT heart, suggesting that the $S1P_1$ receptor in cardiomyocytes may not contribute to cardioprotection from *in vivo* I/R. This finding is compatible with our published evidence that the $S1P_2$ and $S1P_3$ receptors mediate cardioprotection,[47] but contrasts with studies from the Karliner group indicating that the $S1P_1$ receptor mediates cardioprotection.[23] It is important to note that the protective effect of $S1P_1$ against hypoxia was demonstrated in isolated myocytes and against global I/R, while our data and the recent finding from the Levkau group examined *in vivo* I/R. Additional studies will be needed to further investigate the cardioprotective mechanisms downstream of each S1P receptor subtype.

2.2 S1P and hypertrophy

There is evidence that another lysophospholipid, lysophosphatidic acid (LPA), induces hypertrophic growth of cardiomyocytes via activation of G_i -and RhoA-mediated signalling pathways downstream of a family of GPCR's that are related yet distinct from S1P receptors.[52,53] There is conflicting data, however, regarding the role of S1P in hypertrophy. An early study in neonatal rat cardiomyocytes concluded that S1P did not induce hypertrophy, as assessed by atrial natriuretic factor expression and phenylalanine incorporation, although the related sphingolipid, sphingosylphosphorylcholine (SPC), was able to induce hypertrophy through an ERK1/2 dependent pathway.[54] However, the mechanism of this remains unclear as SPC does not act as an agonist at any of the S1P receptor subtypes. Subsequent data from a different group demonstrated that S1P induces

hypertrophy in neonatal rat cardiomyocytes as measured by cell size, phenylanine incorporation, cytoskeletal organization, and expression of brain natriuretic peptide.[55] Importantly, this hypertrophic response appeared to be mediated by the $S1P_1$ receptor as an anti-$S1P_1$ receptor antibody blocked the S1P-mediated hypertrophy. In addition, inhibitor studies revealed that the S1P-mediated hypertrophy involved G_i coupled signalling pathways and activation of MAP kinases, Akt, p70 S6 kinase, and Rho.[55] It should be recognized, however, that the G_i- and RhoA-mediated hypertrophy induced by LPA and S1P occurs more slowly and is less robust than the more canonical hypertrophic responses elicited through the activation of G_q/PLC signalling by norepinephrine, phenylyephrine, and endothelin.[56]

No studies have yet been conducted to determine whether S1P acts as a hypertrophic mediator *in vivo*. We have determined that $S1P_3$ receptor knockout myocytes show a complete loss of S1P-mediated PLC activity, while this response is intact in $S1P_2$ receptor knockout myocytes. This result is in agreement with that obtained using S1P receptor knockout mouse embryonic fibroblast (MEF) cells, which demonstrated that $S1P_3$ receptor deletion prevented PLC activation.[57] In light of the established relationship between G_q signalling and pressure overload induced hypertrophy *in vivo*, [58,59] it is intriguing to postulate that $S1P_3$ receptor/G_q activation may contribute to the cardiac hypertrophic response *in vivo*. On the other hand, preliminary studies from our group indicate that $S1P_3$ receptor knockout mice do not show diminished hypertrophy after transverse aortic constriction (TAC). These data suggest that S1P is not the predominant GPCR agonist responsible for G_q-mediated hypertrophy following TAC.

2.3 S1P in cardiac physiology

S1P is a known mediator of cardiac electrophysiological and contractile responses. Until recently, the mechanisms and receptor subtypes mediating these functions have remained elusive. The first *in vivo* studies demonstrated that S1P treatment of rat or canine hearts resulted in positive chronotropy and vasoconstriction while decreasing inotropy and coronary blood flow. As these responses were not blocked by adrenergic antagonists and S1P did not affect adenylyl cyclase activity, this suggested involvement of a novel signalling pathway which was subsequently explained by discovery of S1P receptors.[60,61] It should also be noted that this *in vivo* negative inotropic response could be explained in part by diminished coronary blood flow.[60,61] However, *in vitro* studies showed that S1P, like acetylcholine, stimulates a receptor-activated inward rectifier K^+ current ($I_{K.Ach}$), which is known to contribute to resting membrane potential and the shape of action potentials in guinea pig,[62–64] rabbit,[65] human, and mouse atrial myocytes.[66] The effects of S1P on $I_{K.Ach}$ were attributed to activation of the $S1P_3$ receptor as suramin, a putative $S1P_3$ receptor antagonist, blocked these effects. This conclusion must be tempered by the knowledge that while suramin can act as an $S1P_3$ receptor antagonist,[67] it also has a myriad of non-specific pharmacological properties.[68] The $S1P_3$ receptor has also been implicated in regulating heart rate and several studies have shown that stimulation of this receptor results in bradycardia in both mice and humans.[69–71] This adverse effect of $S1P_3$ receptor activation, apparently mediated at the level of the SA node, has serious therapeutic implications. Concern about S1P-mediated bradycardia arises because several non-specific S1P receptor agonists (e.g. FTY720) which inhibit lymphocyte egress and reduce the number of circulating lymphocytes are being investigated as potential immunosuppressive agents for transplant procedures or in multiple sclerosis.[72,73]

In more recent studies in which mouse ventricular myocytes were used to examine S1P-mediated changes in contractility, S1P treatment dramatically decreased cell shortening and was able to antagonize isoproterenol-induced increases in *cAMP* and positive inotropy.[24,74] The $S1P_1$ receptor specific agonist, SEW2871, was as efficacious as S1P at antagonizing isoproterenol-induced contractility, and the $S1P_{1,3}$ receptor antagonist/$S1P_4$ agonist, VPC23019, blocked the negative inotropic effects of SEW2871 which was observed in cells lacking $S1P_3$ receptors.[74] Taken together, these results suggest that the $S1P_1$ receptor is the predominant receptor mediating the negative inotropic effects of S1P in mouse ventricular myocytes; although the $S1P_3$ receptor may also play a minor role. The $S1P_4$ receptor is not expressed in cardiac myocytes. These results are corroborated by the unpublished findings from the Levkau group indicating that the ability of S1P to inhibit isoproterenol-stimulated contractility is abolished in the cardiac specific $S1P_1$ receptor knockout myocytes (B. Levkau, personal communication). S1P-mediated negative inotropy was also shown to be significantly, but not completely, blocked by the $I_{K.Ach}$ inhibitor tertiapin, further substantiating the involvement of $I_{K.Ach}$, a G_i ($\beta\gamma$) regulated ion channel, in $S1P_1$-mediated contractile changes. Thus, $S1P_1$ receptor-mediated negative inotropy may result from both the ability of the alpha subunit of G_i to inhibit *cyclic AMP* formation (thus PKA-mediated L-type Ca^{2+} channel activation), and the ability of $\beta\gamma$ subunits of G_i to act on $I_{K.Ach}$[74] (*Figure 1*).

Remarkably, selective coupling of the $S1P_1$ receptor in mediating negative inotropy in ventricular myocytes may be explained by its subcellular localization. The $S1P_1$ receptor has been localized to caveolae in COS cells[75] and more recently in adult mouse ventricular myocytes.[24] Caveolae are known to be enriched in numerous signalling components.[76–78] Our studies examined the ability of S1P to decrease isoproterenol-induced *cAMP* production and positive inotropy in mouse ventricular cardiomyocytes and found these effects to be completely inhibited by treatment with the caveolar disrupting agent methyl-β-cyclodextrin.[24] Thus, it is likely that as a result of its localization near components of the *cAMP* signalling pathway, the $S1P_1$ receptor is uniquely able to antagonize isoproterenol-stimulated *cAMP* production and positive intropy, whereas other S1P receptor subtypes capable of coupling to G_i do not induce this same response.[24]

3. Role of S1P receptors in vascular function

Endothelial cells are found lining the blood vessels of the heart and are involved in a number of important processes including regulation of barrier integrity and angiogenesis. S1P regulates these processes and also stimulates the migration and proliferation of endothelial cells.[79–82]

Aberrant vascular maturation, apparently resulting from the inability of S1P to activate the GTPase Rac, is observed in global $S1P_1$ receptor knockout mice and may be responsible for the impaired development and embryonic lethality that occurs between E12.5 and E14.5.[51,83] The finding that the endothelial specific knockout of the $S1P_1$ receptor phenocopies the embryonic lethality seen in the global knockout demonstrates that $S1P_1$ receptors on endothelial cells play a critical role in this aspect of vascular development.[83]

Endothelial cells express $S1P_1$, $S1P_2$, and $S1P_3$ receptors, the $S1P_1$ receptor being the most abundant subtype, with the others expressed at much lower levels[84–87] (*Table 1*). Most S1P-mediated responses on endothelial cells occur via the $S1P_1$ receptor alone or in combination with the $S1P_3$ receptor. S1P-mediated migration, angiogenesis, and adherens junction formation all require both the G_i mediated activity of the $S1P_1$ receptor and the $G_q/G_{12,13}$ mediated activity of the $S1P_3$ receptor.[85,86,88] The requirement for the $S1P_3$ receptor has been further confirmed in studies demonstrating that a peptide derived from the second intracellular loop of the $S1P_3$ receptor can induce pro-angiogenic responses.[89] S1P also promotes endothelial cell integrity, stabilizes newly formed vessels, and antagonizes the disruptive effects of thrombin on barrier integrity through its effects on $S1P_1$ and $S1P_3$ receptors.[90–92]

The expression pattern for S1P receptors on smooth muscle cells differs from that of endothelial cells (*Table 1*). Smooth muscle cells express $S1P_1$, $S1P_2$, and $S1P_3$ receptors, but levels of the $S1P_1$ receptor are significantly reduced in adulthood such that the $S1P_2$ and $S1P_3$ receptors become the predominant receptor subtypes.[26,93] These distinct receptor expression profiles may explain why smooth muscle cells respond to S1P differently than endothelial cells. Stimulation of either $S1P_1$ or $S1P_3$ receptors leads to activation of Rac, whereas $S1P_2$ receptor stimulation inhibits Rac activation. Interestingly, the mechanism by which the activation of $S1P_2$ receptors inhibits Rac activation is in part through activation of a Rho/Rho kinase pathway.[94] In concordance with this role for SIP_2 receptors, our group showed that S1P-mediated Rho activation is nearly abolished in MEF cells isolated from $S1P_2$ receptor knockout mice and suggested that Rho activation is most effectively coupled to the $S1P_2$ receptor.[95] Due to the greater relative abundance of $S1P_2$ receptors in smooth muscle and $S1P_1$ receptors in endothelial cells, S1P actually inhibits migration of smooth muscle cells, whereas it promotes migration of endothelial cells.[26]

The ability of S1P to activate Rho in smooth muscle cells promotes myosin light chain phosphorylation which in turn contributes to vasoconstriction.[96,97] Recent studies have exploited S1P receptor knockout mice to determine the involvement of individual S1P receptor subtypes in regulating vascular tone. S1P failed to increase vascular tone in basilar arteries isolated from $S1P_3$ receptor knockout mice, while arteries from WT and $S1P_2$ receptor knockout mice showed the expected vasoconstrictor response to S1P treatment.[98] The $S1P_3$ receptor thus appears to be the primary mediator of S1P-induced vasoconstriction, although other studies suggest a role for the $S1P_2$ receptor in regulating resting vascular tone and myogenic responses. S1P can promote the formation of nitric oxide (NO) in endothelial cells and release of NO relaxes smooth muscle cells.[99,100] Whether responses downstream of the $S1P_2$ receptor are mediated solely via smooth muscle cells or also by changes in endothelial function is not yet clear.[101]

4. Role of S1P receptors in cardiac fibroblasts

While myocytes compose the bulk of the ventricular mass, fibroblasts are the most abundant cell type in the heart and function to organize the cardiac myocytes and preserve the ability of cardiac myocytes to respond to many stimuli. Cardiac fibroblasts express predominantly $S1P_3$ receptors with much lower levels of $S1P_1$ and $S1P_2$ receptors as assessed by quantitative PCR.[74] In addition, cardiac fibroblasts have elevated levels of sphingosine kinase activity when compared with cardiomyocytes and thus appear to be an important source of endogenous S1P in the heart.[102] A recent study demonstrates that S1P induces proliferation of cardiac fibroblasts as measured by increased DNA synthesis and alpha-smooth muscle actin expression.[103] How S1P regulates proliferation and transformation of cardiac fibroblasts is unclear, although it appears to involve activation of MAP kinases and of Rho downstream of the $S1P_2$ receptor. S1P also causes proliferation and differentiation in a number of non-cardiac fibroblasts.[104–106] For example, hepatic fibrosis induced by liver injury is diminished in $S1P_2$ receptor knockout mice. Correspondingly, an increase in S1P production by the sphingosine kinase activator, K6PC-5, results in increased fibroblast proliferation and collagen production in the mouse dermis.[107,108]

Conclusions

The heart is a complex organ in which numerous cell types respond to S1P in different manners. Early studies focusing on individual cell types have led to more complex *in vivo* studies to elucidate the physiological consequences of S1P signalling in the cardiovascular system. This has proven challenging as S1P receptor pharmacology is still in its infancy, but has increasingly been aided by the availability of subtype-specific S1P receptor knockout mice. There is now broad consensus that S1P plays a critical role in maintaining cardiac cell survival and function. Further analysis of the actions of each S1P receptor subtype on the constituent cell types within the heart will be necessary for the rational design of potential therapeutics targeting S1P signalling in the desired cell compartment and at the appropriate time.

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