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Mechanisms of Cardioprotection by Lysophospholipids

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Abstract The lysophospholipids sphingosine 1-phosphate (S1P) and lysophosphophatidic acid (LPA) reduce mortality in hypoxic cardiac myocytes. S1P is also cardioprotective in both mouse and rat models of cardiac ischemia/reperfusion (I/R) injury. Although these results are consistent with prior work in other cell types, it is not known what signaling events are critical to cardioprotection, particularly with respect to ceramide and the preservation of mitochondrial function, which is essential for cardiac cell survival. Neither receptor regulation nor signaling has been studied during I/R in the heart with or without the application of S1P or LPA. The role of sphingosine kinase in I/R and in ischemic preconditioning (IPC) has not been defined, nor has the fate or function of S1P generated by this enzyme, particularly during preconditioning or I/R, been elucidated. Whether S1P infused systemically in animal models of myocardial infarction in which survival is an end-point will be hemodynamically tolerated has not been determined. If not, the substitution of agents such as the monosialoganglioside GM-1, which activates sphingosine kinase, or the development of alternative ligands for S1P receptors will be necessary. J. Cell. Biochem. 92: 1095–1103, 2004.

Key words: heart; ischemia/reperfusion injury; sphingosine 1-phosphate; sphingosine kinase; ceramide; mitochondria; lysophosphatidic acid; cardioprotection; preconditioning

Coronary vascular disease is the major cause of death in western countries. For example, in the United States, over 1.1 million patients suffer heart attacks (myocardial infarction) yearly. Although survival rates have been enhanced markedly by a variety of revascularization techniques, such as pharmacological lysis of intracoronary clots, mechanical stenting, and coronary bypass surgery, loss of muscle tissue nevertheless occurs. The resultant scarring and remodeling of the heart often results in congestive heart failure, leading to recurrent hospitalizations, and eventually to a shortened life span. Many heart attacks occur in patients undergoing non-cardiac surgery, and ischemic episodes may go unrecognized in patients under anesthesia or during the postoperative period when patients are unable to complain of symptoms. It has been known for decades that the cause of most myocardial infarctions is partial or complete obstruction to flow through one or more coronary blood vessels. Irreparable damage to heart muscle occurs with complete permanent occlusion, but it is recognized that relief of the occlusion, whether by pharmacologic or mechanical approaches, can result in reperfusion injury that also causes permanent damage. In addition, it is also known that clot lysis may occur spontaneously in patients, and that myocardial injury due to naturally occurring ischemia/reperfusion (I/R) is a common occurrence. Thus, much research has been focused on prevention of such I/R injury.

One common experimental approach for the prevention of I/R injury is called ischemic preconditioning (IPC). It was found that a short, sublethal period of I/R reduced the size of the infarction produced by a longer, lethal period of I/R. IPC is thought to be mediated by a variety of factors, including mitochondrial free radical generation, and the activation of a signaling cascade that involves translocation of εPKC and opening of mitochondrial K_{ATP} channels. Although the initial description of the IPC phenomenon involved early cardioprotection (1–2 h), delayed IPC (48–72 h), possibly

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involving different mechanisms, has also been described. Other approaches to myocardial salvage after I/R include inhibition of free radical generation and preservation of NAD^+ by inhibition of poly(ADP-ribose)polymerase or deletion of the gene for this enzyme.

Pharmacologic preconditioning has also been reported as a result of activation of heptahelical membrane-spanning G-protein-coupled receptors by agents such as adenosine, phenylephrine, and opioid agonists. Among the ligands for G-protein coupled receptors are the lysophospholipids sphingosine 1-phosphate (S1P) and lysophosphatidic acid (LPA). According to current terminology, S1P is thought to act as a ligand for $S1P_{1-5}$ (formerly EDG 1, 5, 3, 6, and 8, respectively), while LPA binds primarily to LPA_{1-3} (formerly EDG 2, 4, and 7). SIP may also act intracellularly as a second messenger, and LPA may also signal intracellularly by activating nuclear transcription factors. The S1P and LPA family of receptors couples to multiple subsets of heterotrimeric G proteins such as G_q, $G_{i/o}$, and $G_{12/13}$. The downstream signals that follow receptor activation are thus varied. Many of the key components of the cardiovascular system besides cardiac myocytes harbor S1P and LPA receptors. These cells and tissues include such important regulators of cardiovascular function as endothelial and smooth muscle cells and platelets. Thus S1P and LPA may contribute to angiogenesis, atherosclerosis, regulation of blood vessel tone, myocardial hypertrophy, and protection against ischemic injury. This review will focus on the potential role of lysophospholipids, especially S1P, in cardioprotection.

S1P RECEPTORS IN THE HEART

We have previously shown that neonatal rat cardiac myocytes express $S1P_2$, $S1P_3$, LPA_1, and LPA_2 receptors, and $S1P_1$ receptors have also been identified in rat cardiac myocytes. In both mouse and human heart tissue, transcripts for $S1P_{1-3}$ (Edg 1, 5, and 3, respectively) have been reported. A recent extensive analysis of cardiovascular tissues using in situ hybridization and immunohistochemistry was carried out by Mazurais et al. [2002], who observed that $S1P_{1-3}$ were expressed in all anatomically distinct subregions of the adult heart examined, i.e., the apex, left and right ventricles, left and right atria, and the aorta. $S1P_1$ (Edg 1) mRNA was equally and strongly expressed in different parts of the heart and in the aorta, whereas the $S1P_3$ (Edg 3) transcript was more abundant in the aorta. $S1P_2$ (Edg 5) was weakly expressed in both types of tissues, but more strongly expressed in fetal samples. $S1P_1$ (Edg 1) mRNA and protein also were strongly expressed in the endothelial layer of cardiac vessels, whereas $S1P_3$ (Edg 3) mRNA was highly expressed in the smooth muscle cell layer of the aorta and cardiac vessels and weakly expressed in ventricular and atrial cardiomyocytes.

No studies of S1P receptor regulation have as yet been reported in the heart. However, Watterson et al. [2002] used hamster fibroblast and HEK293 cell lines to demonstrate dual regulation of S1P receptor phosphorylation and internalization by protein kinase C (PKC) and G-protein-coupled receptor kinase 2. Immediate downregulation evoked by S1P depended on 12 amino acids of the carboxyl terminus, was resistant to PKC inhibition, and was mediated in part by G protein-coupled receptor kinase-2. In contrast, elicitation of immediate downregulation by a phorbol ester did not depend on the carboxyl terminus and was completely suppressed by PKC inhibition. Rapid down-regulation and internalization, but not $S1P_1$ receptor binding and signaling functions, were facilitated by N-linked glycans of $S1P_1$ receptors [Kohno et al., 2002]. We recently showed in T lymphocytes that late recovery of downregulated $S1P_1$ receptors requires εPKC, as receptor function does not return 12–24 h after selective peptide inhibition of εPKC or in εPKC deficient mice [Graeler et al., 2003]. Inhibition of recovery was associated with the inability of S1P to stimulate components of the AP-1 transcription complex 24 h after initial downregulation. How agonist and εPKC regulation of S1P receptors respond in cardiac myocytes under control conditions, or during IPC or I/R remains to be determined.

SYSTEMIC EFFECTS OF S1P

As heart rate, blood pressure, and myocardial contractile state are major determinants of myocardial oxygen demand, and hence of the response to acute insults such as I/R, the systemic effects S1P are of considerable importance. Given the distribution of S1P receptors and the acute regulatory responses described above, it might be predicted that there would be rapid vasoactive responses to S1P given

parenterally. This is indeed the case, but the results are variable. Intravenous injection of S1P (0.1–100 μg/kg equivalent to 0.06–600 nmol/kg) in the rat did not affect mean arterial pressure or heart rate, but reduced renal blood flow transiently [Bischoff et al., 2000]. Direct injection of S1P into the renal artery caused greater blood flow reductions than intravenous administration. In other work, however, it was found that intravenous administration of S1P in the rat decreased heart rate, ventricular contraction, and blood pressure in a dose-dependent manner with the highest dose used (263.5 nmol/kg) having a transient effect, without a significant effect on atrioventricular and intraventicular conduction [Sugiyama et al., 2000a]. Using a different model, a canine isolated sinoatrial node and papillary muscle preparation cross-circulated with heparinized arterial blood of a blood-donor dog, this group reported that S1P increased the sinoatrial rate, while it decreased coronary blood flow by 20–25%, accompanied by a mild decrease in papillary muscle developed tension. In this report, the calculated dose ranged from 2.6 to 26 mM [Sugiyama et al., 2000b]. The concentrations used in these studies cannot be directly compared to steady-state serum levels of S1P in humans, which range from 500 to 900 nM [Okajima, 2002] because tissues are likely receiving much higher concentrations of S1P as the result of rapid bolus injections. Under these circumstances lipoprotein binding, which is a major regulator of effective S1P concentrations under normal conditions [Okajima, 2002], is much less likely to be of importance. In this connection, it should be noted that more than 60% of S1P is bound to lipoproteins [Okajima, 2002]. As measured by a radioreceptor assay, 54% of S1P is bound to high density lipoprotein (HDL), 8% to low density lipoprotein (LDL), 2% to very low density lipoprotein (VLDL), and 36% to lipoprotein deficient serum (LPDP) [Murata et al., 2000].

Recently, Ohmori et al. [2003] reported that S1P-stimulated human coronary artery smooth muscle cell contraction was inhibited by a selective $S1P_2$ receptor antagonist. C3 exoenzyme also inhibited S1P-stimulated contraction, indicating Rho involvement. Lipid phosphate phosphatases were also identified in these human coronary artery smooth muscle cells, and the human form of S1P phosphatase has recently has been cloned [Johnson et al., 2003]. Indeed, the role of S1P phosphohydrolase in the regulation of sphingolipid metabolism has recently been shown in several cell lines. Thus, treatment of mammalian S1P phosphatase transfectants with S1P markedly increased ceramide levels, predominantly in intracellular membranes, diminished survival, and enhanced apoptosis [Le Stunff et al., 2002]. Conversely, in cells in which human S1P phosphatase was reduced by siRNA-induced knockdown of the enzyme, there was a twofold increase of S1P levels. There was a concomitant decrease in sphingosine, as well as increased secretion of S1P into the media, indicating that S1P phosphatase regulates secreted S1P [Johnson et al., 2003]. These observations point to a major role for S1P phosphatase in the regulation of both intra- and extra-cellular S1P levels. No studies of either augmentation or inhibition of S1P phosphatase in simulated or actual I/R have been reported. Although no hemodynamic studies have been carried out in intact animals subjected to IR in which S1P has been infused systemically, GM-1, a monosialoganglioside that augments S1P via activation of sphingosine kinase, has been used in human stroke trials [Argentino et al., 1989; Lenzi et al., 1994; Sass Investigators, 1994].

S1P IN EXPERIMENTAL CARDIOPROTECTION

S1P is known to be a survival factor for a variety of cell types. Spiegel and Milstien [2003] have developed a model describing an intracellular ceramide-S1P "rheostat." This model is founded on observations that increases in the concentration of the pro-apoptotic molecule ceramide can be countered by increases in the intracellular levels of S1P. Thus, inhibition of sphingosine kinase, the final step in S1P synthesis, by dimethylsphingosine induces cell death, whereas activation of sphingosine kinase by stimulation of PKC increases intracellular S1P and enhances cell survival. However, no cell physiology studies had been carried out on cardiac myocytes until it was reported that S1P produced calcium overload in adult rat cardiomyocytes [Nakajima et al., 2000]. If S1P enhances the calcium dysregulation that occurs with I/R, it would be unlikely to function as a useful cardioprotective agent. Accordingly, we set out to determine whether S1P would be cardioprotective or would increase cardiac

myocyte damage in cells and hearts subjected to I/R injury.

To determine the effect of S1P on cardiac myocyte survival, we first used an in vitro culture system [Karliner et al., 2001]. We incubated neonatal rat cardiac myocytes either in room air/1% CO_2 (normoxia) or in an atmosphere of 99% N_2/1% CO_2 (hypoxia) at 37°C for 18–20 h in the absence of glucose. Cell viability was measured using a calcein ester green fluorescence assay. Under normoxic conditions, $88.7 \pm 1\%$ of the cells were viable after 18–20 h. Severe hypoxia reduced viability to $61.3 \pm 4.3\%$ ($n = 6, P < 0.05$). In myocytes preincubated with 10 μM S1P for 2 h, the effects of severe hypoxia on cell viability were prevented, resulting in survival equivalent to normoxia. The mitochondrial K_{ATP} channel antagonist 5-hydroxydecanoic acid had no effect on myocyte survival during severe hypoxia, but completely abolished the ability of S1P to rescue cardiac myocytes from hypoxic cell death, indicating that S1P-mediated cardioprotection, like IPC and other forms of pharmacologic preconditioning, involves signaling mechanisms requiring mitochondrial K_{ATP} channel opening. We also developed an adult mouse myocyte culture system and have found that both S1P and GM-1 protect these cells against hypoxia-induced cell death [Honbo et al., 2001].

Encouraged by these results, we tested the hypothesis that S1P-induced cardioprotection required εPKC activation [Jin et al., 2002]. Previously, it had shown in vitro that membrane translocation (activation) of εPKC is a critical step in preconditioning [Gray et al., 1997]. The model used was an ex vivo Langendorff preparation. We subjected hearts isolated from εPKC knockout mice and wild-type littermate controls to 20 min of global ischemia and 30 min of reperfusion. End-points of the study were left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), a measure of cardiac function obtained by subtracting LVEDP from peak left ventricular pressure, and creatine kinase (CK) release as a measure of myocyte necrosis. Pretreatment with a 2-min infusion of 10 nM S1P significantly improved recovery of LVDP, and reduced the rise in LVEDP and CK release in both wild-type and εPKC knockout hearts. Infarct size measured at the end of the I/R period was reduced by approximately 50%. Coronary flow as measured by collecting the coronary sinus effluent increased significantly compared to control. These data indicate that extracellular S1P induces cardioprotection though a signaling pathway that is independent of εPKC.

We were somewhat surprised that a 10 nM infusion of S1P was cardioprotective in view of the requirement for a much larger concentration requirement (10 μM) in rat cardiac myocyte cell culture. Aside from the use of neonatal cell culture versus an adult ex vivo preparation, these observations should be considered relative to the known serum levels of S1P in humans of 200–900 nM [Okajima, 2002; Deutschman et al., 2003]. These values are much higher than the K_d of S1P for its receptors (2–30 nM); the higher serum levels relative to K_d values may in part be the result of lipoprotein binding of S1P [Okajima, 2002], especially in the HDL fraction as described earlier [Murata et al., 2000]. Thus, a bolus infusion of 10 nM S1P to a buffer perfused heart previously deprived of exposure to circulating S1P in which the total concentration is rapidly delivered to the myocardium appears to be sufficient to produce cardioprotection. A possible explanation is that S1P receptors become acutely saturated and intracellular S1P-mediated signaling is thereby enhanced.

Our observations regarding the dose and timing of S1P administration have been confirmed by Lecour et al. [2002] in an ex vivo rat heart model in which infarct size was markedly reduced by S1P pretreatment. Thus, both rat and mouse heart exhibit virtually identical cardioprotection in response to S1P. In the same paper, these investigators also reported that the benefit conferred by tumor necrosis factor-α (TNF-α), which mimics IPC, was largely prevented by *N*-oleoylethanolamine (NOE, 1 μM), a ceramidase inhibitor. NOE also reversed the benefit of IPC on infarct size. Of note, C2-ceramide also conferred preconditioning-like cardioprotection. In other work, it has been shown that the cystic fibrosis transmembrane regulator (CFTR), a member of the ATP binding cassette family of proteins, augments the cellular uptake of both S1P and LPA [Boujaoude et al., 2001]. The CFTR also has been implicated in the mechanism of IPC as CTFR blockade by gemfibrozil or genetic deletion of the CFTR abolished IPC-induced cardioprotection [Chen et al., 2002]. Taken together, the above results strongly implicate sphingolipid signaling in the mechanism of IPC.

SPHINGOSINE KINASE

Sphingosine kinase catalyses the formation of S1P from sphingosine. Two mammalian isozymes, known as SphK1 and SphK2, have been characterized. SphK1 is abundant in human and mouse heart. It is predominantly a cytosolic enzyme, whereas its substrate sphingosine is generated in membranes. Thus, translocation to membranes is a common feature of SphK1 activation [Spiegel and Milstien, 2003]. SphK (presumably SphK1 in most instances) can be activated by diverse agonists, many of which act through G-protein coupled receptors [for a review, see Maceyka et al., 2002]. Liu et al. [2003] have recently reported that SphK2, despite its sequence similarly to SphK1, promotes apoptosis instead of promoting cell proliferation as does SphK1. This occurs in part by stimulating cytochrome c release from mitochondria along with caspase activation.

As SphK1 is a critical step in the synthesis of S1P, its regulation is of considerable interest. PKC has been implicated in the mechanism of cardioprotection [Gray et al., 1997], and activation of SphK1 in many instances is PKC-dependent. Recently it has been shown that PKC activation results in translocation of SphK1 to the plasma membrane and export of S1P into the media [Johnson et al., 2002]. This process would allow for autocrine/paracrine signaling in response to PKC activation. Olivera et al. [2003] have recently used overexpression of SphK1 in cell culture to demonstrate a role for intracellular S1P in promoting cell growth and survival independent of G-protein-coupled receptors.

SphK1 can also be activated by ERK1/2 or a close relative [Pitson et al., 2003]. It is likely that the latter as well as PKC phosphorylate SphK1. As noted above, TNF-α is cardioprotective, and there is considerable evidence that this molecule activates SphK, presumably SphK1. In a recent report, Xia et al. [2002] identified a TNF receptor-associated factor 2 (TRAF2)-binding motif of SphK1 that mediated the interaction between TRAF2 and SphK1. This resulted in the activation of SphK1, which in turn was required for TRAF2-mediated activation of NF-κB and the anti-apoptotic effect of TNF.

What is the role of SphK in hypoxic or ischemic insults to cardiac cells? There is evidence that SphK activation may be critical for the protection of the ischemic myocardium, presumably by generating S1P which may either act intracellularly [Olivera et al., 2003], or be exported to act in an autocrine/paracrine manner as noted above [Johnson et al., 2002]. The monosialoganglioside GM-1 activates SphK via its stimulation of PKC and protected cardiac fibroblasts against staurosporine- and C2-ceramide-induced cell death [Cavallini et al., 1999]. In the same study, it was shown that GM-1 induced the synthesis of S1P, an effect that was partially blocked by the SphK inhibitor *N*,*N*-dimethylsphingosine (DMS). Subsequently, we treated neonatal rat ventricular myocytes with S1P or GM-1 and found that both prevented DMS-induced cell death [Karliner et al., 2001].

The ability of GM-1, as well as S1P, to prevent cardiac myocyte cell death under in vitro conditions of severe hypoxia prompted us to ask whether these molecules are cardioprotective in an isolated heart preparation subjected to I/R. As noted earlier, S1P is cardioprotective and does not depend on PKC for its action. In contrast, GM-1, which was highly effective in wild-type littermate mouse hearts, was not cardioprotective in hearts from εPKC knockout mice [Jin et al., 2002]. In these experiments, conditions were identical to the S1P experiments described above: GM-1 was infused for 2 min at 10 nM before I/R. In wild-type littermates hemodynamics (left ventricular developed pressure, left ventricular end-diastolic pressure, $\pm$dP/dt), creatine kinase release, and infarct size were improved, but this was not the case in the εPKC knockout mouse. Thus the εPKC KO mouse model provides further evidence that one of the pathways stimulated by translocation of εPKC is generation of S1P via activation of SphK.

In preliminary experiments, we have tested the hypothesis that SphK may be involved in the mechanism of IPC [Jin et al., 2003]. As expected, in isolated Langendorff perfused mouse hearts, IPC significantly reduced myocardial I/R injury. DMS administered before IPC substantially reduced the cardioprotection induced by IPC as measured by hemodynamics and infarct size. SphK activity, as assessed by conversion of [^{3}H]-sphingosine to [^{3}H]-S1P, was augmented by IPC and GM-1 and inhibited by DMS. As both GM-1 and IPC activate PKC, these observations are consistent with the possibility that PKC by phosphorylating SphK indirectly generates

increased intracellular S1P, which can then contribute to the beneficial effect of IPC by mechanisms independent of G-protein coupled receptors that remain to be determined in cardiac myocytes. An alternative or even complementary possibility is that some or most of the S1P so generated could be exported and act as an autocrine/paracrine agonist at S1P receptors on the myocyte cell surface.

CERAMIDE

Ceramide and sphingosine are usually considered to be molecules that promote apoptosis, while the metabolite of sphingosine, S1P, generated by the action of SphK, preserves cell function [Maceyka et al., 2002]. These metabolites are interconvertable, and it is their relative balance at any given time that favors cell survival or death. In neonatal rat ventricular myocytes, one of the earliest responses to hypoxia and reoxygenation is the activation of neutral sphingomyelinase and accumulation of ceramide [Hernandez et al., 2000]. In this study, c-Jun N-terminal kinase (JNK) was also activated and pretreatment with antioxidants quenched sphingomyelinase activation, ceramide accumulation, and JNK activation. The location of ceramide was not specified in this report, but there is abundant evidence that ceramide is located in mitochondria [van Blitterswijk et al., 2003]. Of note is that Birbes et al. [2001] reported that selective hydrolysis of a mitochondrial pool of sphingomyelin produced apoptosis and concluded that ceramide induces cell death specifically when generated in mitochondria. Ceramide can cause reactive oxygen species to be produced at the level of either complex 1 or complex III of the mitochondrial respiratory chain associated with cytochcrome c release [Di Paola et al., 2000]. The mechanism of cytochrome c release may be via the formation of channels in the outer mitochondrial membrane by ceramide [Siskind et al., 2002]. However, it should be noted that there is at least one report in which ceramide was noted to be cardioprotective [Lecour et al., 2002].

Another possible mechanism by which ceramide may act is the vesicular transport of lipid-raft bound GD3, a ceramide-derived ganglioside that may serve to guide pro-apoptotic Bcl-2 family members such as tBid, dephosphorylated Bad or Bax toward their mitochondrial targets [van Blitterswijk et al., 2003]. Ceramide also binds PKCζ, which may result in the inability of this isozyme to activate nuclear transcription factors after I/R [van Blitterswijk et al., 2003].

In several leukemia cell lines, Cuvillier and Levade [2001] reported that S1P antagonizes apoptosis by inhibiting the release of cytochrome c and Smac/DIABLO from mitochondria. In these studies, a phorbol ester which activates PKC had a similar effect, and DMS, which inhibits SphK, sensitized cells to mitochondrial dysfunction by ceramide. Taken together, these studies suggest a key role for the ceramide-S1P "rheostat" in mitochondria. As cardiac myocytes contain abundant amounts of these organelles and are heavily dependent on energy generation for their function, a mitochondrial action of S1P in the heart deserves further investigation.

SIGNALING

As indicated earlier S1P signals via diverse G-proteins depending on which receptor subtype is activated. Most subsequent signaling events in cardiovascular cells have been studied in vascular smooth muscle and endothelial cells [see Levade et al., 2001 for a review]. Regulators of G-protein signaling function as GTPase-activating proteins for the Gα subunit of heterotrimeric G-proteins and are abundant in human heart. They have recently been reported to selectively regulate $S1P_{1-3}$ responses in human aortic smooth muscle cells [Cho et al., 2003]. As virtually no work on S1P signaling has been reported in cardiac cells, we used quiescent cultured adult mouse ventricular myocytes to study signaling pathways activated by S1P and GM-1. In preliminary experiments, we observed that 100 nM S1P stimulated phospho-Akt in a pertussis toxin-sensitive manner [Zhang et al., 2003]. This response was MEK/ERK independent but PI-3 kinase and p38 MAP kinase dependent. GM-1 acted in a similar manner. These responses, which are characteristic of a cell survival pathway, exhibit both similarities and differences from reported data in other cell types. How or if they will be altered following hypoxic or oxidative stress in cell culture or in the whole heart remains to be determined. In addition S1P receptor crosstalk with other G-protein-coupled receptors has not yet been studied in cardiac myocytes, nor has receptor trafficking been investigated.

In summary, there is abundant evidence that S1P is cardioprotective when given exogenously

or generated endogenously via activation of sphingosine kinase. It is effective as a preconditioning mimetic agent in cell culture and ex vivo. PKC, particularly the ε isoform, via its activation of sphingosine kinase, appears to be an important regulator of S1P effects, especially under conditions of I/R injury. εPKC is also an important regulator of $S1P_1$ receptor recycling after receptor downregulation, but this has not yet been studied in cardiac myocytes. The role of S1P phosphatases and S1P lyase has not yet been studied under these circumstances, nor have the intracellular signaling pathways or molecular targets of S1P been identified during I/R injury. Potential systemic effects of S1P at concentrations that are cardioprotective require careful prospective study.

LYSOPHOSPHATIDIC ACID

LPA has been extensively studied in cells and organs other than the heart. Hemodynamic responses to LPA have varied according to species, but blood pressure elevation appears to be prominent. Administration of LPA acutely results in a positive inotropic effect, but an acute antiadrenergic effect resulting in decreased myocardial contractility has also been reported. Like S1P, LPA can increase protein synthesis in cardiomyocytes in culture [Goetzl et al., 2000]. The signaling cascade involved in increased protein synthesis includes activation of ERK1/2 via PKC and PI 3-kinase and was associated with increased phosphotransferase activity of p70S6K. Recent reports indicate that LPA also signals intracellularly by activating nuclear transcription factors [Gobeil et al., 2003; McIntyre et al., 2003]. In neonatal rat ventricular myocytes, we showed that LPA was cardioprotective [Karliner et al., 2001], but further studies in either isolated hearts or whole animal models of myocardial infarction have not been performed.

FUTURE DIRECTIONS

While much has been learned about the importance of lysophospholipids in the cardiovascular system, there are many unanswered questions, particularly regarding cardioprotection. A few of the more outstanding questions are as follows.

1. Will systemic administration of S1P or LPA be hemodynamically tolerated in intact animals subjected to permanent coronary artery ligation or I/R?
2. If not, is GM-1 an acceptable substitute for S1P, or will the development of selective receptor ligands be necessary?
3. What is the role of intracellular S1P in cardioprotection, and how does it work? Or is intracellular S1P largely exported to function at the cell membrane as an external autocrine/paracrine ligand?
4. Where does SphK act—in the cytosol, in the sarcolemma, or in the mitochondria? Or in all three?
5. How is the ceramide/mitochondrial connection related to cardiac myocyte salvage produced by S1P?
6. How are the cardiac S1P and LPA receptors regulated under normoxic conditions, and what signaling cascades are activated when their ligands are present? How are the responses affected by hypoxia, I/R, or oxidative stress?
7. Will serum S1P levels turn out to be a useful predictor of the presence and severity of coronary artery obstruction as has been recently proposed [Deutschman et al., 2003]?

In summary, the role of lysophospholipids in myocardial biology and pathophysiology, and their potential use as therapeutic agents, have become apparent during the past few years. Because of the extensive prevalence of cardiovascular disease, the links between the survival benefit of lysophospholipids that have been amply demonstrated by in vitro experiments have been extended to the whole heart. Future directions that include studies in intact animals, either directly with S1P or with agents that activate sphingosine kinase, will yield much useful information and will point the way to future strategies for myocardial salvage.

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