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Review

Lysophospholipid receptor activation of RhoA and lipid signaling pathways ☆

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ABSTRACT

The lysophospholipids sphingosine 1-phosphate (S1P) and lysophosphatidic acid (LPA) signal through G-protein coupled receptors (GPCRs) which couple to multiple G-proteins and their effectors. These GPCRs are quite efficacious in coupling to the $G\alpha_{12/13}$ family of G-proteins, which stimulate guanine nucleotide exchange factors (GEFs) for RhoA. Activated RhoA subsequently regulates downstream enzymes that transduce signals which affect the actin cytoskeleton, gene expression, cell proliferation and cell survival. Remarkably many of the enzymes regulated downstream of RhoA either use phospholipids as substrates (e.g. phospholipase D, phospholipase C-epsilon, PTEN, PI3 kinase) or are regulated by phospholipid products (e.g. protein kinase D, Akt). Thus lysophospholipids signal from outside of the cell and control phospholipid signaling processes within the cell that they target. Here we review evidence suggesting an integrative role for RhoA in responding to lysophospholipids upregulated in the pathophysiological environment, and in transducing this signal to cellular responses through effects on phospholipid regulatory or phospholipid regulated enzymes. This article is part of a Special Issue entitled Advances in Lysophospholipid Research.

1. Introduction

Sphingosine 1-phosphate (S1P) and lysophosphatidic acid (LPA) are small, bioactive membrane lipid derivatives that play critical roles in many physiological and pathophysiological processes, including development [1], cancer [2], multiple sclerosis [3–5], pain, inflammation, fibrosis [6,7], atherosclerosis, and myocardial ischemia/reperfusion (I/R) [7–9]. Both S1P and LPA act as extracellular signaling molecules by binding to and activating seven-transmembrane spanning G protein-coupled receptors (GPCRs), which exist as multiple subtypes ($S1P_{1-5}$ [10] and LPA_{1-6} [11]). The cloning and identification of the lysophospholipid receptors opened new avenues for research into the signaling pathways and biological processes elicited by the actions of S1P and LPA. Each of the S1P and LPA receptor subtypes signals through specific G-proteins, $G\alpha_{q/11}$, $G\alpha_{12/13}$, $G\alpha_i$ or their associated $G\beta\gamma$ subunits [10,12]. The specificity of receptor subtype coupling to the various G proteins depends on their expression levels and cell type; thus, the downstream signals elicited following receptor activation by S1P and LPA are context dependent. Studies using specific agonists and antagonists as well as cloned receptors and knockout mice have, however, provided considerable impetus to uncovering the significance of lysophospholipid signaling through distinct receptor subtypes [6,7,11,13].

The S1P and LPA receptors are among the most efficacious of the GPCRs shown to couple to $G\alpha_{12/13}$ and hence to activate guanine nucleotide exchange factors (GEFs) for the low molecular weight G protein RhoA [14–17]. RhoA functions as a nodal point in transducing extracellular signals to regulate a wide range of cellular responses [18–20]. In this review, we focus on the ability of S1P and LPA to signal through regulation of RhoA activity, and the molecular mechanism by which RhoA controls enzymes involved in, and regulated through, lipid metabolism. We consider the role of this pathway in myocardial I/R injury and briefly discuss its potential role in neuroinflammation.

2. S1P and LPA Regulates RhoA signaling through $G\alpha_{12/13}$

2.1. RhoA activation

RhoA is a member of the Rho family GTPases and has well-established functions in cytoskeletal organization, inducing the formation of actin stress fibers and focal adhesions [20–22]. Constitutive expression of $G\alpha_{12}$ and $G\alpha_{13}$ has been demonstrated to induce stress fiber and focal adhesion formation, as well as tyrosine phosphorylation of focal adhesion kinase (FAK) and paxillin, all in a Rho dependent manner [23,24]. In response to GPCR agonists like LPA, RhoA also mediates neurite retraction and inhibiting RhoA function using C3 exoenzyme or dominant negative RhoA leads to neurite outgrowth [25,26]. Signaling via S1P and LPA to $G\alpha_{12/13}$ and RhoA has also been linked to cell migration and proliferation as well as transcriptional gene regulation [24,27,28].

The activity of Rho is regulated by GTPase activating proteins (GAPs), GTP exchange factors (GEFs), and GTP dissociation inhibitors (GDIs). GAPs mediate the inactivation of small G proteins by promoting their intrinsic GTPase activity while GEFs promote the exchange

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of GDP for GTP and thereby activate the small G protein. Members of the $G\alpha_{12/13}$ family proteins have been shown to interact directly with and thereby activate several RhoGEFs including p115-RhoGEF, PSD-95/Disc-large/ZO-1 homology (PDZ)-RhoGEF, leukemia-associated RhoGEF (LARG), and lymphoid blast crisis (Lbc)-RhoGEF [17,29]. The p115RhoGEF was also shown to function as a GAP and thereby a signal terminator for $G\alpha_{12/13}$ [30]. All four RhoGEFs are large proteins that are widely expressed in mammals. Dysfunction of the $G\alpha_{12/13}$ RhoGEF pathway can lead to pathophysiological outcomes including cancer, cardiovascular diseases, arterial and pulmonary hypertension [17]. Recent studies demonstrate that $G\alpha_q$ can also regulate RhoGEFs to activate RhoA [15,31,32], however this is a less dedicated pathway since $G\alpha_q$ also couples to phospholipase C-beta (PLCβ) [33–37].

2.2. RhoA effectors

Once activated by GTP binding, RhoA recruits various downstream effectors that relay signals to induce changes in cell shape and movement, proliferation and cell cycle progression, cell survival and gene expression [20,27,38]. Many Rho effectors have been discovered using affinity chromatography, yeast two-hybrid and mutational analysis [39,40]. The most common mechanism for effector activation by GTP-RhoA is the disruption of intramolecular autoinhibition, which exposes the functional domain of the effector protein [20,40]. RhoA effectors include the extensively characterized Rho associated protein kinase (ROCK), a serine/threonine kinase termed protein kinase N (PKN or PRK) and the mammalian diaphanous (mDia) [40–42]. ROCK is a serine/threonine kinase that is activated by binding of GTP-RhoA to its C-terminal coiled-coil domain. Activated ROCK subsequently phosphorylates a number of substrates, including LIM kinase and myosin light chain phosphatase, and thereby modulates actin–myosin cytoskeletal dynamics and contraction [43]. PKN is another serine/threonine kinase that is a direct target of RhoA [39,44,45], which has been shown to be activated by LPA in Swiss 3T3 fibroblasts in a Rho-dependent manner [46,47]. PKN is involved in cytoskeletal regulation, cell adhesion, and cell cycle [48]. A recent report demonstrated that PKN also mediates cardiac myocyte survival in response to oxidative stress or I/R injury [49]. In addition to the canonical effectors mentioned above, RhoA directly or indirectly regulates a number of phospholipid regulatory and regulated enzymes implicated in biological responses. These lipid regulated/regulatory enzymes are discussed in more detail below.

3. Activation of phospholipases by RhoA

3.1. Phospholipase D

The phosphatidylcholine (PC)-selective phospholipase D (PLD) was recognized as a regulated player in cell signaling events beginning in the late 80s. Among the myriad responses that have been subsequently attributed to PLD are cytoskeletal reorganization, membrane trafficking, signal transduction, transformation, proliferation, and cell survival [50–54]. Activated PLD hydrolyzes PC to produce the bioactive lipid phosphatidic acid (PA) and free choline. PA can be further metabolized to generate diacylglycerol (DAG), a second messenger that activates protein kinase C (PKC) and subsequently protein kinase D (PKD). PA can, in addition, be converted to LPA through the actions of secretory type II phospholipase A2 (PLA2) [52,53,55]. Two mammalian PC-selective PLD isozymes, PLD1 and PLD2, have been identified. These isozymes share 53% sequence homology and are subject to different regulatory mechanisms. Abundant evidence indicates that RhoA binds to and activates PLD1, but not PLD2 [51,56,57]. PLD1 has very low constitutive activity and possesses an inactive conformation until bound to its activators. Interestingly, there are multiple and redundant mechanisms by which RhoA regulates PLD1. First, RhoA can activate PLD1 through direct interaction with its C-terminus [56]. In addition, it can act indirectly through its effector PKN, which also interacts with and activates PLD [58]. Another indirect mode of activation is that RhoA regulates phosphatidylinositol (PI) 4-phosphate 5-kinase PIP5K, increasing the synthesis of PI-4, 5-bisphosphate (PIP2), which serve as a critical co-factor for PLD [53,59]. S1P and LPA have been shown to activate PLD in various cell types [60–64]. PLD activation by S1P regulates interleukin-8 secretion in human bronchial epithelial cells and therefore contributes to S1P mediated inflammatory response [60,61]. In human PC-3 prostate cancer cells, LPA was found to activate PLD and induce cell proliferation [63]. Whether RhoA is involved in S1P or LPA mediated PLD activation and downstream effects through mechanisms described above has not been considered.

PLD signaling has been implicated in myocardial protection and there is considerable evidence that PLD is activated and regulated by oxidative stress, a cellular response associated with various cardiac pathologies including coronary heart disease [65]. It has been reported that PLD activation by oxidation involves activities of protein tyrosine kinases and phosphatases [66]. Whether oxidative activation of PLD also occurs through RhoA is not known, but RhoA has a redox-sensitive motif and can be directly activated by reactive oxygen species (ROS) [67,68]. We recently determined that RhoA is rapidly and robustly activated by hydrogen peroxide and by I/R injury [69]. The possibility that RhoA and PLD work together to mediate cardiac protection is supported by the observation that disrupting the direct interaction of RhoA and PLD prevents adenosine-induced cardioprotection from I/R injury [70]. PLD is also activated by ischemic preconditioning and contributes to preconditioning mediated cardioprotection, a paradigm in which brief periods of I/R, applied before the sustained I/R event, protect against the subsequent sustained I/R insult [71,72]. Of particular interest, S1P released by ischemic preconditioning or exogenously applied to the heart can serve as a powerful pharmacological preconditioning stimulus to protect the heart against I/R injury [73,74]. This cardioprotective pathway will be discussed in detail later in Section 5.1 in this review.

3.2. Phospholipase C-epsilon

Rho family GTPases have been shown to regulate phospholipase C (PLC) signaling, and earlier work suggested that this effect was not due to direct PLC activation, but rather through the regulation of PLC substrate PIP2 availability [75,76]. Rho family proteins stimulate the activity of PIP5K, a phospholipid kinase that catalyzes the phosphorylation of the membrane phospholipid phosphatidylinositol 4-phosphate to generate PIP2 [59,77,78]. More recent studies revealed that RhoA actually has direct effect on a family member of PLC, phospholipase C-epsilon (PLCε), through physical interaction with its catalytic domain [79–82]. PLCε is a recent addition to the PLC family (see [83–85] for reviews). Like other PLC enzymes, PLCε hydrolyzes PIP2 to generate two important second messengers, DAG and inositol 1,4,5-triphosphate (IP_3). Generation of DAG is required for the activation of all of the conventional and novel PKC family members as well as other C1 domain containing, DAG sensitive enzymes. IP_3 controls Ca^{2+} release from intracellular IP_3 sensitive Ca^{2+} stores. These second messengers can be generated through any of the more commonly studied PLC isoforms including PLCβ and PLCγ. Unlike PLCβ, which is regulated by binding to the $G\alpha_q$ subunit, and PLCγ, which is regulated by tyrosine phosphorylation, PLCε is unique in being regulated by small GTPases of the Ras family including Ras, Rap1 and RhoA [80,83,85–87]. Ras binds to the Ras-associating (RA) domain, and RhoA to a site in the catalytic domain of PLCε leading to its activation [80,83,85,86,88]. PLCε is also unique in containing an extended N terminus which includes a CDC25 domain not found in other PLC family members. This domain allows it to function as a GEF for Rap1. The activated Rap1 formed in this