New Roads Leading to Ca$^{2+}$ Sensitization

Avril V. Somlyo

G protein–coupled receptors (GPCRs) activated by a wide variety of agonists can switch on myosin light chain phosphorylation and force in smooth muscle. The extent of myosin light chain phosphorylation reflects activity of both the Ca$^{2+}$/calmodulin–dependent myosin light chain kinase (MLCK) and the myosin light chain phosphatase. Thus, at constant Ca$^{2+}$ and MLCK activity, processes that inhibit myosin phosphatase activity cause a leftward shift of the Ca$^{2+}$ force-response curve, a physiological relevant phenomenon known as Ca$^{2+}$ sensitization.\(^1,2\) There are two well-described myosin phosphatase inhibitory pathways. The first is through the small GTPase RhoA, in which GTP-bound RhoA translocates to the membrane and activates Rho-kinase that either directly or indirectly acts on the regulatory phosphatase subunit (MYPT-1) to inhibit phosphatase activity. The second signaling pathway, which is present in only some smooth muscles, is through phosphorylation of a phosphatase inhibitor, CPI-17 that, when phosphorylated, potently inhibits the catalytic subunit of myosin phosphatase.\(^3\) Crosstalk between the two pathways has been implicated from experiments using the Rho-kinase inhibitor Y-27632.\(^4\) A new signaling messenger of Ca$^{2+}$ sensitization, which converges on the RhoA pathway at the level of Rho-kinase, is presented by Kobayashi and colleagues\(^5\) in this issue of Circulation Research: they demonstrate that sphingosylphosphorylcholine (SPC), a product of sphingomyelin deacylation, leads to an increase in force in the absence of an increase in the fura 2 Ca$^{2+}$ signal. This force is inhibited by a Rho-kinase inhibitor, but not by inhibition of conventional or novel PKCs. Interestingly, the Ca$^{2+}$-independent contraction was maintained for \(\approx2\) hours, even after removal of SPC from the bathing medium, and was completely and reversibly relaxed by Y-27632: subsequent stimulation with 118 mmol/L K$^+$/H$^+$1015 induced a normal increase in [Ca$^{2+}$] and contraction. The redevelop-oment of force after a quick-release step during the maintained contraction indicated that SPC did not interfere with the ability of myosin crossbridges to hydrolyze MgATP and cycle normally. Provided that SPC is indeed released into the subarachnoid space, this study, carried out on cerebral arteries, has important implications for the therapy of cerebral vasospasm, a major health risk in some populations. Further studies on SPC production, regulation, and misregulation should establish its significance in normal and/or pathological states.

Apart from their metabolic roles, lysophospholipids such as lyso(sphingophosphoryl)choline (LPA), sphingosine 1-phosphate (SIP), and sphingosylphosphorylcholine have long been known to be associated with cell growth and differentiation but are now also recognized as important signaling molecules functioning in a variety of intracellular pathways that target cell migration, cell growth, and cell survival (antiapoptosis), through effects on such processes as calcium mobilization, inhibition of adenylate cyclase, and activation of mitogen-activated protein kinases.\(^6,9\) SIP has also been implicated in cerebral vasospasm.\(^10\) It has been suggested\(^11\) that lysophospholipids are likely to be as important a class of intracellular signaling molecules as the glycerophospholipid metabolites, which give rise to inositol 1,4,5-trisphosphate (InsP$_3$), diacylglycerol, phosphatidic acid, and arachidonic acid.

A major advance in this still young field has been the identification and cloning of GPCRs for some of the lysophospholipids. Five of the eight GPCRs, commonly called Edg receptors for endothelial differentiation gene, bind SIP with nanomolar affinities and SPC \(\approx10^{-10}\) to 100-fold less efficiently. The remaining three receptors preferentially bind LPA. (For a review of current nomenclature and structure activity relationships of lysophospholipid messengers, see References 9 and 11.) SPC is reported to be a high-affinity ligand \((K_D=33\ \text{nmol}/L)\) for the ovarian cancer G protein–coupled receptor 1 (OG1R1); it is expressed in several tissues and upon activation leads to Ca$^{2+}$ mobilization and activation of p42/44 mitogen-activated protein kinases.\(^12\) A second closely related GPCR, also claimed to be activated by SPC, GPR4, has also been identified.\(^11\) Surprisingly, SPC-mediated Ca$^{2+}$ sensitization in the cerebral artery described in the present study does not appear to signal through a GPCR. This conclusion was largely based on the finding that, unlike agonist-induced Ca$^{2+}$ sensitization, SIP does not require the addition of GTP to the permeabilized smooth muscles, nor was SPC-induced Ca$^{2+}$ sensitization affected by the nonhydrolyzable GDP/JS, which inhibits signaling by GPCRs. Some of these same investigators have also reported similar effects of SPC (1 to 50 \(\mu\text{mol}/L)\) on intact, denuded of endothelium, and permeabilized pig coronary artery smooth muscle.\(^13\) On the other hand, in intact endothelial cells, SPC and SIP increase cytoplasmic [Ca$^{2+}$] through release of intracellular calcium as well as Ca$^{2+}$ influx.\(^14,15\) SPC also increases production of NO, leading to endothelium-dependent vasorelaxation with an \(EC_{50}=5\ \mu\text{mol}/L.\)\(^14\) The different concentrations used by both groups make it probable that Ca$^{2+}$ mobilization is through lysophospholipid GPCRs, whereas the activation of NO may be through direct action of SPC on the eNOS/NO pathway, because SPC readily crosses cell membranes. Evaluation of the contributions of SPC as a signaling molecule in vascular biology will require determination of the intracellular and extracellular concentrations of SPC in normal and pathological states.

If SPC, as suggested, directly activates Rho-kinase, it may be that it interacts with the putative negative regulatory region of Rho-kinase, possibly the pleckstrin homology domain (PH), based on consideration of the effects of arachidonic acid in vitro.\(^16,17\) In this model, the Rho binding domain (RB) and PH domains interact with the catalytic domain to inactivate the kinase (Figure). The enzyme

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is thought to be activated by the binding of RhoA · GTP to RB, thereby releasing the inhibition of the catalytic site. Perhaps SPC and other lipid messengers activate Rho-kinase through a similar mechanism, releasing the inhibitory from the catalytic domain through a conformational change and exposing the catalytic site. Direct activation of Rho-kinase by lipid messengers should not be inhibited by upstream inactivation of RhoA by the bacterial eexoenzyme C3, whereas an inhibitor of SPC production (eg, sphingomyelinase C3) would be expected to inhibit vasospasm due to subarachnoid hemorrhage. These experiments remain to be performed.

In summary, these and other studies revealing the activities of intracellular and extracellular phospholipid mediators point to new mechanisms of signaling and potential new therapeutic targets for pathophysiological states of the cardiovascular system.

References


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