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When S1P meets ATP

Philipp Burow and Fritz Markwardt*
Julius-Bernstein-Institute for Physiology; Martin-Luther-University Halle; D-Halle/Saale, Germany

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*Correspondance to: Fritz Markwardt; Email: fritz.markwardt@medizin.uni-halle.de

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Biological cells possess plenty of mechanisms to maintain intracellular cell volume if they are challenged by changes of osmolarity. For example, if the extracellular space becomes hypoosmolar or the intracellular osmolarity increases due to a massive influx of Na^+ ions, the accompanying water influx leads to cell swelling. The cells compensate for this by extrusion of water together with intracellular organic osmolytes, such as taurine, or by a coupled outward flux of K^+ and Cl^- ions using the electrochemical gradient for K^+. For this, cell swelling activates a K^+-Cl^- cotransport or a K^+-efflux electrically coupled to Cl^- outflow through Cl^--conducting ion channels.[1] The molecular identity of the involved volume-regulated anion channels (VRAC) (or volume-sensitive outwardly rectifying anion channels VSOR) was enigmatic until 2 groups, which performed genome-wide small interfering RNA screens, reported the LRRC8 proteins to be essential parts of VRAC.[2,3] LRRC8 proteins seem to be ubiquitously expressed and the diversity of biophysical properties of VRAC channels may be due to heteromerization of different LRRC8 subunits (LRRC8A to E), with LRRC8A being the most important subunit. VRAC does not only conduct Cl^- but also amino acids such as $glutamate^-$ and $aspartate^-$. Whether VRAC also conducts $MgATP^{2-}$ or ATP^{4-}, i.e., is involved in ATP secretion, is still a matter of debate.[4] Beside the commonly known ATP-release from damaged cells, ATP efflux from cells also occurs during mechanical alteration and hypoxia as well as from platelets during blood coagulation. In the immune system, extracellular ATP serves as a danger-associated molecular pattern (DAMP), informing cells about an upcoming danger. To do this, ATP binds to purinergic G-protein coupled P2Y receptors or ATP-gated ion channels (P2X receptors).[5]

Apart from the purinergic signaling system, sphingolipid metabolites play an important regulatory role in the immune system. A key mediator is sphingosine-1-phosphate (S1P), produced by sphingosine kinases which are activated by several different inflammatory signaling molecules like bacterial lipopeptides and lipopolysaccharide (LPS) from Gram-negative bacteria, platelet-derived growth factor (PDGF), tumor-necrosis factor α (TNFα), thrombin, IgE-bound antigen and, not to mention, ATP. S1P signaling is mediated by 5 different G-protein coupled S1P receptor subtypes (S1PR1-5) and signaling is upregulated in autoinflammatory diseases and sepsis. FTY720 (fingolimod) acts as a functional antagonist of S1PRs and is applied as an immunosuppressant in patients suffering from multiple sclerosis.[6]

In our recent paper, we reported a possible link between the inflammatory sphingolipid and purine signal systems.[7] We observed that apart from cell swelling, VRAC channels can be opened by nanomolar concentrations of S1P via activation of G_i-coupled S1PR1. The strong inhibition of VRAC-currents by the actin-filament disruptor cytochalasin D, points to an involvement of microfilaments in VRAC-activation. The S1P-induced currents are inhibited by osmolarity-induced cell shrinkage and by the same profile of anion blockers acting on swelling-induced currents. This points to VRAC as the S1P-activated ion channel. S1P and, as was already known, cell swelling, induced the secretion of ATP from macrophages. The secretion of ATP was found to be diminished by the same blockers which inhibit the anion currents. Therefore, an autocrine or paracrine activation of purinergic receptors via S1P-induced ATP

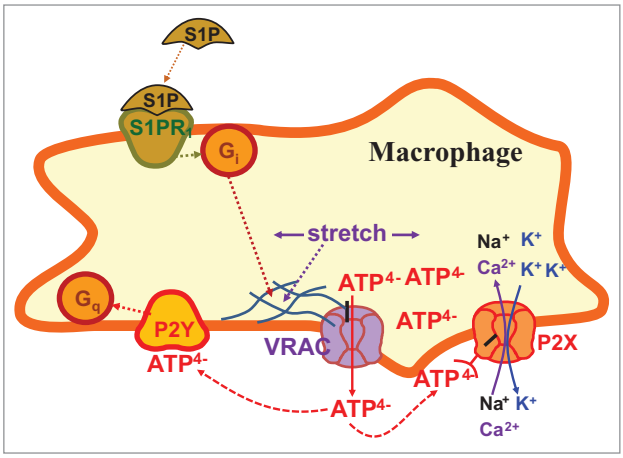

Figure 1. ATP secretion via S1P-dependent activation of VRAC: In macrophages, S1P activates S1P receptor subtype 1 (S1PR1), which signals via a trimeric G_i protein to the cytoskeleton to open VRAC. The efflux of ATP^{4-} may lead to activation of purinergic receptors (G-protein coupled P2Y and/or ligand-gated ion channels P2X).

release in macrophages is conceivable (**Fig. 1**). This mechanism may also work in the earlier-proposed S1P-induced and P2X7 receptor-mediated cortical actin assembly in macrophages used by us.[8]

Whether these processes observed in vitro are of relevance in vivo remains to be established. The molecular mechanism of VRAC activation and its coupling to the cytoskeleton is still unknown. The recent identification of LRRC8 proteins as components of VRAC may offer new tools to investigate this issue. Furthermore, different ATP-secreting mechanisms apart from VRAC may even work in the same cell. Knocking down of LRRC8 subunits may become a valuable means of targeting VRAC more specifically than the notorious unspecific anion channel blockers, especially since LRRC8A is expressed in the RAW macrophages used us (F. Markwardt, unpublished observation). Moreover, a "retrograde" activation of VRAC by massive Na^+ influx via P2X receptors leading to cell swelling is conceivable. Especially the non-desensitizing P2X7 receptor expressed in cells of the immune and inflammatory system can mediate such long-lasting cation influx. An exciting chapter in the investigation of the sphingolipid and purinergic signaling and the involvement of VRAC channels therein may be opened.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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