

Constitutive and agonist stimulated ATP secretion in leukocytes

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Release and reception of extracellular ATP by leukocytes plays a critical role in immune responses to infection, injury and cardiovascular disease. Leukocytes of both the innate, adaptive immune and central nervous system express a repertoire of cell surface receptors for ATP (P2X and P2Y receptors) and its metabolites. ATP acts as a damage-associated molecule pattern (DAMP) released by injured or dying cells. Detection of released ATP by neighboring leukocytes initiates inflammation and wound healing. However, recent evidence from our group and others suggests ATP release by leukocytes themselves serves to regulate homeostatic mechanisms and coordinate responses to external pro-inflammatory cues. Examples include the homeostatic control of intracellular calcium and regulation of migratory guidance during chemotactic response to external cues. Though there has been some progress in elucidating ATP release mechanisms of some mammalian cells types, release conduits and coupling signal transduction machinery remain larger elusive for leukocytes. Our recent studies suggest a role for secretory lysosomes in releasing ATP in monocytes. Though poorly defined, targeting ATP release mechanisms in leukocytes have great anti-inflammatory potential.

Purinergic Signaling in Blood Cells and Leukocytes

Beyond its role of cellular energy currency and phosphate donor, ATP plays a potent signaling role through its extracellular release and activation of cell surface purinergic receptors.^{1,2} Fast responses to ATP are mediated through activation of P2X receptors,³ a family (P2X₁–P2X₇) of

ATP-activated ligand-gated ion channels and metabotropic effects through the activation of P2Y receptors (P2Y₁₋₂, P2Y₄, P2Y₆, P2Y₁₁₋₁₄) which couple to heterotrimeric G proteins. Human leukocytes including monocytes, mast cells, neutrophils and central microglia express a diverse repertoire of P2Y receptors though P2X₁, P2X₄ and P2X₇ are the dominant P2X subtypes present. Activation of purinergic receptors in leukocytes is coupled to the production and secretion of cytokines and other pro-inflammatory molecules including prostaglandin E₂.⁴ Purinergic receptors are associated with inflammation, with some receptors inhibited either directly (P2Y₁₂) or indirectly through the activity of anti-thrombotic, in the case of platelet P2Y₁₂, or anti-inflammatory agents, in the case of the action of statins on monocyte P2X₄.⁵

ATP can act as a non-peptide damage-associated molecular pattern (DAMP) release from injured cells and tissues. In this fashion the release of cellular ATP is unregulated and released due to cell lysis or puncture. Cell surface purinergic receptors are activated by this ATP DAMP signal which serves to initiate an inflammatory response and promote wound healing. However, ATP can be released from cells physiologically and act as a critical signal for painful, inflammatory processes. Cells do release other nucleotides including UTP and UDP-sugars but our focus here is ATP release. Mechanisms of ATP release during physiological processes remain diverse and controversial.

ATP Release Mechanisms in Leukocytes

Routes of ATP release in mammalian cells remain diverse and often controversial.

Keywords: purinergic signaling, ATP release, inflammation, leukocytes, nucleotide

Submitted: 01/14/13

Accepted: 01/15/13

Citation: Campwala H, Fountain SJ. Constitutive and agonist stimulated ATP secretion in leukocytes. *Commun Integr Biol* 2013; 6: e23631; <http://dx.doi.org/10.4161/cib.23631>

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Addendum to: Sivaramakrishnan V, Bidula S, Campwala H, Katikaneni D, Fountain SJ. Constitutive lysosome exocytosis releases ATP and engages P2Y receptors in human monocytes. *J Cell Sci* 2012; 125:4567-75; PMID:22767503; <http://dx.doi.org/10.1242/jcs.107318>.

Investigation into how cellular stress stimulates ATP release in non-leukocytes suggests roles for connexin and pannexin hemichannels,^{6,7} maxi- and volume-regulated anion channels^{8,9} and efflux through the P2X₇ receptor,¹⁰ though release routes and signal transduction mechanism underlying ATP release in leukocytes remain poorly defined.

Agonist Stimulated ATP Release

In neutrophils ATP is released in response to activation of fMLP receptor by bacterially derived *N*-formylmethionine.¹¹ The precise release mechanism is unclear but occurs at the leading edge of migrating neutrophils. Released ATP and its subsequent metabolism to adenosine at the cell surface activate P2Y₂ and A₃ receptors serving to direct cell orientation and promote migration in response to chemotactic signals.¹¹ Bacterially derived lipopolysaccharide can stimulate central microglia to secrete ATP which in-turn activates neighboring astrocytes and modulates excitatory neurotransmission.¹² In endothelial cells and microglia, extracellular ATP itself can stimulate ATP release.^{13,14} This raises the possibility that ATP can act in a feed forward loop possibly amplifying responses to itself or other external cues which couple to ATP secretion. An autocrine feed forward mechanism has been described in epithelial cells.¹⁵

Lysosomes as ATP Release Machines

Our recent study by Sivaramakrishnan et al. (2012) demonstrated that human monocytes secrete ATP in a constitutive fashion.¹⁶ Furthermore, such constitutive secretion leads to activation of cell surface G_q-coupled P2Y receptors which elevate intracellular calcium levels through release of calcium.¹⁶ This mode of constitutive secretion generates a constant pericellular ATP cloud or “halo” which appears to be important in regulating intracellular calcium homeostasis following P2Y receptor activation.¹⁶ In cells of hematopoietic lineage such as monocyte/macrophage, NK

killer and mast cells, secretory lysosomes have evolved as bifunctional organelles which combine classical degradative properties with secretion.¹⁷ Lysosomes accumulate ATP with luminal concentrations in the millimolar range.^{14,18} The mechanism of lysosomal ATP transport remains undefined though a nucleotide transporter (V-NUT) has been characterized for other ATP containing vesicles.¹⁹ There is also evidence that astrocytes release ATP through lysosome exocytosis.¹⁸

Future Investigation

The signal transduction coupling external cues such as cytokines, chemokines and bacterially derived molecules to ATP release is poorly defined. What are the release machines in leukocytes and do they differ from other cell types? It is expected that therapeutic intervention in agonist stimulated ATP release is a potentially novel route to pharmacological modulation of innate immune responses but also in chronic inflammatory disease where normal inflammatory responses are heightened and act deleteriously.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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