Extracellular ATP-mediated purinergic immune signaling in teleost fish

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Objectives: To elucidate the role and molecular mechanisms of extracellular ATP-mediated purinergic immune signaling in teleost fish.

Methods: Combined approaches including gene cloning, real-time PCR, electrophysiological recordings, cell biology, siRNA silencing and pharmacological inhibition were applied both in vitro and in vivo.

Findings: 1) ATP is released from fish immune cells through Pannexin1 (Panx1) and/or Connexin (Cx) channels following inflammatory stimulation and bacterial pathogen infections.

2) A range of ATP-gated P2 receptors including P2X2, P2X4, P2X7 and P2Y2 are functionally expressed in a variety of Japanese flounder (*Paralichthys Olivaceus*) tissues and immune cells.

3) The released ATP binds and activates purinoreceptors with different affinities for ATP, which are coexpressed on fish immune cells, consequently leading to the activation of fish innate immune responses, such as promoting proinflammatory cytokine production, bacterial killing and phagocytosis, enhancing the inducible nitric oxide synthase (iNOS) activity, and increasing the production of inflammatory mediators, including both reactive oxygen species and nitric oxide.

4) Crosstalk between extracellular ATP-mediated purinergic signaling and NLR inflammasome immune signaling has also been found in fish.

5) Extracellular ATP also plays a pro-apoptotic role by promoting the expression and enzyme activities of several cysteine-dependent aspartate-specific cysteine proteases (Caspases) in fish immune cells.

6) The magnitude and duration of extracellular ATP-activated proinflammatory responses in fish depend on the specific ATP-gated purinergic receptors expressed on a given immune cell type and are modified by the ATP-metabolizing ectonucleotidases that control the available concentrations of ATP in the extracellular milieu. A schematic representation of the extracellular ATP and other nucleotide-mediated purinergic immune signaling pathways in fish are summarized in Figure 2 [1].

Conclusions: Extracellular ATP is a potent signaling molecule in the activation of fish innate immune responses, and the dynamic interactions between ATP release channels, ATP-gated purinergic receptors and ATP-metabolizing ectonucleotidases play important roles in regulating extracellular ATP-mediated immune responses in fish.

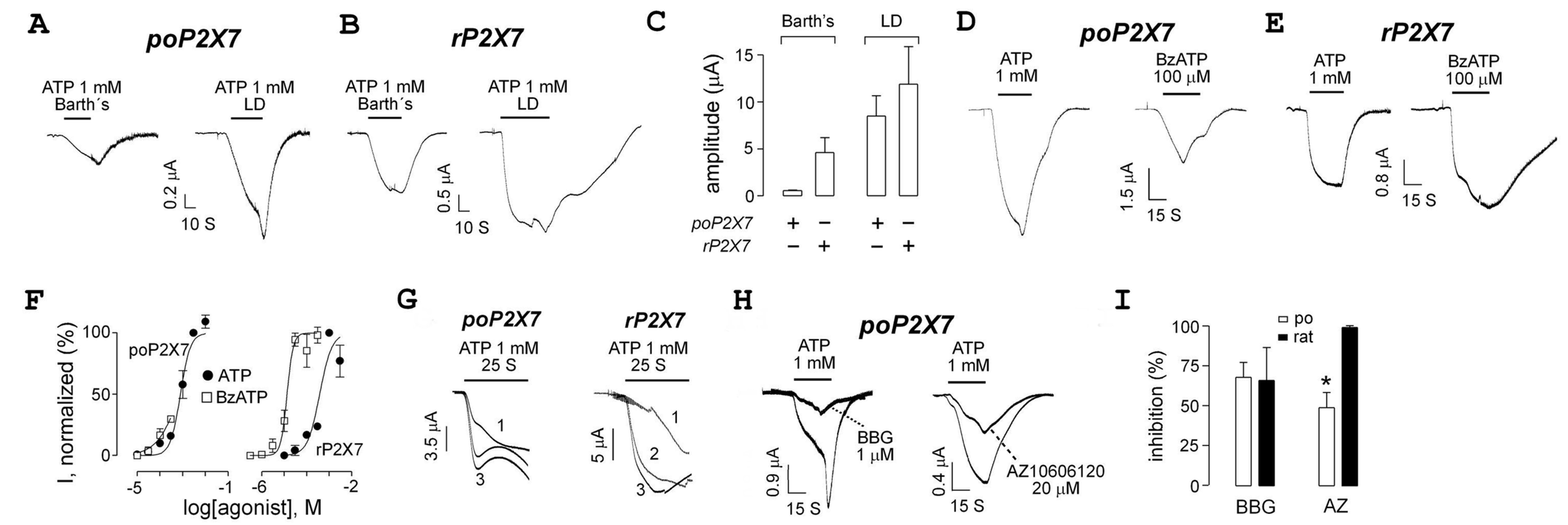

Fig. 1. Comparison of the electrophysiological properties of Japanese flounder ATP-gated P2X7 (poP2RX7) and rat P2X7 (rP2RX7) receptors expressed in Xenopus oocytes. A and B. Representative recordings of individual oocytes expressing poP2RX7 (A) or rP2RX7 (B), currents were gated with 1 mM ATP dissolved in Barth's or low-divalent (LD) media. (C). Summary of the peak amplitudes obtained in Barth's or LD media in oocytes expressing poP2RX7 or rP2RX7. D and E. Currents evoked by 1 mM ATP and 100 μM BzATP from individual oocytes expressing poP2RX7 (D) or rP2RX7 (E), in LD media. (F). Concentration-response curves for BzATP (open squares) and ATP (black circles) for poP2RX7 (left graph) or rP2RX7 (right graph). (G). Current facilitation of poP2RX7 (left recordings) and rP2RX7 (right recordings). In each case, three consecutive ATP pulses were applied to the same oocyte. (H). Representative recording of poP2RX7-expressing oocytes showing the currents evoked by ATP alone and the inhibition induced by pre-application with BBG for 2 min followed by an co-application with 1 μM BBG (left recordings) or 20 μM AZ10606120 (right recordings). (I). Summary of the inhibition induced by BBG or AZ10606120 (AZ) at poP2RX7 (open bars) and rP2RX7 (black bars). *p<0.05, compared with rP2RX7 by Mann-Whitney test, n = 3-5.

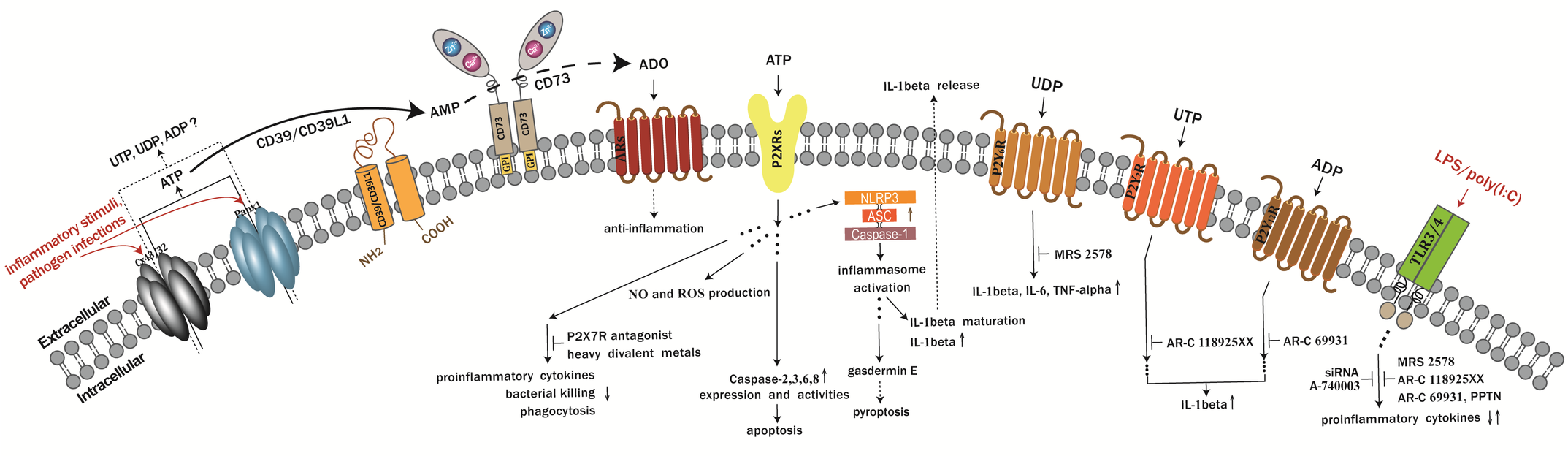

Fig. 2. A schematic representation of the extracellular ATP and other nucleotide-mediated purinergic immune signaling pathways in fish. Under inflammatory stimulation or pathogen infection conditions, ATP and other nucleotides were released into the extracellular environment through Panx1, Cx43 and Cx32 channels in fish. The released extracellular nucleotides, including ATP, ADP, UTP, UDP and their derivatives, bind to and act on the P2 purinergic receptors expressed on the immune cell surface, leading to the activation or inactivation of innate immune responses in fish depending on their binding receptor subtypes and activation of downstream signaling cascades. Extracellular ATP exerts broad proinflammatory effects through activation of various ATP-gated P2XRs that are coexpressed on fish immune cells. Pharmacological blockage of the P2X7 receptor activity significantly reduced phagocytosis, bacterial killing and extracellular ATP- or LPS-induced proinflammatory gene expression in fish macrophages. Fish immune cells also express several functional P2YRs, including $P2Y_2$, $P2Y_6$ and $P2Y_{12}$ receptors. Pharmacological inhibition of $P2Y_2$ and $P2Y_{12}$ receptors' activities with their respective antagonists promotes UTP- and ADP-induced *IL-1beta* gene expression, respectively, but deceased or increased LPS- and poly(I:C)-induced proinflammatory cytokine gene expression in Japanese flounder head kidney macrophages in a receptor-dependent manner. Pharmacological inhibition of endogenous $P2Y_6$ receptor activity greatly upregulated proinflammatory cytokine gene expression in Japanese flounder peripheral blood leukocytes treated with UDP. In addition, pharmacological inhibition of $P2Y_{14}$ receptor activity or downregulated the endogenous expression of $P2Y_{14}$R by siRNA significantly augmented LPS-induced proinflammatory cytokine gene expression in Japanese flounder head kidney macrophages. Activation of fish NLRP3 inflammasome by extracellular ATP not only led to the secretion of mature IL-1beta but also resulted in pyroptotic cell death, possibly via cleavage of gasdermin E by Caspase-1 protein. Immediately after release, the extracellular ATP is enzymatically degraded into ADP and AMP by ectonucleotidases (such as CD39 and CD39L1) that are expressed on the cell surface. AMP was further completely dephosphorylated to adenosine (ADO) by ecto-5'-nucleotidase (CD73). ADO produces anti-inflammatory effects other than ATP, by binding and activating adenosine receptors (ARs) on the plasma membrane and thus may dampen or terminate the extracellular ATP-activated proinflammatory responses. The magnitude and/or duration of extracellular ATP-mediated proinflammatory responses in fish was then balanced by ectonucleotidases CD39 and CD73. Solid-lined arrows indicate the purinergic immune signaling pathways that have been verified in fish, while broken-lined arrows indicate the undetermined pathways. Dots denote the gaps that remain to be determined.

References

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