Reduction of cytokine receptor mediated JNK signalling via purinergic receptor activation: An anti-inflammatory role for G-protein coupled receptors

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Ubiquitously expressed purinergic receptors, which are activated by extracellular nucleotides, have been studied extensively to understand their role in both physiological and diseased conditions.[1] On the surface of endothelial cells, P2Y receptors[2] are exposed to ATP, which is released in response to fluid shear stress, and associated degradation products ADP and adenosine. Thus, we examined the effect of P2Y receptor stimulation in endothelial cells and cross talk with cytokine mediated JNK signalling. In human umbilical vein endothelial cells (HUVECs), ATP alone gave a small transient JNK signal, however pre-treatment of ATP inhibited cytokine (IL-1β) mediated JNK signalling in a concentration dependent manner (% inhibition by 100 μM ATP = 68.9 ± 12.7% and 67.4 ± 6.1%, $p<0.05$, by kinase assay and Western blotting respectively). Responses to sorbitol and anisomycin were not affected by ATP and the inhibition was pathway specific; p38MAP kinase and NF-κB signalling was unaltered. Pre-incubation with the $P2Y_{11}$ antagonist, NF340, reversed the inhibitory effect on IL-1β mediated JNK signalling, however inhibition was unaffected in the presence of the ecto-nucleotidase inhibitor (ARL67156). We then attempted to uncover the signalling mechanisms downstream of $P2Y_{11}$ that is involved in the inhibition of IL-1β mediated JNK signalling. Pre-incubation with the G protein $G_{q/11}$ inhibitor YM-254890, partially reversed the inhibitory action of ATP on IL-1β stimulated JNK (% inhibition of JNK activity: 100 μM ATP= 80.7 ± 6.4%, 100 nM YM+ ATP= 52.9 ± 13.5%). The protein kinase A inhibitor H89 also reversed the ATP inhibitory effect on IL-1β mediated JNK signalling (% inhibition of JNK activity: 30 μM ATP= 55.6 ± 5.3%, 10 μM H89+ ATP= 19.7 ± 5.1%, $p< 0.001$). This inhibition of JNK signalling by ATP was also translated into a physiological outcome as measured by reduction of pro-inflammatory COX-2 production by IL-1β (45.0 ± 4.4 % inhibition by 100 μM ATP). These results collectively support the emerging hypothesis that activation of GPCRs, for example $P2Y_{11}$, can inhibit cytokine mediated JNK signalling via both $G_{q/11}$ and G_s linked pathways. In the context of endothelial cell function, purinergic receptors could exert an anti-inflammatory effect via these mechanisms. Thus selective, high affinity $P2Y_{11}$ receptor agonists could be developed as a therapy for inflammatory mediated diseases. Future studies will focus on the mechanisms downstream of $G_{q/11}$ and G_s that inhibit cytokine receptor activation of JNK.

References:

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