Probenecid, a gout treatment, blocks the human P2X7 receptor

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Activation of the ligand-gated ion channel P2X7 induces uptake of large organic cations and anions, a feature known as secondary permeability and measured by the rapid uptake of dye molecules. The hemichannel pannexin-1 is thought to be responsible for P2X7-induced secondary permeability. Our aim in this study was to determine whether the increased dye uptake response observed in gain-of-function 348Thr-P2X7 receptors was due to increased signalling through pannexin-1 using pharmacological tools.

Methods: ATP-induced dye uptake was measured using a fluorescent plate reader and ethidium/YOPRO-1 dyes. Calcium responses were measured using Fluo-4AM calcium indicator dye and a CoolSnap HQ2 CCD camera coupled to a fluorescent microscope. ATP-induced inward currents were measured by whole cell patch clamp. CD14+ monocytes were isolated from mononuclear cells and primed with LPS for IL-1β secretion assays. Pannexin-1 inhibitors tested were carbenoxolone (CBX), a peptide blocker (^{10}Panx1), flufenamic acid, mefloquine and probenecid.

Results: Unexpectedly we found no pharmacological evidence for pannexin-1 involvement in dye uptake in HEK-293 cells expressing human P2X7 receptors or in primary human monocytes. The pannexin-1 inhibitor carbenoxolone did show an inhibitory effect on ATP-induced dye uptake in J774 mouse macrophages. Probenecid dose-dependently blocked YOPRO dye uptake in HEK cells expressing human P2X7 and blocked ATP-induced IL-1β secretion from primary human monocytes. Probenecid also blocked P2X7-induced calcium responses and ATP-induced inward currents in HEK-hP2X7 cells suggesting that this compound actually interacts with and blocks the P2X7 receptor.