

Purinergic regulation of epithelial sodium channels (ENaC)

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The epithelial sodium channel (ENaC) mediates amiloride-sensitive Na^+ transport in polarised epithelial cells. Defects in ENaC function can cause hypernatremia, hypokalemia and hypertension, and are a significant component in the pathophysiology of cystic fibrosis. Thus it is important to understand the factors that regulate ENaC activity. We have been studying the regulation of ENaC by the purinergic receptor agonist UTP in Fisher Rat Thyroid (FRT) epithelial cells. Our experiments have involved the use of gene transfection and Ussing Chamber electrophysiology. FRT cells were chosen due to their high transfection efficiency compared to other polarised epithelial cells. This characteristic allowed specific genes to be knocked-down, over-expressed or mutated by transfecting cells with siRNA or plasmid DNA. Because, in contrast to native thyroid cells, FRT cells do not express ENaC, this channel also needed to be transfected. Our experiments demonstrate that FRT cells transfected with ENaC display an amiloride-sensitive Na^+ current (Isc_{amil}). This current is inhibited during exposure to UTP, with apical UTP inducing a stronger inhibition than basolateral UTP. Our results further show that the P2Y2 purinergic receptor is responsible for the inhibition of Isc_{amil} by both apical and basolateral UTP. We have found that the P2Y2 receptor mediated inhibition of ENaC involves a pertussis toxin sensitive G-protein and phospholipase C- β 4. Our results also suggest that a second purinergic pathway is involved in the inhibition of Isc_{amil} by apical UTP. Furthermore, we have found that both Cl^- and intracellular Ca^{2+} play a role in the mechanism of UTP inhibition of ENaC. Future studies will aim to further elucidate the signalling pathways involved in the inhibition of ENaC by UTP.