STRUCTURE AND FUNCTION OF $P2Y_2$ NUCLEOTIDE RECEPTORS IN CYSTIC FIBROSIS (CF) EPITHELIUM

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1. INTRODUCTION

Cystic fibrosis (CF) is caused by mutations in the gene encoding the cystic fibrosis transmembrane-conductance regulator (CFTR), a cAMP-dependent chloride transporter expressed in epithelial tissue. A novel treatment being developed for CF utilizes inhaled nucleotides, particularly uridine 5′-triphosphate (UTP), to activate calcium-dependent chloride channels in airway epithelial cells. This therapy is based on earlier studies from the laboratory of Dr. Richard C. Boucher at the University of North Carolina-Chapel Hill, which have shown that luminal administration of UTP[1] and the Na^+ channel blocker amiloride,[2] together overcome the ion transport defects in CF airway epithelial cells by promoting increased Cl^- secretion and decreased Na^+ absorption.[3] UTP stimulates Cl^- secretion in epithelial cells by activating a $P2Y_2$ (formerly named P_{2U}) nucleotide receptor subtype[4,5] that is coupled to phospholipase C via $G_{q\alpha}$ protein resulting in inositol 1,4,5 trisphosphate (IP_3)-dependent increases in the cytoplasmic calcium concentration ($[Ca^{2+}]_i$) that serve to activate calcium-dependent chloride channels.[6] Evidence that activation of this non-CFTR Cl^- secretory conductance may be beneficial in CF therapy was provided by studies with CFTR knockout mice in which the presence of a large, basal, non-CFTR Cl^- conductance was associated with the lack of respiratory disease in this species.[4] Early clinical studies involving administration of aerosolized UTP and amiloride indicate that this therapy has a positive effect on mucociliary clearance in CF nasal epithelia.[7] However, little is known about the mechanisms of nucleotide regulation of epithelial ion transport. It is clear that fundamental infor-

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mation concerning $P2Y_2$ receptor biology in epithelia will be useful in optimizing nucleotide therapy for CF. We have utilized the CFTR knockout mouse and its normal counterpart to isolate cultures of gallbladder epithelial cells that form tight junctions and represent a good model for studying the roles of $P2Y_2$ receptors in mediating signal transduction pathways leading to increased Cl^- secretion. In addition, we have cloned the human airway epithelial cell $P2Y_2$ receptor cDNA and expressed the receptor in a heterologous cell system, human 1321N1 astrocytoma cells, that lack any endogenous nucleotide receptors.[8] In the 1321N1 cell transfectants, the human airway $P2Y_2$ receptor is distinguished pharmacologically by its ability to be activated equipotently by the purine nucleotide ATP and the pyrimidine nucleotide, UTP. The investigation of the molecular and pharmacological properties of the $P2Y_2$ receptor expressed in 1321N1 cells is being undertaken to develop methods to maximize calcium-dependent chloride conductance in CF epithelial cells through stimulation of endogenous $P2Y_2$ receptors.

2. $P2Y_2$ RECEPTOR FUNCTION IN CF EPITHELIAL CELLS

P2 receptors are unique among G protein-coupled receptors (GPCRs) whose activation may be therapeutic in CF. With the exception of a small complement of bradykinin receptors,[9] only nucleotide receptors are prominently localized to the apical membrane of airway epithelial cells[3] where they can signal the mobilization of intracellular calcium that activates calcium-dependent chloride channels.[4] We have investigated the effect of UTP on the activation of IP_3-dependent calcium mobilization coupled to $P2Y_2$ receptors in anion-secreting murine gallbladder epithelial (MGE) cell lines. These cell lines were formed by SV40 transformation of the relatively homogenous epithelial cells of the murine gallbladder and cell lines have been developed from the CFTR knockout mouse[10] (i.e., CFTR negative, MGEN cells) and from normal mice (i.e., CFTR positive, MGEP cells). The MGE cells have been continuously grown on permeable collagen supports to maintain their polarity. The cells form electrically-resistive tight junctions and have not changed their morphological or bioelectrical properties in over 70 passages. We have demonstrated a UTP-dependent transepithelial short-circuit current (Isc) in these cells that can be attributed to electrogenic anion secretion.[5,11–13] The inability of cAMP agonists to increase anion secretion was shown to be characteristic of the absence of functional CFTR in MGEN cells.[11]

The anion secretory response to UTP in the MGE cells has a similar profile to changes in intracellular Ca^{2+} mobilization and is characterized by a rapid increase in the Isc, followed by a decline to a steady-state level within minutes.[5,12,13] An EC_{50} of 2×10^{-6} M was obtained for $P2Y_2$ receptor activation by UTP of the peak Isc response in MGEP cells (Figure 1). The role of calcium signaling in this Isc response is supported by experiments showing that UTP stimulated IP_3 production and Ca^{2+} mobilization in MGEP cells (Figure 1).

In our studies, the kinetics and dose-dependence of these responses in MGEP and MGEN cells were comparable to the activation of a recombinant $P2Y_2$ receptor expressed in 1321N1 cells that is coupled to IP_3-mediated Ca^{2+} mobilization.[8,11,12,15] In addition to the Ca^{2+}-dependent Isc response in MGE cells, 10^{-4} M UTP also stimulated a small, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS)-inhibitable, increase in Isc in the continued presence of a maximally effective concentration of a Ca^{2+} ionophore, indicative of the co-activation of a Ca^{2+}-independent Cl^- conductance.[12] These results suggest that in addition to CFTR, MGE cells have both Ca^{2+}-dependent and Ca^{2+}-independent secretory pathways coupled to $P2Y_2$ receptor activation. Recently, we have found that UTP evokes a