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Calcium entry is regulated by Zn^{2+} in relation to extracellular ionic environment in human airway epithelial cells

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ABSTRACT

The extracellular pH, sodium and divalent cation concentrations influence the ATP-induced changes in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). This elevation of $[Ca^{2+}]_i$ and activation of Ca^{2+}-dependent Cl^- channels represent a possible therapeutic approach in cystic fibrosis (CF). We investigated the changes of $[Ca^{2+}]_i$ in different external ionic environment, and P2X purinergic receptors (P2XRs) expression in the control and CF airway epithelial cells. The parallel removal of Na^+ and alkalinization of the extracellular solution increased the amplitude of sustained ATP-induced Ca^{2+} signals independent of wild-type or mutant CFTR expression. The ATP-induced Ca^{2+} entry was either inhibited or stimulated by Zn^{2+} depending on the extracellular Na^+ concentration. In Na^+-free environment, Zn^{2+} and other divalent cations elicited a biphasic Ca^{2+} signal. Immunohistochemical data suggest that, multiple subtypes of P2XRs are expressed in these airway epithelial cells. In conclusion, Ca^{2+} entry is finely regulated by external ionic environment. Therefore, we speculate that properly compiled aerosols could influence efficacy of zinc-based therapy in CF.

1. Introduction

Calcium is a crucial intracellular second messenger that can regulate many different cellular functions (Berridge et al., 2003). An increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) is triggered by release of Ca^{2+} from intracellular stores and/or entry of Ca^{2+} from the extracellular space. In non-excitable cells, Ca^{2+} entry may occur mainly through agonist-dependent and a voltage-independent manner that includes receptor channel-mediated and store-operated mechanisms (Elliott, 2001). In airway epithelial cells, modulation of calcium signaling could be of clinical importance because activation of Ca^{2+}-dependent anion channels have been proposed to substitute for defective protein kinase A/cAMP-dependent transepithelial Cl^- and/or HCO_3^- transport in cystic fibrosis (CF) (Kunzelmann et al., 2007).

ATP stimulates both G-protein-coupled P2Y metabotropic receptors (P2YRs) and P2X ligand-gated receptor channels (P2XRs). P2YRs elicit Ca^{2+} release from intracellular stores and stimulate store-operated Ca^{2+} channels whereas P2XRs allow Ca^{2+} entry only from the extracellular space (Burnstock, 2004). The targeting of P2YRs with a stable UTP analog, INS37217 (denufosol), is currently in Phase 3 clinical trials for CF therapy. Denufosol is designed to enhance the lung's innate mucosal hydration and mucociliary clearance through the stimulation of the $P2Y_2$ receptors (http://www.inspirepharm.com/pipeline.html#37217resp). We have demonstrated previously that the stimulation of P2XRs lead to anion secretion in CF mouse nasal epithelial cells *in vivo* (Zsembery et al., 2004).

Silberberg and his colleagues have shown that the extracellular Na^+ regulates airway ciliary motility by inhibiting P2XRs; lowering external Na^+ enhanced ciliary beat and P2X receptor channel activity on ciliated airway epithelial cells (Ma et al., 1999). Recently, the same research group has reported that Na^+ both inhibits and permeates P2XRs in rabbit airway ciliated cells suggesting that extracellular Na^+ concentration may play a fundamental role in P2XR-mediated Ca^{2+} influx (Ma et al., 2006). They also suggested that $P2X_{cilia}$ might be assembled from $P2X_4$ and $P2X_7$ subunits (Ma et al., 2006). However it is important to note that activity of P2XRs are also modulated by extracellular H^+ and divalent cations such as Zn^{2+}, Cu^{2+}, Mg^{2+} and Ca^{2+} (Negulyaev and Markwardt, 2000;

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Khakh et al., 2001). These ions may either potentiate or inhibit P2XRs depending on composition of different subtypes present in the cells. Interestingly our previous data indicate that, in alkaline and Na^+-free environment Zn^{2+} acts as a full agonist of P2XRs in human control and CF airway epithelial cell lines (Zsembery et al., 2004). Small interference RNA fragments specific to $P2X_4$ and $P2X_6$ (but not $P2X_5$) applied into IB3-1 CF human airway epithelial cells inhibited markedly extracellular Zn^{2+}-induced Ca^{2+} entry suggesting that multiple P2X receptor subtypes were involved (Liang et al., 2005). Thus, $P2X_4$ and $P2X_6$ may also co-assemble in airway epithelial cells offering a possible new target for CF therapy.

Besides interacting with P2XRs, extracellular Zn^{2+} can bind to zinc sensing receptors (ZnRs). The ZnRs are coupled to $G\alpha_q$-protein which triggers release of intracellular Ca^{2+} and activates ion transport in colonocytes (Hershfinkel et al., 2001). Furthermore, in renal epithelial cells, heavy metals, such as Cd^{2+}, Ni^{2+} and Zn^{2+}, are potent inducers of IP_3 generation and Ca^{2+} mobilization by stimulating a cation-sensing receptor (CSR) (Faurskov and Bjerregaard, 2002). In contrast to effects elevating $[Ca^{2+}]_i$, Zn^{2+} can also inhibit Ca^{2+} entry by blocking store-operated Ca^{2+} channels (SOCs) (Huang et al., 2006). In fact, a high affinity binding site for Zn^{2+} has been identified on SOCs (Gore et al., 2004). Therefore ATP-induced sustained Ca^{2+} signal may be either potentiated or inhibited by Zn^{2+} depending on relative contributions of P2XRs and SOCs in relation to Ca^{2+} entry. This study was designed to explore the features of Ca^{2+} entry induced by ATP and divalent metal cations in airway epithelial cells (CFBE41o$^-$) expressing wild-type CFTR (wt-CFTR) or delF508-CFTR (ΔF-CFTR).

2. Methods

2.1. Materials

ATP, Thapsigargin, U-73122, BZ-ATP, suramin, hexokinase and apyrase were purchased from Sigma Chemical (St. Louis, MO). Fluo-3/AM was purchased from Invitrogen Inc. (Carlsbad, CA). All other chemicals were purchased from Reanal Inc. (Budapest, Hungary).

2.2. Culturing and maintenance of CFTR-transfected CFBE41o$^-$ cells

The human airway epithelial cell line (CFBE41o$^-$) was a generous gift from Dr. Erik M. Schwiebert and Dr. Zsuzsa Bebők (University of Alabama at Birmingham, Birmingham, AL). The CFTR expressing CFBE41o$^-$ cells were stably transfected with either wild-type or delF508 CFTR cDNA and selected by puromycin resistance. Details of the transfections are described by Bebok et al. (2005). Cells were grown in plastic tissue culture flasks in Dulbecco's modified Eagle's/Ham-F12 (1:1) medium supplemented with 10% fetal bovine serum and 100 μg/ml penicillin/streptomycin. Two days before the experiments cells were plated and grown on a round glass coverslip at 37 °C.

2.3. Recording solutions

At the beginning of each experiment we superfused the cells with a standard solution (Na–Ca–7.4-sol) containing NaCl 145 mM, KCl 5 mM, $CaCl_2$ 3 mM, $MgCl_2$ 1 mM, HEPES 10 mM, glucose 10 mM, (pH 7.4). Sodium was substituted with either equimolar N-methyl-D-glucamine (NMDG–Ca–7.4-sol) or lithium (Li–Ca–7.4-sol). The Ca^{2+} depleted solutions were prepared by simply omitting $CaCl_2$ (Na–7.4-sol or NMDG–7.4-sol). The effects of alkaline external conditions were tested at pH 7.9. In some experiments, to empty intracellular Ca^{2+} stores 0.1 μM thapsigargin was used for 15 min prior to the recording. Experiments were performed at room temperature under a continuous flow of solutions with a speed of 3 ml/min. The volume of the chamber was 1.5 ml therefore complete change of the bath solution took approx. 30 s.

2.4. Measurement of intracellular calcium

CFBE41o$^-$ cells were loaded with Fluo-3/AM (4 μM) in the Na–Ca–7.4-sol for 60 min at room temperature. The cells were then washed with Na–Ca–7.4-sol, and the coverslips were mounted into a perfusion chamber equipped with an inverted microscope. Recordings were made with a confocal laser scanning microscope, Axiovert 200 M Zeiss LSM 510 Meta (Carl Zeiss, Jena, Germany) equipped with a 20× Plan Apochromat (NA = 0.80) DIC objective which is able to acquire data in real-time enabling Ca^{2+} dynamics of individual cells *in vitro*. We used a 488-nm argon-ion laser for the excitation. The emitted light was collected with BP 505–570 band pass filter. A few cells apparently demonstrated high or low basal level of cytosolic Ca^{2+} that we excluded from our experiments. Studies were conducted preferentially with clones emitting medium fluorescence as judged by the eye. This was done by selecting region of interests (ROIs). Each measurement was stored in the form of digital video recording. The average fluorescence of the chosen area was obtained at a rate of 0.5 Hz.

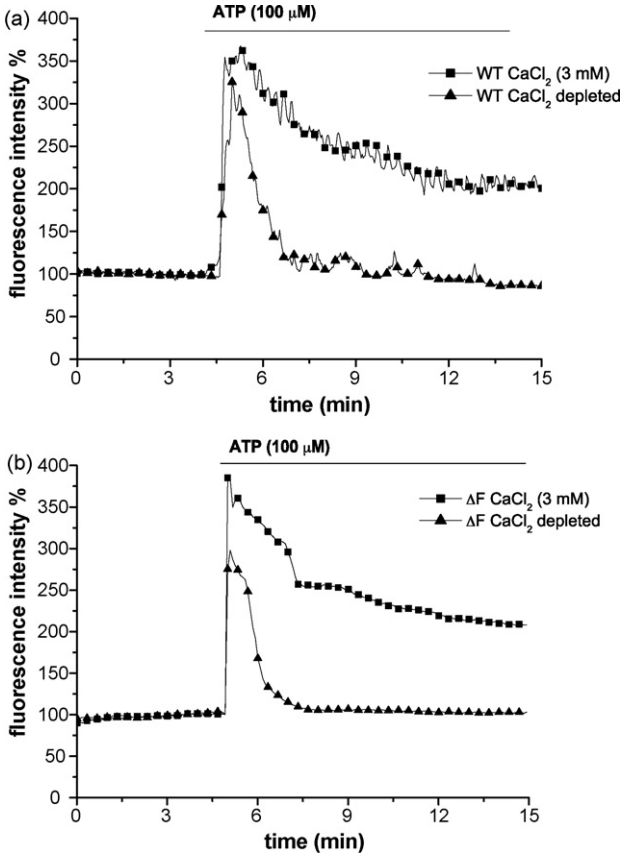

Fig. 1. Effects of ATP on $[Ca^{2+}]_i$ in CFBE cells. (a) Original traces showing the effect of ATP (100 μM) on $[Ca^{2+}]_i$ of WT cells perfused with Na^+-containing solution at pH 7.4 in presence of $CaCl_2$ (3 mM) and following extracellular Ca^{2+} depletion. (b) Effect of ATP (100 μM) on $[Ca^{2+}]_i$ in ΔF cells perfused with Na^+-containing medium at pH 7.4 in the presence of $CaCl_2$ (3 mM) and following extracellular Ca^{2+} depletion. The average of fluorescence intensity between the second and the third minute of each experiment was considered as 100%. Each experiment was performed 10 times using cells from at least two different passages with similar results.