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Calcium homeostasis is abnormal in cystic fibrosis airway epithelial cells but is normalized after rescue of F508del-CFTR

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Abstract

Retention of F508del-CFTR proteins in the endoplasmic reticulum (ER) is dependent upon chaperone proteins, many of which require Ca^{2+} for optimal activity. Here, we show in human tracheal gland CF-KM4 cells, that after correction of F508del-CFTR trafficking by miglustat (*N*-butyldeoxynojirimycin) or low temperature (27 °C), the Ca^{2+} mobilization is decreased compared to uncorrected cells and becomes identical to the Ca^{2+} response observed in non-CF MM39 cells. In CF-KM4 and human nasal epithelial CF15 cells, we also show that inhibiting vesicular trafficking by nocodazole prevents not only the rescue of F508del-CFTR but also the Ca^{2+} mobilization decrease. Finally, experiments using the CFTR inhibitor $CFTR_{inh}$-172 showed that the presence but not the channel activity of F508del-CFTR at the plasma membrane is required to decrease the Ca^{2+} mobilization in corrected CF cells. These findings show that correction of the abnormal trafficking of F508del-CFTR proteins might have profound consequences on cellular homeostasis such as the control of intracellular Ca^{2+} level.

Keywords: Cystic fibrosis; Calcium signalling; Pharmacology; F508del-CFTR; Trafficking; Miglustat

1. Introduction

In airway epithelial cells, calcium mobilization can be elicited by selective autocrine and/or paracrine activation of apical or basolateral membrane heterotrimeric G protein-coupled receptors linked to phospholipase C (PLC) stimulation, which generates inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG) and induces Ca^{2+} release from endoplasmic reticulum (ER) stores [1,2]. Three structurally and functionally different isoforms of the IP3 receptors (IP3R) expressed in a cell-type specific manner have been identified [3,4] including in epithelial cells [5,6]. IP3 mobilizes intracellular Ca^{2+} and DAG activates protein kinase C [7–9].

In airway epithelia of patients suffering of cystic fibrosis (CF), the functional absence of the cystic fibrosis transmembrane conductance regulator (CFTR) at the apical plasma membrane results in a diminished periciliary liquid layer depth [10] and a reduction in mucus clearance. The most common CF mutation F508del in CFTR induces trapping of the protein in the ER, misfolding and proteasomal degradation of CFTR proteins [11]. For most CF patients, life expectancy is limited (≈32 years) due to the progressive loss of functional lung tissue. Already very early in life a persistent neutrophylic inflammation is present in the airways [12]. Histamine, one of the mediator of inflammation [13] causes in human airway a transient elevation of intracellular free Ca^{2+} concentration which is due to release from intracellular stores [14]. In airway tissue, most cells express at least one subtype of histamine receptors, and in particular the H_1-receptor in epithelial cells [7,13]. Stimulation of H_1-receptors leads to the hydrolysis of phosphatidyl 4,5-biphosphate and the formation of IP3 and DAG. Previous works showed that the pro-inflammatory mediator bradykinin triggers Ca^{2+} mobilisation [15] and induces interleukin-8 secretion in non-CF and CF human airway epithelia [16,17].

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We recently found that the α1,2-glucosidase inhibitor miglustat (*N*-butyldeoxynojirimycin, NB-DNJ) prevents the interaction between F508del-CFTR and calnexin in the ER and partially corrects the abnormal processing of functional F508del-CFTR in cystic fibrosis epithelial cells [18]. Here, we studied the consequence of rescuing F508del-CFTR by miglustat and low temperature [19] on the Ca^{2+} homeostasis of the ER in the human CF airway epithelial CF15 cells or in the human CF tracheal gland serous CF-KM4 cells [20] compared to the human non-CF tracheal gland serous MM39 cells [21].

2. Materials and methods

2.1. Cells

The human nasal epithelial JME/CF15 cell line F508del/F508del-CFTR was grown at 37 °C in 5% CO_2 under standard culture conditions [18,22]. Human CF and non-CF tracheal gland serous CF-KM4 and MM39 cell lines were cultured as previously described [20,21].

2.2. Functional assay

CFTR Cl^- channel activity was assayed on a cell population by the iodide (^{125}I) efflux technique as described [18].

2.3. Resting intracellular calcium concentration measurements

Intracellular free calcium measurements were performed by means of a ratiometric fluorescence method using an OSP100 microscopic photometry system (Olympus, Tokyo, Japan) and the calcium fluorescent probe Indo-1. The ratiometric method and the calibration procedure have been published elsewhere [23]. The ratio (R) of the dual emission fluorescence of the Ca^{2+}-free and Ca^{2+}-bound forms of Indo-1 (at 485 and 405 nm, respectively) were separated, filtered, and collected by two photomultipliers. The intracellular free calcium concentrations were calculated from the following equation [23]: $[Ca^{2+}]_c = K_d \times \beta \times [(R - R_{min})/(R_{max} - R)]$. All the experiments were performed at room temperature. Briefly, cells were rinsed with a buffer solution containing: (in mM) 130 NaCl, 5.4 KCl, 2.5 $CaCl_2$, 0.8 $MgCl_2$, 5.6 glucose, 10 Hepes, pH 7.4 (adjusted with Tris base). Cells were incubated for 45 min at room temperature in the same solution supplemented with 3 μM (final concentration) of the acetoxy-methyl ester form of Indo-1 (Indo-1/AM, Sigma Chemicals, St Louis, MO, USA). We determined the resting intracellular calcium concentration in CF15 cells: 62.2 ± 25.9 nM ($n = 73$), in CF-KM4 cells: 142.7 ± 27.2 nM ($n = 80$) and in MM39 cells: 144.5 ± 30.9 nM ($n = 80$).

2.4. Recording calcium signals

Cells were loaded with 3 μM Fluo-4 acetoxymethyl ester (FluoProbes®) for 20 min at room temperature and Ca^{2+} activity was recorded by confocal laser scanning microscopy using Bio-Rad MRC 1024. All the experiments were performed at minimum on two different cell passages. Other details are as described [24].

2.5. Statistics

Results are expressed as mean ± S.E.M. of n observations. Sets of data were compared with a Student's *t*-test. Differences were considered statistically significant when $P < 0.05$. ns: non significant difference, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All statistical tests were performed using GraphPad Prism version 4.0 for Windows (Graphpad Software) and Origin version 5.0.

2.6. Chemicals

Forskolin, genistein and thapsigargin are from LC Labs. Histamine, ATP, 2-APB, caffeine, La^{3+}, ionomycin, nocodazole are from Sigma. Miglustat, NB-DGJ are from Toronto Research Chemicals. $CFTR_{inh}$-172 is from Calbiochem.

3. Results

We investigated the effect of histamine and ATP on the intracellular Ca^{2+} mobilization in non-CF (MM39 cells) and CF (CF-KM4) cells, two paired human tracheal gland serous cells [20,21]. In the F508del/F508del CF-KM4 cells, following the addition of histamine (Fig. 1A) or ATP (Fig. 1B) to the bath perfusing cells, we recorded a biphasic Ca^{2+} response: an initial Ca^{2+} peak was followed by a sustained rise of intracellular Ca^{2+} that persisted during the 5 min stimulation. Quantification of the area under the curve (AUC) is presented for each cell type (Fig. 1C). Whatever the stimulation, we observed a significant difference of Ca^{2+} mobilization between CF and non-CF cells (Fig. 1C). The histamine-dependent Ca^{2+} response was decreased by ∼60 (CF-KM4 cells) and 50% (MM39 cells) by 2-aminoethyoxydiphenyl borate (2-APB, 100 μM preincubated 15 min), an inhibitor of IP3 receptors (IP3R) (Fig. 1C) demonstrating the implication of these receptors in the ER Ca^{2+} store release produced by histamine. Incubation with 100 μM 2-APB also inhibited the ATP-dependent Ca^{2+} response by ∼40% for MM39 cells and ∼70% for CF-KM4 cells (Fig. 1C). On the contrary, caffeine (10 mM) did not mobilize intracellular Ca^{2+} in any of the airway cells studied suggesting the absence of ryanodine receptors (data not shown). These results demonstrate that the intracellular Ca^{2+} mobilization in response to histamine or ATP is abnormal in CF airway epithelial cells and that the IP3R but not the ryanodine receptors are likely to be responsible for the Ca^{2+} store release in these cells.