Histamine-stimulated Cl⁻ transport in HeLa cells.

DAVID RUSSELL, MARGARET A. McPHERSON and ROBERT L. DORMER

Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

The molecular basis of cystic fibrosis (CF) is characterized by defective regulation of Cl⁻ transport and exocytotic protein secretion [1]. The CF gene protein, cystic fibrosis transmembrane conductance regulator (CFTR), has inherent cyclic AMP-activated Cl⁻ channel activity [2] but regulates the activity of other Cl⁻ channels [3] and secretion of serous and mucous proteins [4-7]. We have shown in CF submandibular cells [4,5] and by introduction of a CFTR antibody into living rat submandibular acini [6], that defective β-adrenergic stimulation of mucin secretion is CFTR-mediated and can be partially restored by 3-isobutyl-1-methylxanthine (IBMX), suggesting the use of selective phosphodiesterase inhibitors in pharmacological therapy for CF [8]. A drug therapy might work either by activating mutant CFTR directly or by stimulating a CFTR-independent secretory pathway to bypass the defect. Hela cells are an epithelial cell which do not express CFTR but can be transfected with wild type or mutant forms of CFTR [9]. Thus, they are an important model cell for studying alternative Cl⁻ secretory pathways and the effects of mutant CFTR on their pharmacological activation. The aim of the present study is to determine whether HeLa cells possess an agonist stimulated, Ca²⁺-dependent Cl⁻ secretory pathway.

Cl⁻ transport was assayed by ¹²⁵¹ efflux [10]. HeLa cells, cultured in DMEM to 70-80% confluence, were transferred into Hepes-buffered Ringer solution (HR) immediately before loading with ¹²⁵¹ (2.5pCi/ml) for 1h at 37°C. After washing cells 5 times, ¹²⁵¹ efflux was measured by medium replacement.

Fig. 1. Effect of hypotonicity or histamine on the rate of ¹²⁵¹ efflux from Hela cells.

Followed by 125¹ efflux, 5 medium samples were taken at various intervals before agonist addition (i) and further sampling at 0.5 min intervals. (o) basal flux.

As shown in Fig.1, 125¹ efflux was stimulated by hypotonicity, attained by reducing the NaCl concentration by 25%. The data also show that histamine (100µM) markedly stimulated the rate of 125¹ efflux. The total stimulated efflux was calculated from the area under the curve, corrected for the equivalent basal efflux (prestimulated) rate. 100µM histamine evoked a response which was 382±83.6% of control (n=7). In HR containing no added calcium + 0.1µM EGTA, the response was reduced to 137±24.3% of control (n=4), indicative of a requirement for Ca²⁺ entry. Histamine (0.1-100µM) has been shown to stimulate Ca²⁺ mobilization and generation of Ins(1,4,5)P₃ in HeLa cells [11,12]. However, at lower histamine concentrations (0.1-1µM) the ¹²⁵¹ efflux response was variable, being approx. 122-144% of control. In conclusion, it has been demonstrated that, in addition to the previously described volume-activated Cl⁻ channel [13], Hela cells possess a histamine-stimulated Cl⁻ transport activity which is 80-90% inhibited by removal of extracellular calcium. This response to a high concentration of histamine may be relevant in vivo, where in inflammatory situations, histamine is released from mast cells which degranulate in close proximity to the epithelium [14]. The HeLa cell model system will allow investigation of the action of pharmacological agents, acting via Ca²⁺ to bypass CFTR-dependent Cl⁻ transport, which could be useful in development of a drug therapy for CF.

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