Abstract

Cystic fibrosis is caused by a genetic defect in the CFTR protein, whose main function is chloride transport across epithelial cells. The measurement of CFTR ability to transport chloride is considered a good, and perhaps, the only practical method to assess its activity. In this thesis, the transport of chloride ions across the CFTR channel was studied using airway epithelial cell lines of healthy patients (NuLi-1) and patients with cystic fibrosis (CuFi-1). A fluorescent method using a fluorescent chloride-sensitive probe N-(ethoxycarbonylmethyl)-6-methoxyquinolinium (MQAE) was chosen and optimized. This compound is providing fluorescence in the blue part of the spectrum and has the greatest sensitivity to chloride ions. In the development of an optimal method two approaches of chloride transport measurement were used. In the first experiment the secretion of the chloride ions to the buffer containing MQAE was measured. In the second one the dye had to be loaded into cells before performing experiment. Then, the MQAE fluorescence quenched by intracellular chloride was monitored by a change in the fluorescence intensity of the probe. The second method was considered as a usefull and more reproducible to study chloride transport across cell membranes. Moreover, the influence of the CFTR modulator forskolin was studied. Forskolin increases intracellular concentration of cAMP, which stimulates CFTR activity. Forskolin-stimulated Cl^- loss is a direct approach to assess whether CFTR Cl^- channel activity could be measured. However, influence of forskolin was not clearly demonstrated and it will be a subject of the further studies.

Key word

Cystic fibrosis, epithelial cells, CFTR channel, loading of a fluorescent probe, MQAE, forskolin