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Dysfunction of the non-neuronal cholinergic system in the airways and blood cells of patients with cystic fibrosis

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Abstract

The non-neuronal cholinergic system is widely expressed in human airways, skin and immune cells. Choline acetyltransferase (ChAT), acetylcholine and nicotine/muscarine receptors are demonstrated in epithelial surface cells, submucosal glands, airway smooth muscle fibres and immune cells. Moreover, acetylcholine is involved in the regulation of cell functions like proliferation, differentiation, migration, organization of the cytoskeleton, cell–cell contact, secretion and transport of ions and water. Cystic fibrosis (CF), the most frequent genetic disorder, is known to be caused by a mutation of the CF-gene coding for the cystic fibrosis transmembrane regulator protein (CFTR). CFTR represents a regulating transport protein for ion channels and processes involving endo- and exocytosis. Despite the identification of the genetic mutation knowledge of the underlying cellular pathways is limited. In the present experiments the cholinergic system was investigated in the peripheral blood and in the lung of CF patients undergoing lung transplantation ($n=7$). Acetylcholine content in bronchi and lung parenchyma of CF was reduced by 70% compared to controls (tumor-free tissue obtained from patients with lung tumor; $n=13$). In contrast, ChAT activity was elevated to some extent ($p>0.05$) in CF, and esterase activity did not differ from control. Acetylcholine content extracted from peripheral leucocytes (30 ml) was also reduced by 70% in CF ($n=13$) compared to healthy volunteers ($n=9$). Double labelling experiments with anti-CF antibodies and anti-ChAT antibodies showed a co-localization in peripheral lymphocytes, giving first evidence that CFTR may be linked with the intracellular storage/transport of non-neuronal acetylcholine. It is concluded that the non-neuronal cholinergic system is involved in the pathogenesis of CF. A reduced content of non-neuronal acetylcholine could contribute to the deleterious changes of epithelial ion and water movements in CF, because acetylcholine stimulates apical Cl^- secretion, inhibits apical Na^+ and water absorption and therewith facilitates mucociliary clearance.
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Keywords: Cystic fibrosis; Non-neuronal acetylcholine; Choline acetyltransferase (ChAT); CFTR; co-localization

Introduction

Acetycholine has been demonstrated in the vast majority of human non-neuronal cells, for example epithelial, endothelial, mesothelial, immune cells as well as smooth muscle fibres (Grando, 1997; Klapproth et al., 1997; Kawashima and Fujii, 2000, 2003; Wessler et al., 1999, 2003; Grando et al., 2006). In addition, nicotinic and muscarinic receptors are widely expressed on these non-neuronal cells (for references see Wessler and Kirkpatrick, 2001; Kawashima and Fujii, 2003; Grando et al., 2006). Non-neuronal acetylcholine can act as a local cell molecule via paracrine and autocrine mechanisms to control basic cell functions such as proliferation, differentiation, maturation, migration, secretion, organization of the cytoskeleton and cell–cell contact (Grando, 1997; Wessler et al., 1998, 2001a,b; Kawashima and Fujii, 2000, 2003; Grando et al., 2006). These findings have only been established in recent years, so that our knowledge about a possible involvement of

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the non-neuronal cholinergic system in the pathogenesis of diseases is limited. It is important to identify diseases, in which the non-neuronal cholinergic system is involved in pathophysiological cellular mechanisms. This will allow new diagnostic, therapeutic and preventive approaches.

Cystic fibrosis (CF) is the most frequent genetic disorder. The genetic basis of CF is well characterized, showing a mutation of the CF-gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR; Riordan et al., 1989). The diagnosis of CF is made on the basis of the genetic status and on functional tests (sweat chloride concentration >60 mEq/L). However, despite these milestones in CF research our understanding of the underlying cellular mechanisms is limited. Efforts have been undertaken to illuminate the role of the CFTR channel which represents a Cl^- channel (Anderson et al., 1991; Davis et al., 1996). Later on it became evident that the CFTR not only represents a Cl^- channel but a transporter protein which is involved in the regulation of other ion channels (epithelial Na channel [ENa^+ C]; outwardly rectifying Cl^- channel; voltage-gated K^+ channel) and cellular mechanisms such as endocytosis and exocytosis (Greger et al., 2001). However, the role of CFTR in mediating airway epithelium dysfunction like reduced chloride secretion (see Fig. 1, effect 1), enhanced apical sodium and water absorption (epithelial Na channel; ENaC; effect 2), enhanced basolateral Na^+/K^+ATPase activity (effect 3), impaired mucociliary activity and enhanced infections is only poorly understood. Acetylcholine represents an important regulator of epithelial ion and water movements. For example, in sheep tracheal epithelium acetylcholine stimulates apical Cl^- secretion (effect 1) and basolateral potassium conductance (driving force for Cl^- secretion; step 1) and as a sustained effect, acetylcholine abolishes apical Na^+ absorption (effect 2; Acevedo, 1994). Consequently, fluid secretion into the airways and mucociliary clearance are stimulated (Acevedo, 1994; Ballard et al., 2002). Both functions are critically impaired in CF (see Fig. 1). Therefore, the present study was designed to investigate the cholinergic system in CF patients. It was postulated that dysfunction of epithelial acetylcholine or of the non-neuronal cholinergic system may mediate or at least contribute to these impaired epithelial ion movements.

Methods

Patients

The protocol for obtaining human tissue was approved by the local ethical review board for human studies (Landesärztekammer Rheinland-Pfalz, Germany). Lung tissue was obtained at surgery of CF patients with lung transplantation. Lung tissue of 7 CF patients (4 female, 3 male; mean age 23 [range 16–36 years] was available for investigation. Tumor-free lung tissue from patients with lung cancer was used as control (5 female, 8 male; mean age 61 [range 22–77 years]). Within 1 h after pneumectomy or lobectomy bronchi were dissected down to a diameter of 4 mm and placed in oxygenated, ice-cold salt solution (composition in mmol/L: 125 NaCl, 23.8 $NaHCO_3$, 5.05 glucose, 2.68 KCl, 1.80 $CaCl_2$, 1.04 $MgCl_2$, 0.54 NaH_2PO_4, 0.057 ascorbic acid, and 0.001 choline chloride). Large (diameter 9–6 mm) and small (5–4 mm) bronchi were washed several times in the oxygenated salt solution, carefully cleaned from adhering parenchyma, and cut into pieces of 100–200 mg for analysis of ChAT, esterase and acetylcholine content. Likewise, pieces of lung parenchyma (about 50 mg) were isolated and washed with the salt solution.

Heparinized blood (30 ml) was taken from CF patients (13 females, 9 males; mean age 27 [range 5–40 years] and healthy

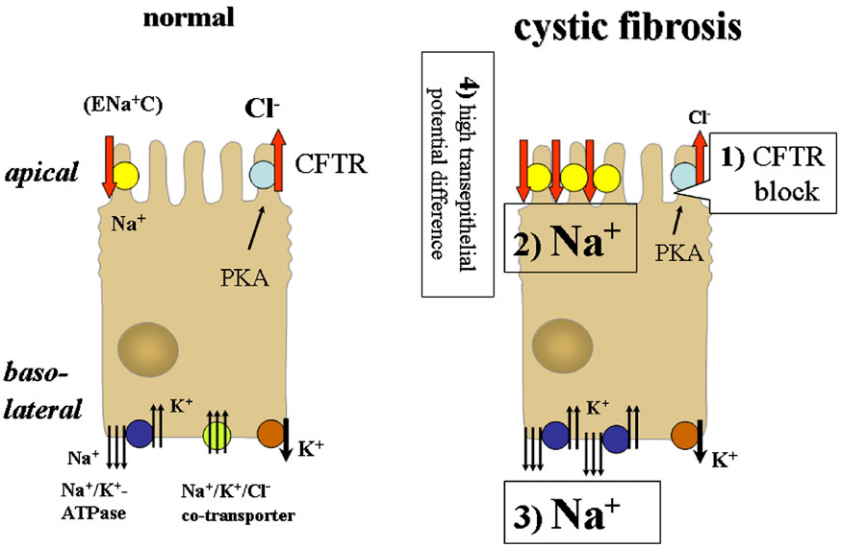

Fig. 1. Model of ion and water movements in polar epithelial cells (according to Knox and Peckham, 1995). Basolateral potassium conductance and sodium/potassium ATPases generate the driving force for apical chloride secretion which can be also directly stimulated by secretagogues or proteinkinase A activation. Epithelial sodium conductance at the apical part mediates transport of sodium and water from the lumen into the cell (ENa^+ C). In CF epithelial cells show the following changes: reduced apical chloride secretion (1), 3-fold enhanced apical sodium reabsorption (2), enhanced basolateral sodium secretion and consequently a high transepithelial potential difference (4).

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