

The Role of Ion Channels to Regulate Airway Ciliary Beat Frequency During Allergic Inflammation

Marta Joskova, M. Sutovska, P. Durdik, D. Koniar, L. Hargas, P. Banovcin, M. Hrianka, V. Khazaei, L. Pappova, and S. Franova

Abstract

Overproduction of mucus is a hallmark of asthma. The aim of this study was to identify potentially effective therapies for removing excess mucus. The role of voltage-gated (Kir 6.1, K_{Ca} 1.1) and store-operated ion channels (SOC, CRAC) in respiratory cilia, relating to the tracheal ciliary beat frequency (CBF), was compared under the physiological and allergic airway conditions. *Ex vivo* experiments were designed to test the local effects of Kir 6.1, K_{Ca} 1.1 and CRAC ion channel modulators in a concentration-dependent manner on the CBF. Cilia, obtained with the brushing method, were monitored by a high-speed video camera and analyzed with ciliary analysis software. In natural conditions, a Kir 6.1 opener accelerated CBF, while CRAC blocker slowed it in a concentration-dependent manner. In allergic inflammation, the effect of Kir 6.1 opener was insignificant, with a tendency to decrease CBF. A cilio-inhibitory effect of a CRAC blocker, while gently reduced by allergic inflammation, remained significant. A K_{Ca} 1.1 opener turned out to significantly enhance the CBF under the allergic OVA-sensitized conditions. We conclude that optimally attuned concentration of K_{Ca} 1.1 openers or special types of bimodal SOC channel blockers, potentially given by inhalation, might benefit asthma.

M. Joskova (✉), M. Sutovska, V. Khazaei, L. Pappova, and S. Franova
Department of Pharmacology, BioMed Martin, Jessenius Faculty of Medicine, Comenius University in Bratislava, 4C Mala Hora St, 036 01 Martin, Slovakia
e-mail: joskova@jfmmed.uniba.sk

P. Durdik and P. Banovcin
Department of Children and Adolescents, Jessenius Faculty of Medicine, Comenius University in Bratislava and Martin University Hospital, 2 Kollarova St, 036 01 Martin, Slovakia

D. Koniar, L. Hargas, and M. Hrianka
Department of Mechatronics and Electronics, Faculty of Electrical Engineering, University of Zilina, 1 Univerzitna St, 010 26 Zilina, Slovakia

Keywords

Asthma • Ciliary beat frequency • Mucociliary transport • Store-operated ion channels • Voltage-gated ion channels

1 Introduction

Ion channels are transmembrane proteins through which ions pass according to their electrochemical gradient. They are gated by voltage, second messengers, and other intracellular/extracellular mediators and are implicated in a multitude of pathophysiological processes, including respiratory system diseases (Valverde et al. 2011).

Out of the large spectrum of ion channels listed in the IUPHAR nomenclature, we focused our attention on voltage-gated and store-operated ion channels (SOC), particularly on the inwardly rectifying potassium channels (Kir 6.1), the calcium-activated potassium channels (potassium large conductance calcium-activated channels K_{Ca} 1.1), and the calcium release-activated calcium ion channels (CRAC).

In the airways, potassium channels contribute to the bronchodilator responses and control neuronal reflexes, the production of mucus and its secretion from goblet cells, the reduction of microvascular permeability, and the modulation of mucociliary clearance and epithelial cell restoration (Manzanares et al. 2011, 2014; Sutovska et al. 2013). All beneficial features of the potassium channel openers could be advantageous in the therapy of chronic pulmonary diseases if they are bronchoselective. In pulmonary medicine, inhalation is the preferred route of administration with the lower risk of systemic side effects. Only have a few experimental studies been devoted to the relationship of inhaled potassium channel openers and bronchodilation (Kidney et al. 1996). Cumulative single doses of potassium channel openers have been studied in adult patients with mild-to-moderate non-allergic asthma, but without confirmation of their bronchodilator efficacy (Faurschou et al. 1994). Purkey et al. (2014) have investigated the association between chronic rhinosinusitis and human genetic

variants in two airway epithelial potassium channels (K_{Ca} 1.1 and voltage-dependent Kv 7.5). K^+ channel genes have been confirmed in a greater number in *Paramecium*, a genus of ciliated protozoan, a representative of the ciliate group, than in humans (Haynes et al. 2003).

Many medications can alter the potassium channel function. Hypoglycemic agents derived from sulfonylurea, vasodilatory drug used to treat angina, nicorandil, and antiarrhythmic and inotropic (levosimendan) agents are known as the channel modulators. The ion channel expression differs under pathophysiological conditions. Asthma is associated with the loss of K_{Ca} 1.1 channel function and the upregulation of sensor (STIM1) and structural (Orai1) protein components of CRAC ion channels (Spinelli et al. 2012).

These latter ion channels respond to the depletion of endoplasmic reticulum (ER) calcium stores as a consequence of inositol triphosphate signal transduction, followed by the store-operated calcium entry from extracellular space *via* Orai1. This plasma membrane channel, through coupling with translocated STIM sensor protein, replenishes ER calcium stores. CRAC blocker regulate changes in defense mechanisms of the airways, e.g., cough reflex and bronchoconstriction under the asthma experimental conditions (Sutovska et al. 2013). They are also involved in secretory functions of mast cells, T cells, and eosinophils (Di Capite et al. 2011).

The inflammatory cells mentioned above and the structural cells of the airways, including epithelial cells, are major sources of mediator-driven chronic inflammation in asthma, the pathological features of which include bronchospasm, plasma exudation, mucus secretion, airway hyperreactivity and structural changes (Barnes 2003). Mucus overproduction makes expectoration more difficult. Therefore, stimulation of ciliary beating may increase and support mucociliary transport

to prevent airway obstruction by viscous mucus plugs. Several experimental studies have documented changes in the ion channel expression and function of the airway epithelium (Galiotta et al. 2004; Anagnostopoulou et al. 2010). Therefore, we hypothesized that the modulation of ion channels might affect the motor component of mucociliary clearance as well. We addressed the issue by examining the function of the Kir 6.1, K_{Ca} 1.1. and CRAC ion channels in the airway ciliary movement in the physiological condition and experimental allergen-induced airway inflammation.

2 Methods

The study protocol was approved by a local Ethics Committee of the Jessenius Faculty of Medicine in Martin, Slovakia. The experiments were in accord with the EU criteria for experimental animal welfare (EK 996/2012).

Experiments were performed in adult Trix strain male guinea pigs, weighing 250–300 g after a minimum 1-week quarantine period. The animals were obtained from the Department of Experimental Pharmacology, Slovak Academy of Sciences, Dobra Voda, Slovakia, and they were kept in an animal house with the recommended temperature, humidity, ventilation rate, noise levels and 12:12 h day/night cycles. The animals were housed in groups of maximum four *per* cage, with ready access to fresh water and a proper diet.

2.1 Study Design

The guinea pigs were randomly divided into the following experimental groups, consisting of eight animals each:

- Control – healthy, treated with 0.9 % NaCl for 21 days
- OVA – sensitized to ovalbumin allergen, treated with 0.9 % NaCl for 21 days

The following ion channel modulators were used:

- Pinacidil – Kir 6.1 channel opener
- Glibenclamide – Kir 6.1 channel blocker
- NS1619 – K_{Ca} 1.1 channel opener
- TEA – K_{Ca} 1.1 channel blocker
- FPCA – CRAC channel blocker

The groups with administration of substances to the tracheal cilia of healthy animals:

- P – pinacidil 10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$
- P + G – pinacidil 10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$ added to glibenclamide 10^{-6} , 10^{-5} , and 10^{-4} mol.l $^{-1}$
- NS – NS1619 10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$
- NS + TEA – NS1619 10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$ added to TEA 10^{-6} , 10^{-5} , and 10^{-4} mol.l $^{-1}$
- FPCA – FPCA 10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$
- DMSO – 10 % DMSO
- SB – positive control – salbutamol 10^{-4} mol.l $^{-1}$

The groups with administration of substances to the tracheal cilia of allergen-sensitized animals:

- PS – pinacidil 10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$ (PS7, PS6, PS5)
- NSS – NS1619 10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$ (NSS7, NSS6, NSS5)
- FPCAS – FPCA 10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$ (FPCAS7, FPCAS6, FPCAS5)
- DMSOS – 10 % DMSO
- SBS – positive control – salbutamol 10^{-4} mol.l $^{-1}$

2.2 Chemicals

The following chemical agents were purchased from Sigma-Aldrich (Hamburg, Germany): DMSO – dimethyl sulfoxide, pinacidil monohydrate – ($\pm$)-N-cyano-N'-4-pyridinyl-N''-(1,2,2-trimethylpropyl) guanidine monohydrate, glibenclamide – 5-chloro-N-[4-(cyclohexylureidosulfonyl) phenethyl]-2-methoxybenzamide, glyburide – N-p-[2-(5-chloro-2-methoxybenzamido) ethyl] benzenesulfonyl-N'-cyclohexylurea, NS1619 – 1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl) phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one, TEA – tetraet

hylammonium chloride. FPCA – 3-fluoropyridine-4-carboxylic acid was purchased from Alfa Aesar (Karlsruhe, Germany) and RPMI 1640 medium from Invitrogen/Life Technologies Gibco (Waltham, MA). Pinacidil, glibenclamide, and NS1619, were dissolved in 10 % DMSO before they were diluted to a definite concentration of 10^{-5} mol.l⁻¹, 10^{-6} mol.l⁻¹, and 10^{-7} mol.l⁻¹. TEA was dissolved in 0.9 % NaCl to a concentration of 10^{-5} mol.l⁻¹, 10^{-6} mol.l⁻¹, and 10^{-7} mol.l⁻¹. FPCA was dissolved in water for injection prior to its dilution in the saline to a concentration of 10^{-5} mol.l⁻¹, 10^{-6} mol.l⁻¹, and 10^{-7} mol.l⁻¹.

2.3 Ovalbumin-Induced Allergic Inflammation of Airways

The ovalbumin allergen causes airway reactivity changes *via* immunological mechanisms. Aluminium hydroxide, Al(OH)₃, adjuvant is known as a Th₂ inducer. A suspension of ovalbumin of 10^{-5} mol.l⁻¹ in Al(OH)₃ was administered over a period of 21 days. Guinea pigs received concurrent 0.5 ml OVA intraperitoneal and subcutaneous injections on Day 1 of sensitization, 0.5 ml OVA intraperitoneally alone on Day 4, and 0.5 ml OVA subcutaneously on Day 14. The degree of sensitization was confirmed by responses to allergen (1 % OVA), given by inhalation, once a day for 1–3 min on Days 15–21 through a PARI Jet Nebulizer (Paul Ritzau, Pari-Werk GmbH; Starnberg, Germany; output 5 l.s⁻¹, particle mass median diameter 1.2 µm) attached to a double-chamber whole body plethysmograph (HSE type 855, Hugo Sachs Elektronik, Germany). All animal experiments were initiated 1 week after the last allergen exposure.

2.4 Ciliary Beat Frequency Analysis

An analysis of ciliary beat frequency was carried out in an *in vitro* laboratory air-conditioned setting, with controlled temperature (21–24 °C) and

relative humidity (approximately 55 ± 10 %). Temperature of the cilia RPMI 1640 medium (ThermoFisher Scientific; Waltham, MA) and the microscopic glass slide was maintained in a range of 37–38 °C by a PeCon 2000–2 Temp Controller (PeCon GmbH; Erbach, Germany). After sacrificing the animals, a transverse access to the anterior tracheal wall was made by the midline incision of neck tissues. Ciliated samples were obtained by brushing the tracheal wall, with a cytology brush of 2.5 mm in diameter. The brush was dipped into the saline, gently rotated on the mucosal surface of the trachea and then removed. Cilia were suspended in 1 ml of RPMI 1640 Medium and used to make a microscopic preparation. Only were undisrupted beating ciliated cells recorded with a digital high-speed video camera (Basler A504kc; Basler AG, Germany) at a frame rate of 256–512 *per* sec. The camera was connected with both inverted phase contrast microscope (Zeiss Axio Vert. A1; Carl Zeiss AG, Göttingen, Germany) and a computer. There were approximately 10–12 sequential image recordings, each approximately 10 s in duration, of the same preparation performed at 1 min intervals.

Video records were analyzed using ciliary analysis software (LabVIEW™) to generate the ciliary region of interest (ROI), the intensity variation in selected ROIs, and the intensity variance curve. The curve was subjected to the fast Fourier transform (FFT) algorithm. The Fourier spectrum of each intensity variance curve was then equal to the frequency spectrum of beating in selected ROIs. ROIs were finally compared with the relevant video record to filter out artefacts.

2.5 Statistical Analysis

The median of ciliary beat frequency for each ROI and the arithmetic mean of a set of ROI values for each sample were used to determine the ciliary beat frequency (CBF) expressed in Hertz (Hz). All data were expressed as means $\pm$ SE. Statistical significance of differences was assessed with one-way ANOVA with *post-hoc* Bonferroni test. A p-value of <0.05 was used to define significant differences.

3 Results

In healthy guinea pigs, the opener of Kir 6.1 potassium ion channels pinacidil (10^{-5} mol.l $^{-1}$, 10^{-6} mol.l $^{-1}$), caused a significant increase in CBF (* p <0.05). This effect was concentration-dependent and was abolished in the presence of the non-specific Kir 6.1 blocker glibenclamide (10^{-4} mol.l $^{-1}$ and 10^{-5} mol.l $^{-1}$) (Fig. 1a). In contrast, during airway allergic inflammation, the opening of Kir 6.1 channels by pinacidil (10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$) was associated with a tendency to a decrease in CBF (Fig. 1b).

The opener of K $_{Ca}$ 1.1 channels NS1619 (10^{-7} , 10^{-6} , and 10^{-5} mol.l $^{-1}$) had no effect on the CBF in the physiological condition (Fig. 2a), but its highest concentration (10^{-5} mol.l $^{-1}$) caused a significant enhancement of CBF in the airway inflammatory condition (Fig. 2b).

The selective blocker of SOC (CRAC) ion channels FPCA (10^{-7} mol.l $^{-1}$, 10^{-6} mol.l $^{-1}$, 10^{-5} mol.l $^{-1}$) significantly reduced the ciliary movement, almost in a concentration-dependent

manner (* p <0.05, ** p <0.01, *** p <0.001, respectively) in the physiological condition (Fig. 3a). This decrease was mitigated but still significant by FPCA concentration of 10^{-5} mol.l $^{-1}$ in allergic inflammatory conditions (Fig. 3b).

4 Discussion

In this study we demonstrate the role of potassium (Kir 6.1 and K $_{Ca}$ 1.1) and calcium (CRAC) ion channels in the regulation of tracheal ciliary beat frequency (CBF) in healthy and ovalbumin-sensitized guinea pigs. We confirmed the crucial role of Kir 6.1 and CRAC ion channels in the modulation of the CBF in both experimental conditions. Kir 6.1 channels were engaged in the tracheal ciliostimulation in the healthy condition, which is in line with the findings of Ohba et al. (2013), who have demonstrated a relationship between pharmacological K $_{ATP}$ stimulation and acceleration of ciliary movement in mice. These authors show that activation of

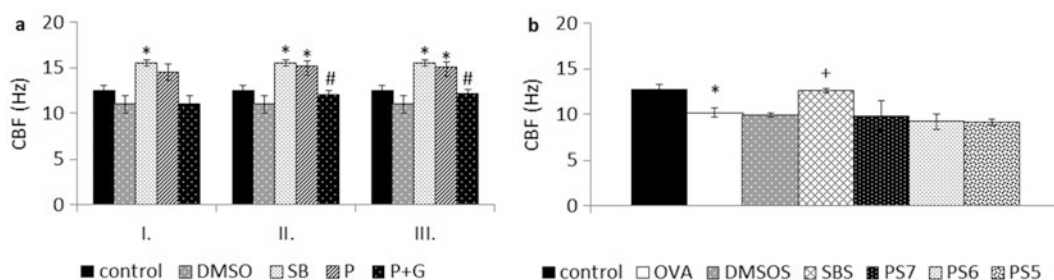


Fig. 1 The role of Kir 6.1 ion channels in the regulation of ciliary beat frequency (CBF) in unsensitized and ovalbumin (OVA)-sensitized animals after local application of DMSO, salbutamol, and pinacidil and glibenclamide in *in vitro* condition. Tracheal cilia were exposed to the agents always after brushing. (a) physiological conditions: I – pinacidil (10^{-7} mol.l $^{-1}$), glibenclamide (10^{-6} mol.l $^{-1}$); II – pinacidil (10^{-6} mol.l $^{-1}$), glibenclamide (10^{-5} mol.l $^{-1}$); and III – pinacidil (10^{-5} mol.l $^{-1}$), glibenclamide (10^{-4} mol.l $^{-1}$). Control group – cilia of healthy guinea pigs exposed to saline; DMSO – cilia of healthy guinea pigs exposed to 10 % DMSO; SB – cilia of healthy guinea pigs exposed to salbutamol (10^{-4} mol.l $^{-1}$); P – cilia of healthy guinea pigs exposed to pinacidil (10^{-7} mol.l $^{-1}$; 10^{-6} mol.l $^{-1}$; 10^{-5} mol.l $^{-1}$), an opener of Kir 6.1; P + G – cilia of healthy guinea pigs exposed to pinacidil (10^{-7} mol.l $^{-1}$; 10^{-6}

mol.l $^{-1}$; 10^{-5} mol.l $^{-1}$) plus glibenclamide (10^{-6} mol.l $^{-1}$; 10^{-5} mol.l $^{-1}$; 10^{-4} mol.l $^{-1}$); (b) allergic condition consisting of OVA-sensitized guinea pigs treated for 21 days with 0.9 % NaCl – control bar is same as in Panel A; DMSOS – cilia of OVA-sensitized group exposed to 10 % DMSO; SBS – cilia of OVA-sensitized group exposed to salbutamol (10^{-4} mol.l $^{-1}$); PS7 – cilia of OVA-sensitized group exposed to pinacidil (10^{-7} mol.l $^{-1}$), an opener of Kir 6.1; PS6 – cilia of OVA-sensitized group exposed to pinacidil (10^{-6} mol.l $^{-1}$); PS5 – cilia of OVA-sensitized group exposed to pinacidil (10^{-5} mol.l $^{-1}$) (Data are expressed as means $\pm$ SE; n = 8; * p < 0.05 compared with the control group; + p <0.05 compared with the OVA group; # p < 0.05 compared with the P group)

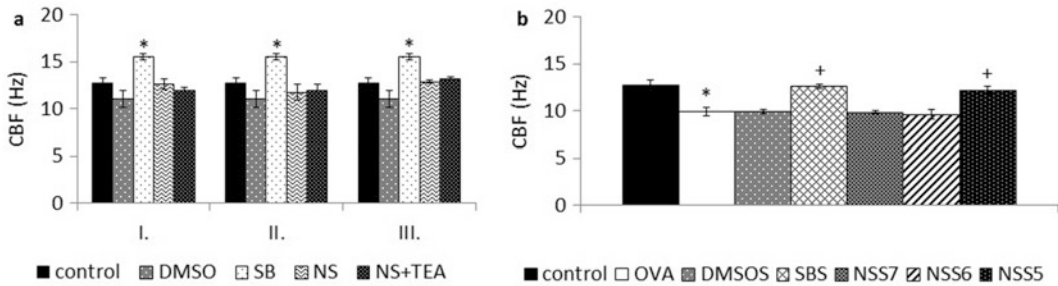


Fig. 2 The role of K_{Ca} 1.1 ion channels in the regulation of ciliary beat frequency (CBF) in unsensitized and ovalbumin (OVA)-sensitized guinea pigs after local application of DMSO, salbutamol, and NS1619 and tetraethylammonium chloride (TEA) in *in vitro* condition. Tracheal cilia were exposed to the agents always after brushing. (a) physiological condition: I – NS1619 (10^{-7} mol.l $^{-1}$), TEA (10^{-6} mol.l $^{-1}$); II – NS1619 (10^{-6} mol.l $^{-1}$), TEA (10^{-5} mol.l $^{-1}$); and III – NS1619 (10^{-5} mol.l $^{-1}$), TEA (10^{-4} mol.l $^{-1}$); Control group – cilia of healthy guinea pigs exposed to saline; DMSO – cilia of healthy guinea pigs exposed to 10 % DMSO; SB – cilia of healthy guinea pigs exposed to salbutamol (10^{-4} mol.l $^{-1}$); NS – cilia of healthy guinea pigs exposed to NS1619 (10^{-7} mol.l $^{-1}$; 10^{-6} mol.l $^{-1}$; 10^{-5} mol.l $^{-1}$), an opener of K_{Ca} 1.1; NS + TEA – cilia of healthy guinea pigs exposed to NS1619

(10^{-7} mol.l $^{-1}$; 10^{-6} mol.l $^{-1}$; 10^{-5} mol.l $^{-1}$) plus TEA (10^{-6} mol.l $^{-1}$; 10^{-5} mol.l $^{-1}$; 10^{-4} mol.l $^{-1}$), a blocker of K_{Ca} 1.1; (b) allergic condition consisting of OVA-sensitized guinea pigs treated for 21 days with 0.9 % NaCl – control bar is same as in Panel A; DMSO – cilia of OVA-sensitized group exposed to 10 % DMSO; SBS – cilia of OVA-sensitized group exposed to salbutamol (10^{-4} mol.l $^{-1}$); NSS7 – cilia of OVA-sensitized group exposed to the NS1619 (10^{-7} mol.l $^{-1}$), an opener of K_{Ca} 1.1; NSS6 – cilia of OVA-sensitized group exposed to NS1619 (10^{-6} mol.l $^{-1}$); NSS5 – cilia of OVA -sensitized group exposed to NS1619 (10^{-5} mol.l $^{-1}$) (Data are expressed as means $\pm$ SE; n = 8; *p < 0.05 compared with the control group; +p < 0.05 compared with the OVA group)

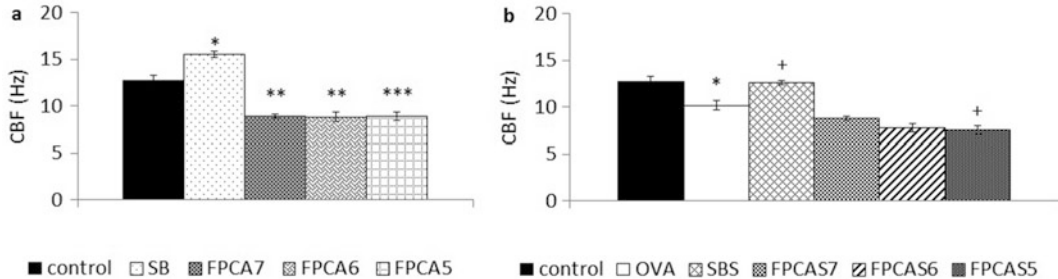


Fig. 3 The role of SOC (CRAC) ion channels in the regulation of ciliary beat frequency (CBF) in unsensitized and ovalbumin (OVA)-sensitized animals after local application of 3-fluoropyridine-4-carboxylic acid (FPCA) in *in vitro* condition. Tracheal cilia were exposed to the agents always after brushing. (a) physiological conditions: Control group – cilia of healthy guinea pigs exposed to saline; SB – cilia of healthy guinea pigs exposed to salbutamol (10^{-4} mol.l $^{-1}$); FPCA7/FPCA6/FPCA5 – cilia of healthy guinea pigs exposed to FPCA (10^{-7} mol.l $^{-1}$; 10^{-6} mol.l $^{-1}$; 10^{-5}

mol.l $^{-1}$), a blocker of CRAC; (b) allergic condition consisting of OVA-sensitized guinea pigs treated for 21 days with 0.9 % NaCl – control bar is same as in Panel A; SBS – cilia of OVA-sensitized group exposed to salbutamol (10^{-4} mol.l $^{-1}$); FPCAS7/FPCAS6/FPCAS5 – cilia of OVA-sensitized group exposed to FPCA (10^{-7} mol.l $^{-1}$; 10^{-6} mol.l $^{-1}$; 10^{-5} mol.l $^{-1}$), a blocker of CRAC (Data are expressed as means $\pm$ SE; n = 8; *p < 0.05, **p < 0.01, and ***p < 0.001 compared with the control group; +p < 0.05 compared with the OVA group)

non-voltage dependent calcium channels (non-VDCC) is a consequence of membrane hyperpolarization induced by a K_{ATP} opener. We demonstrate in the present study that ciliary movement slows down when Kir 6.1 ion channels

become open in allergic inflammation. That, in turn, seems in line with an argument that CBF decreases with increasing external mucus viscosity until it reaches a plateau (Liedtke and Heller 2007).

There have only been a few reports on the identification of potassium ion channels in ciliates (Haynes et al. 2003). Past research has established an association between calcium-activated potassium channels and swimming behavior in cilia (Valentine et al. 2012). In contrast, our present results demonstrate that large-conductance K_{Ca} 1.1 channels were not crucial in affecting the tracheal CBF in healthy guinea pigs, but became important in pathology. These channels are abundant in normal smooth muscle cells of airways, where they regulate membrane potentials and the process of muscle contraction. In human bronchial epithelium, apical K_{Ca} 1.1 channels regulate surface liquid volume (Manzanares et al. 2011). However, these channels have not been identified in the mouse tracheal epithelium (Schreiber et al. 2002). It is of interest that cytokines, typical for allergic asthma, may have diverse effects on K_{Ca} 1.1 channel activity. Whereas interleukin-4 (IL-4) provides a stimulatory input, IL-13 partly antagonizes the effect of IL-4 (Martin et al. 2008). A human study of Laoukili et al. (2001) reported a time- and dose-dependent inhibitory effect of the Th_2 cytokine, IL-13, on the nasal CBF. Different sensitivities of individual subfamilies of calcium-activated potassium channels to inhibitors have also been determined. The sequence homology of transmembrane hydrophobic cores has revealed the following differences: BK channels are large conductance K_{Ca} 1.1 channels inhibited by TEA, SK channels are small conductance K_{Ca} 2.1, 2.2, and 2.3 channels, IK channels are intermediate conductance K_{Ca} 3.1 channels, and the other subfamilies – K_{Ca} 4.1, 4.2, and K_{Ca} 5.1 are structurally related to K_{Ca} 1.1 channels, but insensitive to internal Ca^{2+} (Perez-Zoghbi et al. 2009). It is possible that other subtypes of potassium channels, such as K_{Ca} 3.1, could be involved in the alteration of CBF during natural conditions.

In the present study we also demonstrate the importance of SOC ion channels of airway cilia in the control of CBF in the healthy condition. A possible explanation of this role may be a deficit in the calcium replenishment of empty stores in the endoplasmic reticulum resulting from SOC ion channel inhibition. Calcium ions belong to

the intracellular signals that mediate changes in CBF in response to different stimuli. SOC ion channels have also been implicated in the regulation of brain ependymal cilia (Nguyen et al. 2001). In the present study, inhibitory effect on ciliary beating of SOC antagonism persisted, although mitigated, in ovalbumin-driven allergic inflammation. The SOC channels could participate in maintaining ciliary movement during inflammation as a result of action of inflammatory mediators. Prostaglandins and histamine released during allergic inflammation influence mucociliary clearance, acting alone or in combination with other mediators. Prostaglandin E1 (PGE1) increases tracheal CBF in the guinea pig and enhances the stimulatory effect of histamine on CBF in the rabbit maxillary sinus. Nonetheless, histamine does not appreciably influence CBF in the guinea pig, due likely to interspecies differences in responsiveness to inflammatory mediators (Khan et al. 1995; Dolata 1990).

CRAC channels in airway cilia play a notable role in the pathophysiology of allergic airway inflammation (Di Capite et al. 2011). The tracheal surface in guinea pigs is abundant in ciliated cells, which makes these animals much suitable for airway epithelium studies (Li et al. 2011). The concentration of the CRAC ion channel blocker used in the present study was identical to that used in other experiments that confirmed the blocker's ability to weaken the cough reflex and airway resistance (Sutovska et al. 2013). Therefore, allergic inflammation might modify the expression and function of CRAC channels.

Respiratory infections often evoked by ciliary dysfunction might reduce the amount of intact cilia and, along with hypersecretion of mucus, they can intensify a vicious inflammatory cycle. We thus submit that modulation of CBF by potassium (Kir 6.1, K_{Ca} 1.1) and SOC ion channels could plausibly be an expression of airway defense mechanisms. Medicines with potential ciliostimulating effects might act beneficially by aiding the mucociliary defense mechanism. As CRAC channels exhibit bimodal concentration-dependent responses to their

blockade (Jairaman and Prakriya 2013), nebulization of specific CRAC blocking agents, yet to be designed, could benefit the management of airway inflammatory conditions. On the other side, Kir 6.1 channel openers, known as potential bronchodilators, do not appear to have a positive effect on CBF in asthma. By contrast, K_{Ca} 1.1 openers could ameliorate a disturbed natural cleaning function of airway cilia in inflammation, providing these agents are given directly to the airways, which would also help avoid systemic side effects. A better understanding of the ion channels' peculiarities would be of therapeutic potential in the pathological states characterized by deranged function of cilia in the tracheal epithelial cells in airway allergic inflammatory conditions.

Acknowledgement This research was conducted within the project 'Measurement of Respiratory Epithelium Ciliun Kinematics' and was supported by the grants VEGA 1/0165/14; MZ 2012/35-UK MA-12; APVV-0305-12; BioMed Martin (ITMS 26220220187); Center of Experimental and Clinical Respiratory II, and 'The increasing of opportunities for career growth in research and development in the medical sciences', co-financed from EU sources. The authors would like to thank Katarina Jesenska for technical help during the experiments.

Conflict of Interests All authors declared no conflicts of interest in relation to this article.

References

- Anagnostopoulou P, Dai L, Schatterny J, Hirtz S, Duerr J, Mall MA (2010) Allergic airway inflammation induces a pro-secretory epithelial ion transport phenotype in mice. *Eur Respir J* 36:1436–1447
- Barnes PJ (2003) Pathophysiology of asthma. *European Respiratory Monograph* 23:84–113
- Di Capite JL, Bates GJ, Parekh AB (2011) Mast cell CRAC channel as a novel therapeutic target in allergy. *Curr Opin Allergy Clin Immunol* 11:33–38
- Dolata J (1990) Prostaglandin E1 enhances the histamine induced stimulation of the mucociliary activity in the rabbit maxillary sinus. *Eur Respir J* 3:559–565
- Faurschou P, Mikkelsen KL, Steffensen I, Franke B (1994) The lack of bronchodilator effect and the short-term safety of cumulative single doses of an inhaled potassium channel opener (bimakalim) in adult patients with mild to moderate bronchial asthma. *Pulmonary Pharmacology Therapeutics* 7:293–297
- Galiotta LJV, Folli C, Caci E, Pedemonte N, Taddei A, Ravazzolo R, Zegarra-Moran O (2004) Effect of inflammatory stimuli on airway ion transport. *Proc Am Thorac Soc* 1:62–65
- Haynes WJ, Ling KY, Saimi Y, Kung C (2003) PAK paradox: paramecium appears to have more K^+ -channel genes than humans. *Eukaryotic Cell* 2:737–745
- Jairaman A, Prakriya M (2013) Molecular pharmacology of store-operated CRAC channels. *Channels* 7:402–414
- Khan R, Dolata J, Lindberg S (1995) Effects of inflammatory mediators on ciliary function in vitro. *Rhinology* 33:22–25
- Kidney JC, Lotvall JO, Lei Y, Chung KF, Barnes PJ (1996) The effect of inhaled K^+ channel openers on bronchoconstriction and airway microvascular leakage in anaesthetised guinea pigs. *Eur J Pharmacol* 296:81–87
- Laoukili J, Perret E, Willems T, Minty A, Parthoens E, Houcine O, Coste A, Jorissen M, Marano F, Caput D, Tournier F (2001) IL-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells. *J Clin Invest* 108:1817–1824
- Li Y, Wang J, He HY, Ma LJ, Zeng J, Deng GC, Liu X, Engelhardt JF, Wang Y (2011) Immunohistochemical demonstration of airway epithelial cell markers of guinea pig. *Tissue Cell* 43:283–290
- Liedtke WB, Heller S (eds) (2007) The TRP ion channel function in sensory transduction and cellular signaling cascades. The TRPV4 channel in ciliated epithelia. CRC Press Taylor and Francis Group, USA, Boca Raton, Chapter 30. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK5252/>. Accessed on 20 Jan 2016
- Manzanares D, Gonzalez C, Ivonnet P, Chen RS, Valencia-Gattas M, Conner GE, Larsson HP, Salathe M (2011) Functional apical large conductance, Ca^{2+} -activated, and voltage-dependent K^+ channels are required for maintenance of airway surface liquid volume. *J Biol Chem* 286:19830–19839
- Manzanares D, Srinivasan M, Salathe ST, Ivonnet P, Baumbach N, Dennis JS, Conner GE, Salathe M (2014) IFN- γ -mediated reduction of large-conductance, Ca^{2+} -activated, voltage-dependent K^+ (BK) channel activity in airway epithelial cells leads to mucociliary dysfunction. *Am J Physiol Lung Cell Mol Physiol* 306:L453–L462
- Martin G, O'Connell RJ, Pietrzykowski AZ, Treisman SN, Ethier MF, Madison JM (2008) Interleukin-4 activates large-conductance, calcium activated potassium (BKCa) channels in human airway smooth muscle cells. *Exp Physiol* 93:908–918
- Nguyen T, Chin WC, O'Brien JA, Verdugo P, Berger AJ (2001) Intracellular pathways regulating ciliary beating of rat brain ependymal cells. *J Physiol* 531 (Pt 1):131–140
- Ohba T, Sawada E, Suzuki Y, Yamamura H, Ohya S, Tsuda H, Imaizumi Y (2013) Enhancement of Ca^{2+} influx and ciliary beating by membrane

- hyperpolarization due to ATP-sensitive K^+ channel opening in mouse airway epithelial cells. *J Pharmacol Exp Ther* 347:145–153
- Perez-Zoghbi JF, Karner C, Ito S, Shepherd M, Alrashdan YA, Sanderson MJ (2009) Ion channel regulation of intracellular calcium and airway smooth muscle function. *Pulmonary Pharmacology Therapeutics* 22:388–397
- Purkey MT, Li J, Mentch F, Grant SFA, Desrosiers M, Hakonarson H, Toskala E (2014) Genetic variation in genes encoding airway epithelial potassium channels is associated with chronic rhinosinusitis in a pediatric population. *PLoS ONE* 9:e89329. doi:[10.1371/journal.pone.0089329](https://doi.org/10.1371/journal.pone.0089329)
- Schreiber R, Mürle B, Sun J, Kunzelmann K (2002) Electrolyte transport in the mouse trachea: no evidence for a contribution of luminal K^+ conductance. *J Membr Biol* 189:143–151
- Spinelli AM, González-Cobos JC, Zhang X, Motiani RK, Rowan S, Zhang W, Garrett J, Vincent PA, Matrougui K, Singer HA, Trebak M (2012) Airway smooth muscle STIM1 and Orai1 are upregulated in asthmatic mice and mediate PDGF-activated SOCE, CRAC currents, proliferation, and migration. *Pflugers Arch* 464:481–492
- Sutovska M, Adamkov M, Kocmalova M, Mesarosova L, Oravec M, Franova S (2013) CRAC ion channels and airway defense reflexes in experimental allergic inflammation. *Adv Exp Med Biol* 756:39–48
- Valentine MS, Rajendran A, Yano J, Weeraratne SD, Beisson J, Cohen J, Koll F, van Houten J (2012) *Paramecium* BBS genes are key to presence of channels in Cilia. *Cilia* 1:16
- Valverde MA, Cantero-Recasens G, Garcia-Elias A, Jung C, Carreras-Sureda A, Vicente R (2011) Ion channels in asthma. *J Biol Chem* 286:32877–32882