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Nicotine-induced activation of soluble adenylyl cyclase participates in ion transport regulation in mouse tracheal epithelium

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ABSTRACT

Aims: Functional nicotinic acetylcholine receptors (nAChR) have been identified in airway epithelia and their location in the apical and basolateral membrane makes them targets for acetylcholine released from neuronal and non-neuronal sources. One function of nAChR in airway epithelia is their involvement in the regulation of transepithelial ion transport by activation of chloride and potassium channels. However, the mechanisms underlying this nicotine-induced activation of ion transport are not fully elucidated. Thus, the aim of this study was to investigate the involvement of adenylyl cyclases in the nicotine-induced ion current in mouse tracheal epithelium.

Main methods: To evaluate the nicotine-mediated changes of transepithelial ion transport processes electrophysiological Ussing chamber measurements were applied and nicotine-induced ion currents were recorded in the absence and presence of adenylyl cyclase inhibitors.

Key findings: The ion current changes induced by nicotine (100 μM, apical) were not altered in the presence of high doses of atropine (25 μM, apical and basolateral), underlining the involvement of nAChR. Experiments with the transmembrane adenylyl cyclase inhibitor 2'5'-dideoxyadenosine (50 μM, apical and basolateral) and the soluble adenylyl cyclase inhibitor KH7 (10 μM, apical and basolateral) both reduced the nicotine-mediated ion current to a similar extent. Yet, a statistically significant reduction was obtained only in the experiments with KH7.

Significance: This study indicates that nicotine binding to nAChR in mouse tracheal epithelium activates transepithelial ion transport involving adenylyl cyclase activity. This might be important for novel therapeutic strategies targeting epithelial ion transport mediated by the non-neuronal cholinergic system.

Introduction

Nicotinic acetylcholine receptors (nAChR) are pentameric receptors that can be assembled by different combinations of subunits that define the properties of the particular nAChR subtypes. Until now 10 α subunits (α1-α10), 4 β subunits (β1-β4), 1 δ subunit, 1 ε subunit and 1 γ subunit have been identified that serve as a pool for the formation of the various nAChR (Lustig, 2006). Different subtypes of these receptors can be found in a wide variety of tissues and cell types, such as neurons, muscle cells, immune cells and also epithelial cells (Kummer et al., 2008; Lustig, 2006).

During recent years evidence for the presence of nAChR in the airway epithelium accumulated. Transcripts for several subunits of nAChR were found in human bronchial epithelial cells (Maus et al., 1998) as well as in rat and mouse tracheal epithelium (Kummer et al., 2008; Hollenhorst et al., 2012). Moreover, the α7 and the α5 nAChR subunits are present in the embryonic lung and play an important role in lung development (Reynolds and Hoidal, 2005; Reynolds et al., 2010). A recent study in mice additionally identified the α7 nAChR as playing an essential role during alterations of lung development induced by prenatal nicotine exposure (Wongtrakool et al., 2012). Additionally, functional nAChR have been described in human nasal epithelium (Blank et al., 1997), in human bronchial epithelium (Maus et al., 1998) and in monkey bronchial epithelial cells (Fu et al., 2009). Furthermore, we have recently been able to detect the presence of functional nAChR on the apical and the basolateral side of the mouse tracheal epithelium (Hollenhorst et al., 2012). By this we provided evidence for a possible targeting of nAChR by ACh released from non-neuronal sources such as airway epithelial cells and immune cells as well as by ACh released from cholinergic nerve endings.

It was further shown that administration of nicotine to the mouse tracheal epithelium evokes an apical chloride secretion that is dependent on a basolateral potassium secretion (Hollenhorst et al., 2012). The mechanisms behind this nicotine-induced effect on transepithelial ion transport are so far not fully understood. There are hints for an involvement of cAMP-dependent signaling in addition to Ca^{2+}-dependent activation

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of Ca^{2+}-regulated ion channels (Hollenhorst et al., 2012). Increased intracellular cAMP levels are generated by cAMP-synthesizing adenylyl cyclases. These enzymes can be grouped in transmembrane adenylyl cyclases (tmAC) and soluble adenylyl cyclases (sAC) according to their location in the cells. Both adenylyl cyclases, tmAC (tmAC1 and tmAC8) and sAC, can be activated by Ca^{2+} (Dunn et al., 2009; Ferguson and Storm, 2004), providing a possible connection between Ca^{2+}- and cAMP-dependent signaling processes. Therefore, we here investigate the involvement of adenylyl cyclases in the nicotine-induced transepithelial ion transport.

Materials and methods

Animals and tissue preparation

Female and male 10 to 12 week old C57Bl/6 mice were either obtained from the local animal breeding facility (Justus-Liebig-University, Giessen, Germany) or purchased from Charles River (Sulzfeld, Germany). The mice were kept under standard conditions and sacrificed by exposition to an overdose of the narcotic isoflurane (Abbot, Wiesbaden, Germany) followed by aortic exsanguination. The trachea was immediately removed and placed in an ice-cold buffer solution until further preparations were started. The buffer was composed of (in mM): 145 NaCl, 1.6 K_2HPO_4, 1.3 Ca^{2+}-gluconate, 1 $MgCl_2$ and 0.4 KH_2PO_4, 5 glucose, 5 HEPES (pH 7.4 with TRIZMA base). All experiments were carried out in accordance with the current law on the protection of animals in Germany.

Ussing chamber recordings

The transepithelial tracheal ion transport of C57Bl/6 mice was investigated electrophysiologically with the Ussing chamber technique. The freshly isolated tracheae were mounted into a modified Ussing chamber with a circular aperture of 1.8 mm^2. Before mounting, the surrounding connective tissue covering the cartilage was removed and the tracheae were cut open longitudinally on the ventral side under the dissection microscope. The apical and the basolateral compartments were continuously perfused with a buffer solution (see "animals and tissue preparations" for composition) that was heated to 37 °C.

The Ussing chamber was connected to a voltage-clamp amplifier via Ag/AgCl electrodes that were linked with bridges of 2% agar and 3 M KCl to the bathing compartments. The spontaneously generated transepithelial potential (V_T) was clamped to 0 V after an equilibration period of approx. 5 min. All experiments were carried out under short-circuit conditions. The short-circuit current (I_{SC}) was recorded on a strip chart recorder (Philips, Amsterdam, The Netherlands) and acquired via a MacLab/2e interface and the Chart program (ADInstruments, Bella Vista, Australia) on a Macintosh LCII computer (Apple, USA).

Chemicals

NaCl, D-glucose, $MgCl_2$, and Ca^{2+}-gluconate were obtained from Fluka (Taufkirchen, Germany). KH_2PO_4 and K_2HPO_4 were purchased from Serva (Heidelberg, Germany). HEPES, nicotine-d-di-tartrate-salt and atropine were ordered from Sigma (Taufkirchen, Germany). KH7 was obtained from Cayman Chemical Company (Ann Arbor, MI, USA) and 2',5'-dideoxyadenosine from Enzo Life Sciences (Lörrach, Germany).

Data analysis and statistics

All values are presented as mean ± standard error of the mean (SEM) and were normalized to 1 cm^2 measuring area. The number of the carried out experiments is marked by "n." All data were first analyzed for normal distribution with the Kolmogorov–Smirnov test. Afterwards statistical significance was evaluated with the paired Student's *t*-test. Values with $p<0.05$ were considered as significantly different and marked with asterisks (*). The nicotine-induced ion current is designated I_{NIC}.

Results

It has previously been described that some ACh receptors display mixed nicotinic-muscarinic pharmacological receptor properties that can be activated and/or inhibited by both, nicotinic and muscarinic agonists and/or antagonists (Verbitsky et al., 2000; Cox, 1993; Lustig, 2006). Further, an upregulation of both, nAChR and muscarinic receptors due to nicotine treatment has been described in the rat bladder (Yamamoto et al., 2011). Therefore, we performed experiments in which we examined the influence of the muscarinic receptor antagonist atropine on the nicotine-mediated ion current (I_{NIC}) to evaluate the potential contribution of muscarinic receptors to I_{NIC}. In all experiments nicotine has been applied on the apical side of the epithelium, whereas atropine (25 μM) was applied simultaneously to the apical and the basolateral side of the epithelium. Application of nicotine (100 μM, apical) under control conditions (without atropine) resulted in a transient current increase (Fig. 1A). After nicotine washout, epithelia were incubated with atropine and subsequently exposed to nicotine in the presence of atropine. The nicotine-mediated current change was not significantly affected by the presence of atropine judged by comparison of I_{NIC} after atropine to the control effect ($p=0.40$, $n=5$). These findings indicate that the nicotine-induced current changes are completely mediated by nAChR that are not sensitive to atropine.

Activation of nAChR can result in an increase of intracellular Ca^{2+} levels (Blank et al., 1997). Furthermore, there are hints that cAMP-mediated signaling processes are also involved in nicotinic receptor signaling (Hollenhorst et al., 2012). One possibility for the interaction between Ca^{2+}-dependent and cAMP-dependent pathways is represented by Ca^{2+}-dependent adenylyl cyclases. Therefore, we performed experiments with the adenylyl cyclase inhibitor 2'5'-dideoxyadenosine (DDA), which has been described as a cell permeable inhibitor that preferentially blocks tmAC (Landa et al., 2005; Dunn et al., 2009), and KH7, a specific inhibitor of sAC (Schmid et al., 2007). Application of DDA (50 μM, apical and basolateral) had no effect on the baseline current ($p=1$, $n=8$, Fig. 2A). Further, although a trend towards reduction was visible, the nicotine-induced ion current (100 μM, apical) was not significantly decreased in the presence of DDA ($p=0.1$, $n=8$, Fig. 2B). In contrast to this, a significantly reduced I_{NIC} ($p<0.05$, $n=6$) was observed when the tissue was pre-incubated with 10 μM KH7 (apical and basolateral side of the epithelium, Fig. 2C, D). Similar to the effect observed with DDA, KH7 did not significantly alter the baseline current ($p=0.28$, $n=6$). Thus, these results suggest that nicotine mediates cAMP-dependent activation of I_{NIC} and that this effect involves activation of sAC.

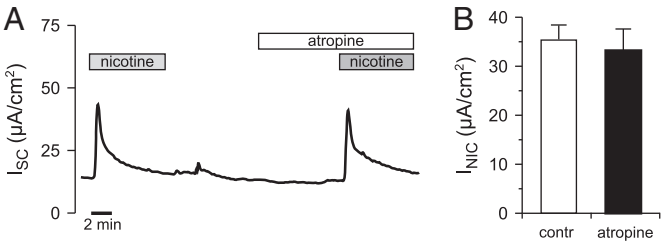

Fig. 1. A. Representative current trace showing the typical current increase that is induced by application of nicotine (100 μM, apical) in the absence (control effect) and the presence of atropine (25 μM, apical and basolateral). **B.** Statistical analysis revealed no significant changes between the nicotine-induced current (I_{NIC}) in the presence of atropine compared to control conditions ($p=0.40$, $n=5$).