J. Membrane Biol. 189, 143–151 (2002)
DOI: 10.1007/s00232-002-1009-5

The Journal of
Membrane
Biology

Electrolyte Transport in the Mouse Trachea: No Evidence for a Contribution of Luminal K^+ Conductance

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Received: 8 March 2002/Revised: 29 May 2002

Abstract. Recent studies on frog skin acini have challenged the question whether Cl^- secretion or Na^+ absorption in the airways is driven by luminal K^+ channels in series to a basolateral K^+ conductance. We examined the possible role of luminal K^+ channels in electrolyte transport in mouse trachea in Ussing-chamber experiments. Tracheas of both normal and CFTR (−/−) mice showed a dominant amiloride-sensitive Na^+ absorption under both, control conditions and after cAMP-dependent stimulation. The lumen-negative transepithelial voltage was enhanced after application of IBMX and forskolin and Cl^- secretion was activated. Electrolyte secretion induced by IBMX and forskolin was inhibited by luminal glibenclamide and the blocker of basolateral $Na^+2Cl^-K^+$ cotransporter azosemide. Similarly, the compound 293B, a blocker of basolateral KCNQ1/KCNE3 K^+ channels effectively blocked Cl^- secretion when applied to either the luminal or basolateral side of the epithelium. RT-PCR analysis suggested expression of additional K^+ channels in tracheal epithelial cells such as Slo1 and Kir6.2. However, we did not detect any functional evidence for expression of luminal K^+ channels in mouse airways, using luminal 293B, clotrimazole and Ba^{2+} or different K^+ channel toxins such as charybdotoxin, apamin and α-dendrotoxin. Thus, the present study demonstrates Cl^- secretion in mouse airways, which depends on basolateral $Na^+2Cl^-K^+$ cotransport and luminal CFTR and non-CFTR Cl^- channels. Cl^- secretion is maintained by the activity of basolateral K^+ channels, while no clear evidence was found for the presence of a luminal K^+ conductance.

Key words: CFTR — Mouse airways — Trachea — K^+ conductance — Cystic fibrosis — Epithelial transport

Introduction

According to well-established models, ion transport in airway and colonic epithelial cells is driven by basolateral K^+ channels, which maintain a hyperpolarized membrane voltage during absorption or secretion of electrolytes [27, 34, 43]. A hyperpolarized membrane voltage is essential to drive luminal Cl^- secretion by CFTR and Ca^{2+}-activated Cl^- channels, or to maintain Na^+ absorption via the epithelial Na^+ channel ENaC. Mouse trachea is a Na^+-absorbing epithelium under resting conditions, but is able to secrete electrolytes when stimulated with cAMP or Ca^{2+}-dependent agonists [18, 28, 30]. Cl^- secretion in the mouse trachea via CFTR and with the help of the basolateral $Na^+2Cl^-K^+$ cotransporter has been questioned recently [37]. In this in situ hybridization study, neither expression of CFTR nor $Na^+2Cl^-K^+$ cotransporter could be detected. These results are in agreement with immunocytochemical data, indicating a lack of CFTR expression in mouse trachea [45]. However, in previous studies by our group and by others, evidence for functional expression of both CFTR and basolateral $Na^+2Cl^-K^+$ cotransporter in mouse trachea has been found [18, 28]. We therefore reexamined the secretory transport in this tissue. Electrolyte transport in mouse trachea is different from that in other tissues, due to the presence of an alternative

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Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; IBMX, 3-isobutyl-1-methylxanthine; DIDS, 4,4′-diisothiocyano-2,2′-stilbene disulfonate; cAMP, 5′ cyclic adenosine monophosphate; ENaC, epithelial Na^+ channel; $I_{sc} = V_{te}/R_{te}$, equivalent short circuit current; V_{te}, transepithelial voltage; $I_{sc\text{-}Amil}$, amiloride-sensitive short circuit current; $I_{sc\text{-}Secretion} = I_{sc} - I_{sc\text{-}Amil}$, secretory short circuit current.

Ca^{2+}-activated Cl^- channel, which compensates for the CFTR Cl^- channel defect in CFTR (−/−) knockout mice [21]. Since stimulation with IBMX and forskolin also increases intracellular Ca^{2+} in this tissue and activates Ca^{2+}-dependent Cl^- secretion, gene complementation and successful expression of wtCFTR is difficult to prove [20]. However, functional analysis of ion transport in the present study identified CFTR and $Na^+2Cl^-K^+$ dependent secretion and therefore suggests some use of this tissue, even in gene complementation studies and CF gene therapy trials [16, 36].

In a previous patch-clamp study, maxi K^+ channels have been identified, colocalized with CFTR Cl^- channels in the apical membrane of exocrine gland acini isolated from frog skin [40]. Similar K^+ channels have been identified on the luminal side of the surface epithelium of the rat colon and rabbit collecting duct [7, 23]. A luminal cAMP-activated K^+ conductance may also exist in the human colon [35]. A modulating effect of luminal K^+ channels on Cl^- secretion has been suggested in previous studies [10, 40]. The large luminal Cl^- conductance is typically due to the activity of CFTR Cl^- channels and is activated by cAMP-dependent stimulation of secretory epithelia. Accordingly, luminal Cl^- exit is not rate limiting for Cl^- secretion, but is largely dependent on the K^+ conductance, supplying the electrical driving force for luminal Cl^- exit. It has been demonstrated that locating up to 20% of the total cellular K^+ conductance in the luminal membrane increases Cl^- secretion, by enhancing the driving force for transcellular Cl^- secretion [10]. A previous study has demonstrated activation of K^+ secretion across cultured airway epithelial cells by stimulation with the purinergic agonist ATP [9]. However, little is known about the role of luminal K^+ channels for Cl^- secretion in the airway epithelium. We therefore examined expression of K^+ channels in mouse trachea and tried to identify a luminal K^+ conductance. The results show a lack of K^+ channels in the luminal membrane of mouse trachea and supply evidence that Cl^- secretion completely relies on the driving force generated by the basolateral K^+ conductance.

Materials and Methods

Cell Isolation and RT-PCR Analysis

Six- to 8-week old Quackenbush mice were fed on normal diet and were sacrificed by cervical dislocation. For isolating airway epithelial cells, tracheas were incubated for 15 min at 37°C in a Ca^{2+}-free solution containing (in mmol/l): 127 NaCl, 5 KCl, 5 D-glucose, 1 $MgCl_2$, 5 pyruvate, 10 HEPES, 5 EDTA, pH 7.4. The epithelial layer was then removed under a dissection microscope [18]. Total RNA was isolated from tracheal epithelial cells and from the airway epithelial cell lines Calu-3 and 16HBE using NucleoSpin RNAII columns (Macherey and Nagel, Australia). Calu-3 and 16HBE cells were cultured as described previously [26, 41]. The total RNA was reverse-transcribed at 37°C for 1 hr using random primers and reverse transcriptase (Superscript II; Life Technologies). cDNA encoding the K^+ channels ROMK [4], KIR6.1, KIR6.2 [1] and KCNMA1 (Slo1) [38] as well as the sulfonylurea receptor (SUR) [1] were amplified by PCR (94°C for 2 min; 35 cycles: 94°C for 30 sec, 56°C for 30 sec and 72°C for 60 sec; 72°C for 10 min) using the following sense (s) and antisense (as) primers: i) mouse ROMK: (s) 5′-ATGTTCAAACATCTTCGAAGATG-3′ (as1; 177 bp), 5′-CCTCCATTTCAGGTCAAGTAC-3′ (as2; 407 bp), 5′-GTGGCACACTGTTCTGTCAC-3′. ii) mouse SUR (498 bp) (s), 5′-ATGAGCCTTTCTTTTTGTGGG-3′ (as) 5′-GCG CAGGTCTGACACTCC-3′. iii) Human SUR (494 bp) (s) 5′-GAGCCTTTCATTTTGTGGTAAC-3′ (as) 5′-GCAGGTTTGA-TATGTCCAAGC-3′. iv) Mouse KIR6.1 (546 bp) (s) 5′-CTGG AGTCCGCTGTCTGTG-3′ (s) 5′-GAGAATCACTATGACCT CCAG-3′. v) Human KIR6.1 (548 bp) (s) 5′-GTTTGGAGTC-CACTGTGTGTG-3′ (as) 5′-CAGAATAACTATGACCTCCA AG-3′. vi) Mouse KIR6.2 (521 bp) (s) 5′-CCGGAGAGGG CACCAATG-3′ (as) 5′-GCAGGTCACTAGGAGCCAG-3′. vii) Human KIR6.2 (520 bp) (s) 5′-CACTGCTGAGCCCTGTGTC-3′ (as) 5′-GTGGTGGTGCAGGTCGCTG-3′. viii) Mouse and human KCNMA1 (505 bp) (s) 5′-GGTCCACGAGCCCAAGATG-3′ (as) 5′-GTAGAGGAGGAAGAACACGTTG-3′. All PCR products were sequenced using a Big Dye Terminator Cycle Sequencing Kit (Perkin-Elmer Applied Biosystem, Boston, MA).

Ussing-Chamber Experiments

Tracheas were taken from sacrificed animals and surrounding connective tissue was removed under a dissection microscope. Tracheas were opened along the anterior side and mounted under a dissection microscope into a micro Ussing chamber with an exposed surface area of 0.79 mm^2 and the cartilage-free mucosa facing the opening of the Ussing-chamber insert. Both sides of the epithelium were continuously perfused (5 ml/min) with buffer solution at 37°C and were allowed initially to equilibrate for 30 min. Transepithelial resistances (R_{te}) were calculated from the voltage deflection (ΔV_{te}) due to pulsed current injection (1 sec) of 0.5 μA and subtraction of the empty chamber resistance. Equivalent short-circuit currents (I_{sc}) were determined from V_{te} and R_{te} according to Ohm's law. The polarity of I_{sc} and V_{te} was referred to the luminal side of the epithelium [29].

Compounds and Data Analysis

Amiloride, glibenclamide, acetazolamide, DIDS and IBMX were all from Sigma (Australia). 293B, forskolin and azosemide were gifts from Dr. M. Bleich (Aventis Pharma, Frankfurt, Germany). Cromakalim was from Tocris (Australia). All chemicals used were of highest grade of purity available. Data are shown as individual recordings or as mean ± SEM (n = number of tissue samples). Statistical analysis was performed using paired Student's t-test. P values < 0.05 were accepted to indicate statistical significance.

Results

Luminal Cl^- and Basolateral K^+ Conductances

Mouse tracheal epithelium is dominated by amiloride-sensitive Na^+ absorption, independent of stimulation by the secretagogues forskolin (2 μmol/l) and

IBMX (100 μmol/l). Unlike in human respiratory epithelium [32], amiloride-sensitive transport was not reduced after cAMP-dependent stimulation (Fig. 1*A*,*B*). However, Cl^- secretion is activated upon stimulation with forskolin and IBMX, thereby further increasing lumen-negative V_{te}. Activation of Cl^- secretion ($I_{sc\text{-}Secretion}$; lower trace Fig. 1*B*) becomes apparent after subtracting amiloride-sensitive Na^+ transport ($I_{sc\text{-}Amil}$) from the total short circuit current I_{sc} (Fig. 1*B*). Cl^- secretion was also induced and an I_{sc} of 183 ± 28 μA/cm^2 ($n = 5$) was activated when luminal $P2Y_2$ receptors were stimulated by 100 μmol/l ATP. This Ca^{2+}-dependent Cl^- conductance was reduced to 74 ± 9.3 μA/cm^2 ($n = 4$) in the presence of the inhibitor of Ca^{2+}-activated Cl^- channels, 4,4′-diisothiocyano-2,2′-stilbene disulfonate (DIDS; 200 μmol/l), similar to previously published results [28]. Basolateral application of 293B (10 μmol/l), which blocks KCNQ1/KCNE3 K^+ channels, was inhibitory on both $I_{sc\text{-}Amil}$ as well as $I_{sc\text{-}Secretion}$, suggesting that KCNQ1/KCNE3 K^+ channels maintain both Na^+ absorption as well as Cl^- secretion. Similar to basolateral 293B, also luminal application of glibenclamide (10–100 μmol/l) inhibited Cl^- secretion, which is probably due to inhibition of cystic fibrosis transmembrane conductance (CFTR) Cl^- channels (Fig. 1*C*). In most experiments, luminal glibenclamide caused a brief initial increase of I_{sc}, which was followed by a second stable inhibition of I_{sc}. Simultaneous application of both glibenclamide and 293B almost completely abolished IBMX/forskolin-induced Cl^- secretion. Somewhat unexpectedly, glibenclamide also inhibited amiloride-sensitive Na^+ absorption. Since glibenclamide is known to bind to sulfonylurea receptors (SUR) [1], RT-PCR analysis of microdissected mouse tracheal epithelial cells was performed. However, expression of SUR could neither be detected in isolated native tracheal epithelial cells from mouse trachea nor in cultured airway epithelial cells (Fig. 2*A*). An RT-PCR signal for SUR was obtained when total trachea was used as a positive control (Fig. 2*B*). However, this signal is not derived from epithelial cells, but from tissue surrounding the trachea. In contrast, transcripts for the ATP-sensitive K^+ channel Kir6.2 were clearly identified in mouse tracheal epithelial cells as well as in the human airway epithelial cell lines Calu-3 and 16HBE (Fig. 2*A*,*C*).

An inhibitory effect of glibenclamide on $I_{sc\text{-}Amil}$ was also detected in tracheas of cftr$^{(G551/G551D)}$ mice (Fig. 3*A*). However, an inhibitory effect on Cl^- secretion was not observed, since these mice lack expression of functional CFTR Cl^- channels [13]. Since a high concentration of glibenclamide (100 μmol/l) was used to inhibit CFTR [39], we suggest a rather nonspecific inhibitory effect on Na^+ absorption. Lower concentrations of glibenclamide (1–10 μmol/l) were without any clear effects on ion transport (*data not shown*). We never observed an activation of Na^+ transport by glibenclamide, as suggested recently [8]. In addition, cromakalim (100 μmol/l), an activator of K_{ATP} channels, had no effect on $I_{sc\text{-}Amil}$ but also inhibited Cl^- secretion (Fig. 3*B*). Both glibenclamide and cromakalim did not show any clear effect on V_{te} when applied to the basolateral side of the epithelium. We are therefore unable to assess a functional role of Kir6.2 in mouse trachea from the present experiments.

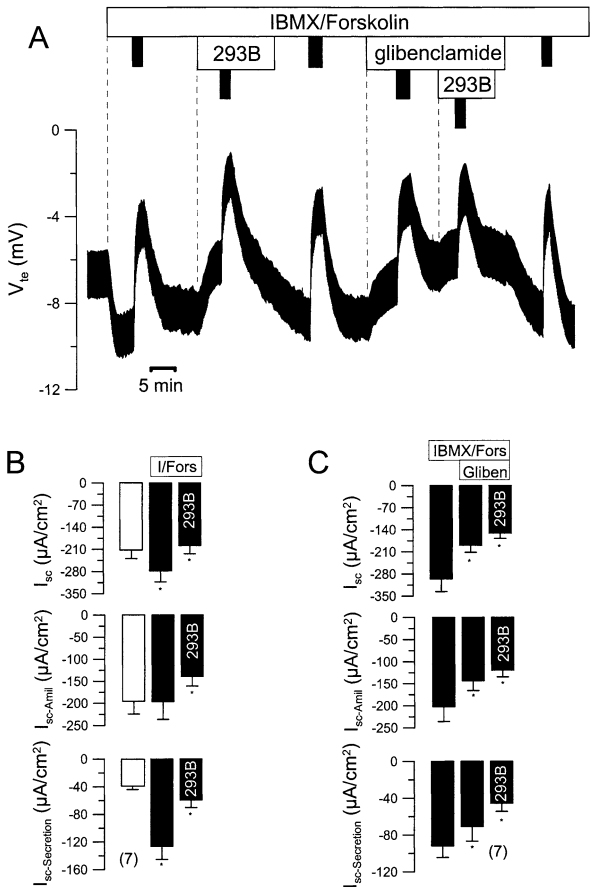

Fig. 1. Ussing-chamber recordings of the transepithelial voltage (V_{te}) in mouse trachea. (*A*). Original continuous recording of V_{te} obtained in a perfused micro Ussing chamber. Effects of amiloride (10 μmol/l; black bars), the K^+ channel blocker 293B (10 μmol/l) and the CFTR blocker glibenclamide (100 μmol/l) on V_{te}. Amiloride leads to inhibition of the lumen-negative V_{te}, even after stimulation of the tissue with IBMX (100 μmol/l) and forskolin (2 μmol/l). The IBMX/forskolin-induced lumen-negative potential is inhibited by either 293B or glibenclamide, with an additive effect of both blockers. (*B*,*C*) Summary of the calculated equivalent short circuit currents (I_{sc}), the amiloride-sensitive I_{sc} ($I_{sc\text{-}Amil}$) and the I_{sc} that is left after subtraction of $I_{sc\text{-}Amil}$ ($I_{sc\text{-}Secretion}$). Black columns indicate stimulation with IBMX/forskolin. Both $I_{sc\text{-}Amil}$ and $I_{sc\text{-}secretion}$ are inhibited by 293B and the inhibitory effects of both blockers are additive. Asterisk indicates significant difference when compared to control (paired *t*-test). Number of experiments in parentheses.

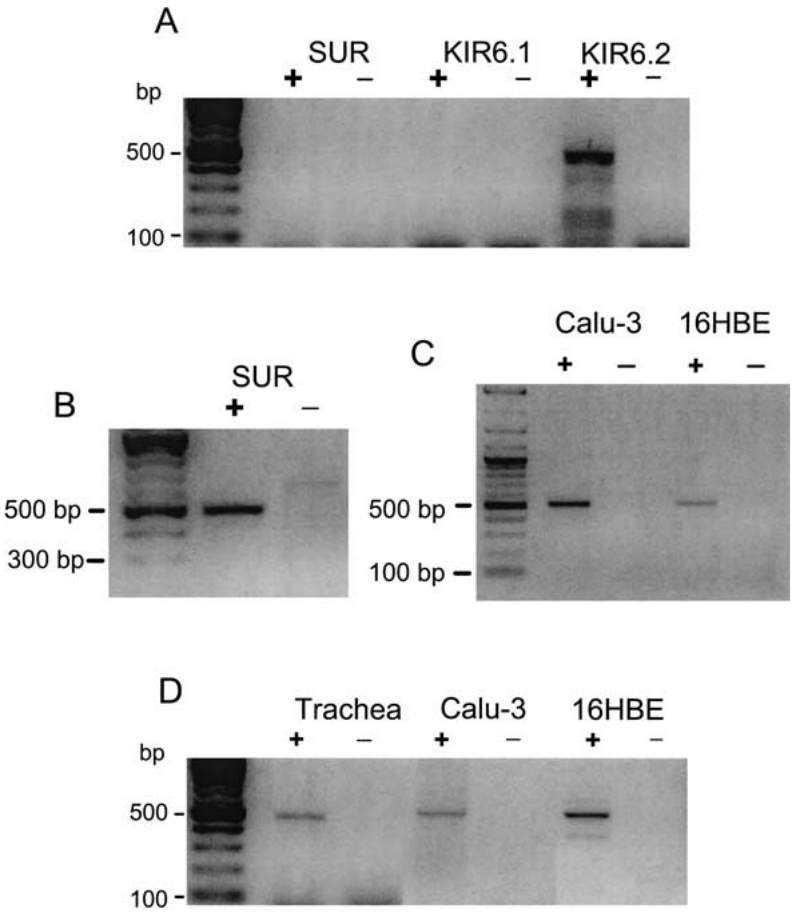

Fig. 2. RT-PCR analysis of RNA extracted from microdissected tracheal epithelial cells as well as two airway epithelial cell lines (Calu-3 and 16HBE). (*A*) Expression of the K_{ATP} channel Kir6.2 (520 bp) but not Kir6.1 (546 bp) or the sulfonylurea receptor (SUR; 498 bp) in isolated tracheal epithelial cells. (*B*) Expression of the sulfonylurea receptor (SUR; 498 bp) in total trachea. (*C*) Expression of Kir6.2 in mouse tracheal epithelial cells was confirmed for both airway epithelial cell lines Calu-3 and 16HBE. (*D*) Transcripts for the BK K^+ channel KCNMA1 (Slo1; 505 bp) were detected in tracheal epithelial cells as well as Calu-3 and 16HBE cells. +/– indicates the presence or absence of reverse transcriptase.

No Clear Evidence for Luminal K^+ Channels in Mouse Trachea

We also checked for expression of other K^+ channels in mouse trachea. As demonstrated recently, cAMP-activated KCNQ1/KCNE3 and Ca^{2+}-activated SK4 K^+ channels are expressed in mouse and human airway epithelial cells [17, 18, 34]. KCNQ1/KCNE3 K^+ channels are blocked by the chromanol 293B and are presumably located only in the basolateral membrane of airway epithelial cells. When applied to the luminal side of mouse trachea, 293B reduced the lumen-negative transepithelial voltage and inhibited short-circuit currents under baseline conditions and after stimulation with IBMX/forskolin (Fig. 4). 293B is probably entering the cells and is acting on basolateral KCNQ1 K^+ channels rather than luminal KCNQ1 channels, which should have otherwise led to an increase in lumen-negative V_{te} [18]. We also tested for the presence of luminal intermediate-conductance K^+ channels (KCNN4, SK4, IK1, hKCa4) by using the potent inhibitor clotrimazole (10 μM) [14]. However, results similar to those for the KCNQ1 blocker 293B were obtained, and thus no clear functional evidence was found for expression of KCNN4 in the luminal membrane of mouse tracheal epithelial cells (Fig. 4*C*). A similar conclusion can be reached from experiments with Ba^{2+} (5 mmol/l). When Ba^{2+} was applied to the luminal side, the lumen-negative V_{te} was inhibited and I_{sc} was reduced from -114.97 ± 18.42 μA/cm^2 to -87.46 ± 20.5 μA/cm^2 (control) and from -171.74 ± 30.9 μA/cm^2 to -138.79 ± 28.21 μA/cm^2 ($n = 7$) (IBMX/forskolin), respectively. This suggests inhibition of basolateral rather than luminal K^+ channels.

Large-conductance K^+ channels have been reported to be coexpressed together with CFTR in the luminal membrane of exocrine gland acini from frog skin and rat distal colon [7, 40]. In the present study we found transcripts for the BK channel Slo1 (KCNMA1) in isolated mouse tracheal epithelial cells as well as Calu-3 and 16HBE cell lines (Fig. 2*D*). In contrast, transcripts for ROMK K^+ channels could not be identified (*data not shown*). Because RT-PCR analysis showed evidence for Slo1 expression, we examined luminal effects of charybdotoxin and other toxins known to block K^+ channels (100 nmol/l). However, all toxins including charybdotoxin, apamin and α-dendrotoxin were without any effect on electrolyte transport in mouse trachea (Fig. 5). In additional experiments, none of the toxins exerted any effect on ion transport when applied to the basolateral side of the epithelium, independent of stimulation with IBMX and forskolin (*data not shown*).

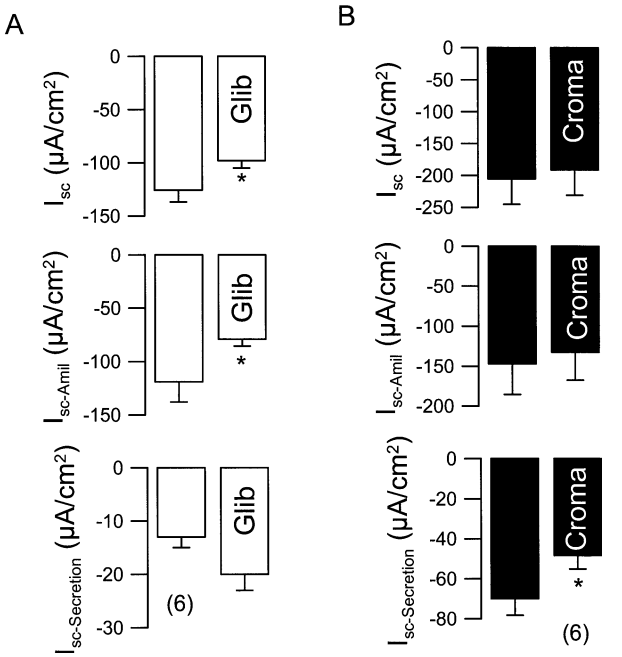

Fig. 3. (*A*). Summary of the effects of glibenclamide (Glib) on short-circuit currents measured in tracheas of G551D (−/−) mice. (*B*) Summary of the effects of the K_{ATP}-channel opener cromakalim (100 μmol/l) after stimulation of the tissues with IBMX/forskolin. * indicates significant difference when compared to control (paired *t*-test). Number of experiments in parentheses.

Taken together, the current experiments do not deliver any conclusive evidence for expression of K^+ channels in the luminal membrane of mouse tracheal epithelium.

Ion Secretion Is Blocked by Inhibition of CFTR, NKCC1 and Basolateral K^+ Channels

As mentioned above, ion transport in mouse trachea is dominated by amiloride-sensitive Na^+ absorption. However, after stimulation with IBMX and forskolin, Cl^- secretion is induced, as indicated by an increase in the lumen-negative transepithelial voltage and a decrease in the transepithelial resistance (Fig. 6). Blockage of the basolateral $Na^+2Cl^-K^+$ cotransporter by azosemide (100 μmol/l) did not change basal I_{sc} but decreased Cl^- secretion significantly (Fig. 6*B*). Additional inhibition of luminal CFTR Cl^- channels by glibenclamide (100 μmol/l) and basolateral KCNQ1 K^+ channels by 293B (10 μmol/l) completely inhibited forskolin-induced ion transport. Luminal application of 293B had no further impact on ion secretion. Any residual current could be due to secretion of HCO_3^-. However, it is unlikely that HCO_3^- secretion participates in the ion transport observed under the present HCO_3^--free conditions. This is further confirmed by additional experiments, in which we applied the carbonic anhydrase inhibitor acetazolamide (100 μmol/l), in order to block endogenous production of bicarbonate. In 4 experiments we did not observe any inhibitory effects of acetazolamide on short-circuit currents when applied in either absence or presence of stimulation with IBMX/forskolin.

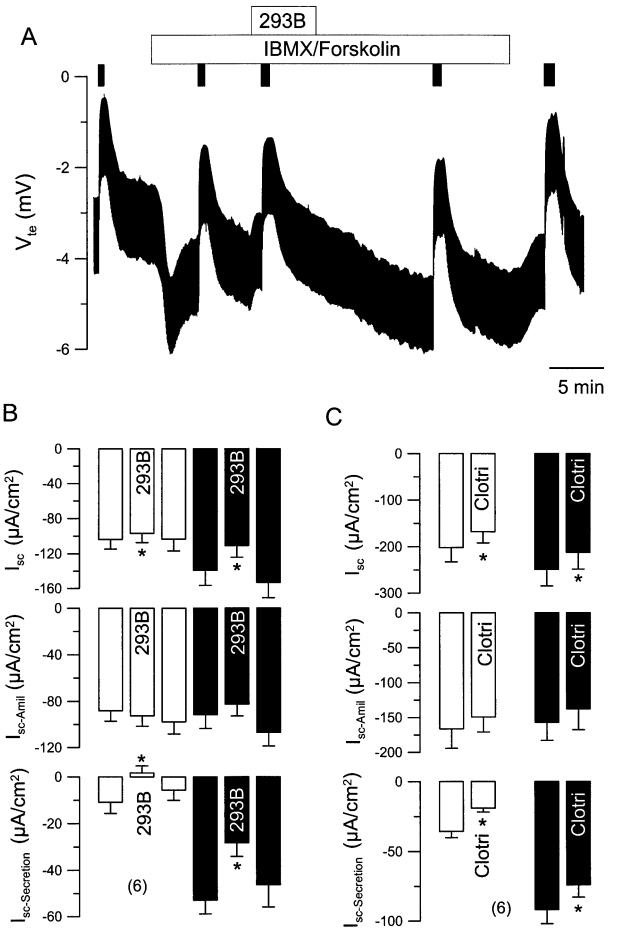

Fig. 4. Original continuous recording of V_{te} obtained in a perfused micro Ussing-chamber. Effects of amiloride (10 μmol/l; black bars) in absence or luminal presence of the K^+-channel blocker 293B (10 μmol/l) and stimulation by IBMX (100 μmol/l) and forskolin (2 μmol/l). Luminal 293B partially inhibits the lumen-negative V_{te} and reduces the effect of amiloride. (*B*) Summary of the calculated short-circuit currents (I_{sc}). Luminal 293B inhibits $I_{sc\text{-}Secretion}$. In both absence (white bars) or presence (black bars) of IBMX/forskolin. (*C*) Summary of the effects of luminal clotrimazole on calculated short-circuit currents (I_{sc}). Clotrimazole inhibits $I_{sc\text{-}Secretion}$, In both absence (white bars) or presence (black bars) of IBMX/forskolin. * indicates significant difference when compared to control (paired *t*-test). In parentheses, number of experiments.

Taken together, the present experiments show clear activation of ion secretion in mouse trachea, which is blocked by inhibitors of basolateral $Na^+2Cl^-K^+$ cotransport, basolateral K^+ channels and luminal CFTR Cl^- channels. No clear evidence was found for luminal K^+ secretion in mouse trachea.

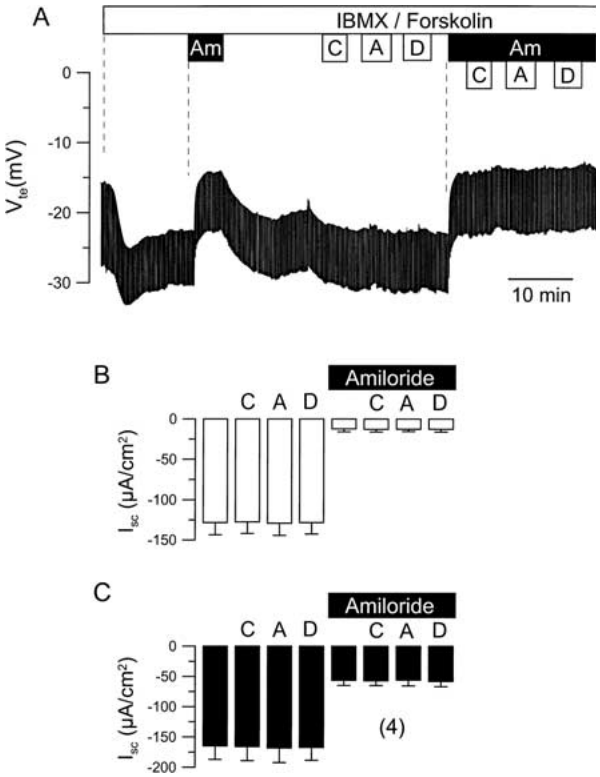

Fig. 5. (*A*) Original continuous recording of V_{te} obtained in a perfused micro Ussing-chamber. Effects of amiloride (10 μmol/l; *Am*) and the K^+ channel-inhibiting toxins (all 100 nmol/1) charybdotoxin (*C*), apamin (*A*) and α-dendrotoxin (*D*), when applied to the luminal side of the epithelium, in the presence of IBMX/forskolin. (*B*, *C*) Summaries of the calculated short circuit currents (I_{sc}) and the effects of luminal charybdotoxin, apamin and α-dendrotoxin in the absence (white bars) or presence (black bars) of IBMX/forskolin. In parentheses, number of experiments.

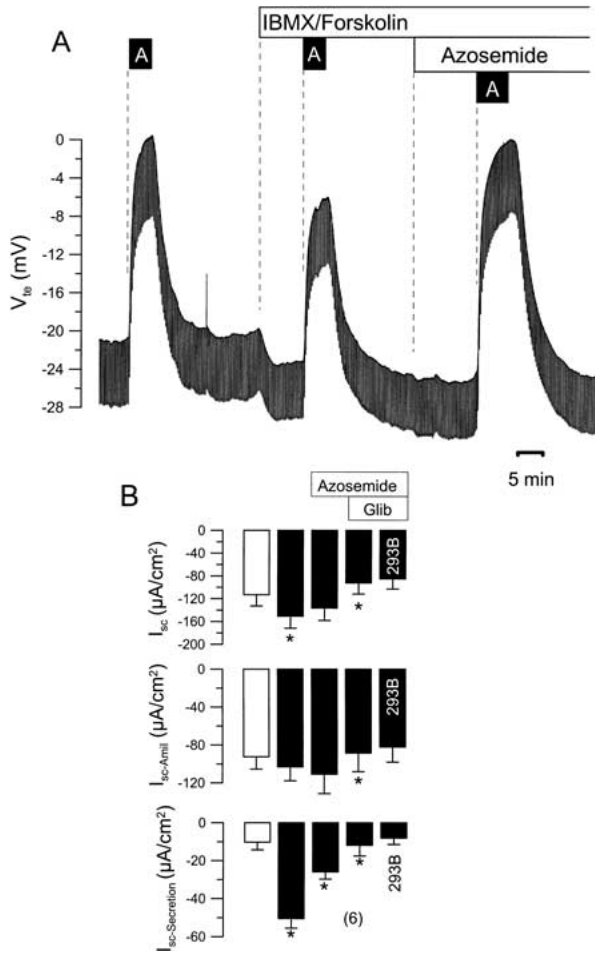

Fig. 6. (*A*). Original continuous recording of V_{te} obtained in a perfused micro Ussing-chamber. Effects of amiloride (10 μmol/l; black bars) in absence or presence of IBMX/forskolin and azosemide (100 μmol/l). The inhibitory effect of azosemide on the lumen-negative V_{te} is masked by the activatory effects on $I_{sc\text{-}Amil}$. (*B*) Summary of the calculated short-circuit currents. Azosemide and glibenclamide (Glib) inhibit the short-circuit current that is activated by IBMX/forskolin. * indicates significant difference when compared to control (paired *t*-test). In parentheses, number of experiments.

Discussion

Evidence For Functional Expression of CFTR and NKCC1 in Mouse Trachea

The present paper demonstrates activation of electrolyte secretion in mouse trachea by stimulation with IBMX and forskolin. It has been demonstrated in a previous study that stimulation of mouse trachea leads to a transient increase in intracellular Ca^{2+}. It was concluded that this Ca^{2+} increase is the cause for forskolin-induced Cl^- secretion in mouse trachea [20]. In the same paper, a relatively small contribution of amiloride-sensitive Na^+ transport has been detected, which is in contrast to the results of the present study. Here, a large and dominating amiloride-sensitive Na^+ conductance of about 200 μA/cm² was detected under control conditions. Stimulation of the tracheas with IBMX and forskolin induced a pronounced secretory response of approximately 120 μA/cm². As in the previous report [20], stimulation of ion secretion by IBMX and forskolin was stable and not transient. This argues somewhat against Ca^{2+} as the main mediator of Cl^- secretion in mouse trachea, since forskolin-induced Ca^{2+} increase was transient and not stable [20]. In the present study, IBMX/forskolin-induced ion secretion was almost completely blocked by basolateral 293B, an inhibitor of cAMP-activated K^+ channels. It is known from studies on native colonic and airway epithelia that intermediate-conductance SK4-type K^+ channels are active under baseline conditions and after Ca^{2+}-dependent stimulation [18, 34, 43, 44]. Upon stimulation with IBMX and forskolin and increase in intracellular cAMP, basolateral KCNQ1/KCNE3 K^+ channels are activated, which maintain the electrical driving force for luminal Cl^- exit [18, 34]. In addition to cAMP, these channels are also activated

by increase in intracellular Ca^{2+} [6, 18]. We therefore conclude that the sustained Cl^- secretion observed in mouse trachea upon IBMX/forskolin-dependent stimulation, is predominantly due to activation of basolateral cAMP-dependent K^+ channels.

As shown here and also in a previous study, basolateral $Na^+2Cl^-K^+$ cotransporters are expressed in the basolateral membrane of mouse trachea [18]. This is clearly shown by the action of azosemide [22], which reversibly inhibits IBMX/forskolin-activated Cl^- secretion (Fig. 6). The present results also suggest expression of CFTR Cl^- channels in mouse trachea. Evidence is derived through the fact that IBMX/forskolin induced stable secretion, which should not be expected if all Cl^- transport would rely only on luminal Ca^{2+}-activated Cl^- channels [20, 25, 33]. In some tissues, we observed a transient component in the IBMX/forskolin-induced Cl^- secretion (*data not shown*). The main part of secretion, however, was always non-transient and was inhibited reversibly by the CFTR Cl^- channel blocker glibenclamide (Fig. 6). In contrast, IBMX/forskolin-induced Cl^- secretion was only slightly inhibited by 100 μmol/l DIDS, a blocker of Ca^{2+}-activated Cl^- channels (*data not shown*). Glibenclamide had also a small inhibitory effect on Cl^- secretion under baseline conditions, an effect that was not observed in CF tracheas (Fig. 3). Even after stimulation with IBMX/forskolin and activation of a Cl^- secretion, glibenclamide was without effect in CF tracheas (*data not shown*).

Taken together, the present results supply reasonable evidence for functional expression of CFTR Cl^- channels in mouse trachea. Along with alternative non-CFTR Cl^- channels, they form the luminal exit pathway for Cl^- in this epithelium. It has been shown in previous reports that very low copy numbers of CFTR are expressed in airway epithelial cells and that only low copy numbers are required for expression of a CFTR Cl^- conductance [3, 15, 42]. Difficulties in demonstrating CFTR expression in this tissue by immunocytochemistry are therefore not surprising, and negative results from in situ hybridization experiments are almost expected [37]. However, in contrast to human airways, where activation of CFTR inhibits Na^+ absorption [31], this is not observed in mouse trachea. This may point to an either limited role of CFTR in this tissue or it indicates solely expression of CFTR in the rare submucosal glands, which don't express epithelial Na^+ channels. The lack of inhibition of ENaC by CFTR may also be caused by a missing signaling intermediate that is not present in mouse trachea. In this regard, studies on tracheas of *cftr*$^{(-/-)}$ mice have failed to demonstrate increase of ENaC-dependent Na^+ absorption [2, 19]. Despite a clearly detectable Cl^- secretion, mouse trachea is primarily a Na^+ absorbing epithelium. This is clearly indicated by the fact that the largest part of the transport under resting conditions is due to amiloride-sensitive Na^+ absorption. This is in good agreement with the lack of expression of luminal K^+ channels in mouse trachea, as shown in this paper. One may speculate that a luminal K^+ conductance would only be required in case of a substantial Cl^- secretion.

No Evidence for Luminal K^+ Channels in Mouse Trachea

Coexpression of luminal K^+ channels together with CFTR Cl^- channels has been nicely demonstrated in a detailed study on frog skin gland acini [40]. Using various blockers we were unable to detect a luminal K^+ conductance in the present Ussing-chamber study. A luminal K^+ conductance is likely to support epithelial Cl^- secretion and eventually Na^+ absorption, at least in some epithelia [10]. The K^+ concentration in the luminal airway surface liquid (ASL) of human and canine trachea is around 25–30 mmol/l, which suggests luminal K^+ secretion [5, 24]. This is in marked contrast to rodent trachea, which is covered by an ASL containing plasma isotonic K^+ concentrations of around 4.5 mM [11, 12]. These measurements in rat and mouse trachea correspond well to our results, indicating a lack of luminal secretory K^+ channels in mouse trachea. A problem of the present experiments is caused by diffusion of luminally applied K^+ channel blockers such as Ba^{2+}, 293B or clotrimazole to the basolateral side and inhibition of basolateral K^+ channels. Inhibition of basolateral K^+ channels could mask a potential inhibition of luminal K^+ channels [18]. Therefore, Ussing-chamber experiments may not be the best tool to study luminal K^+ conductance in mouse trachea. Since patch-clamp studies on native tracheal epithelial cells are notoriously difficult, additional microimpalement studies in parallel with transepithelial voltage measurements may be applied to further examine a putative contribution of luminal K^+ channels to Cl^- secretion and Na^+ absorption in mouse trachea.

Taken together, the present study does not supply any evidence for expression of luminal K^+ channels in mouse trachea, which explains why the K^+ concentration in mouse ASL is isotonic to the plasma K^+ concentration.

We thank Dr. M. Bleich (Aventis Pharma, Frankfurt, Germany), Prof. Dr. D. Gruenert (Colchester Research Facility, University of Vermont, USA) and Dr. M. Hug (Department of Physiology, University of Münster, Germany) for supplying the compounds 293B and azosemide, forskolin and the cell lines 16HBE and Calu-3, respectively. We thank Prof. Dr. B. Wainwright for supplying the cftr$^{(G551D/G551D)}$ mice. The study was supported by the Deutsche Forschungsgemeinschaft grant DFG Ku756/4-1, German Mukoviszidose e.V., Cystic Fibrosis Australia and ARC Grants 00/ARCS243 and ARC A00104609.

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