Paradoxical Effects of Hydrogen Peroxide on Human Airway Anion Secretion

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ABSTRACT

The present study concerns intriguing effects of hydrogen peroxide (H$_2$O$_2$) on cAMP-mediated anion secretion in polarized human airway epithelia. Although H$_2$O$_2$, applied to the apical and basolateral membrane increases short-circuit currents (I$_{sc}$) with analogous properties, it has opposite effects on subsequent cAMP-activated I$_{sc}$ responses. Namely, forskolin (FK)-induced I$_{sc}$ responses were down-regulated by the apical presence of H$_2$O$_2$, whereas they were up-regulated by its basolateral presence. Despite this contrasting effect, oxidative stimuli from either aspect of the monolayer hindered FK-induced increments in cytosolic cAMP levels and apical membrane Cl$^-$ conductance. The site-dependent effects of H$_2$O$_2$ were reproduced in the responses to 8-bromo-cAMP. Estimation of the anionic composition of the I$_{sc}$ revealed that the FK up-regulated both bumetanide [an Na$^+-$K$^+-2Cl$^-$ cotransporter (NKCC1)] inhibitor]-sensitive and 4,4'-dinitrostilbene-2,2'-disulfonic acid [an HCO$_3^-$-dependent anion transporter (NBC1/AE2)]-sensitive I$_{sc}$ in the control, whereas the up-regulation evidently favored bumetanide-sensitive I$_{sc}$ in the basolateral presence of H$_2$O$_2$. The FK-induced NKCC1 augmentation after exposure to basolateral H$_2$O$_2$ was counteracted by cytochalasin D, an inhibitor of microfilament function, but not by charbdotoxin, a blocker of the intermediate conductance Ca$^{2+}$-activated K$^+$ channel, whose activation could be related to NKCC1-mediated Cl$^-$ secretion. These observations suggest that basolaterally but not apically applied H$_2$O$_2$ potentiates subsequent cAMP-mediated Cl$^-$ secretion by an increase in Cl$^-$ uptake via basolateral NKCC1, whose sensitivities to cAMP/protein kinase A are up-regulated, overcoming the H$_2$O$_2$-induced inhibition of cAMP-mediated anion conductance. The basolateral membrane-specific effects of H$_2$O$_2$ may be relevant to the basolateral cytoskeleton, which is believed to interact with NKCC1.

Morphologic and physiologic alterations in the human organism are often associated with increases in production of reactive oxygen species (ROS), such as peroxynitrite (ONOO$^-$) and hydrogen peroxide (H$_2$O$_2$) (Bebok et al., 2002). It is well known that ROS damage tissue via direct oxidation of protein, DNA, or lipids (Okayama, 2005). Because of the toxicological effects of ROS, their production by inflammatory cells during episodes of infection and inflammation is responsible for the pathogenesis of a number of respiratory diseases, including bronchial asthma, cystic fibrosis, and chronic obstructive pulmonary disease (Ricciardolo et al., 2006). These ROS-related airway diseases share aspects of mucous congestive diseases (Kellerman, 2002), in which excessive and tenacious mucus secretion causes airway obstruction, and the resultant dysfunction of mucociliary clearance is involved in the morbidity and mortality of these diseases (Rogers, 2005). In vivo, bronchial gland cells contribute to maintenance of effective mucociliary clearance by regulating salt and water secretion via the vectorial ion transport system, thereby forming low-viscosity mucus (Shimura, 2000). Thus, the relationship between ROS and airway ion transport is of considerable interest. The purpose of this work is to elucidate this point, using polarized Calu-3 cells, which may be a model of human airway submucosal gland serous cells (Shen et al., 1994). This cell line expresses high levels of the cystic fibrosis transmembrane conductance regulator (CFTR), a representative anion exit pathway, on
the apical membrane (Haws et al., 1994) and several anion uptake transporters on the basolateral membrane (Loffing et al., 2000). In the present study, we examined the effects of oxidant stress caused by H$_2$O$_2$ on cAMP-dependent anion secretion in polarized human airway serous cell epithelia and found the paradoxical phenomena that the oxidative stimuli applied from the apical or basolateral membrane had opposite effects on cAMP-activated anion secretion.

Materials and Methods

Cell Culture. Calu-3 human airway cells (American Type Culture Collection, Manassas, VA) at passages 29 through 35 were grown confluent were rinsed with physiological saline solution (PSS) with clearly defined nadirs immediately after exposure to H$_2$O$_2$. The PSS was composed of 115 mM NaCl, 5 mM KCl, 1 mM CaCl$_2$, 2 mM MgCl$_2$, 10 mM glucose, 10 mM Hepes, and 25 mM NaHCO$_3$ at 37°C. The PSS was made and sonicated for 30 s just before use.

Bioelectric Responses to Apically and Basolaterally Applied H$_2$O$_2$. The basal $I_{SC}$ and $R_t$ in our experiments using Calu-3 cells were 12.6 ± 0.4 μA/cm$^2$ and 474.4 ± 16.7 Ωcm$^2$, respectively (n = 181). Previous studies have reported that relatively high concentrations of H$_2$O$_2$, ranging from 0.1 to 5 mM, were required to induce barrier dysfunction and anion secretion because airway epithelial cells, including Calu-3, have a strong antioxidant defense capacity (Waters et al., 1997; Zhao and Davis, 1998; Cowley and Linsell, 2002). As shown in Fig. 1, A and B, the $R_t$ of cells decreased with clearly defined nadirs immediately after exposure to oxidative stimulation of H$_2$O$_2$ (5 mM) from either the apical (A) or basolateral (B) aspect of the membrane, and the parameter gradually returned to its basal level. In a polarized epithelial monolayer, an irreversible increase in monolayer conductance is suggestive of cell damage and loss of viability (Alvarez et al., 1998; Ito et al., 2001). The reversible changes in monolayer $R_t$ observed in the present study indicate that the cells are tolerant of H$_2$O$_2$ at this concentration. Concomitant with the $R_t$ changes, the corresponding peak values in $I_{SC}$ were 73.0 ± 3.6 (n = 5), to apical H$_2$O$_2$, and 49.0 ± 3.3 μA/cm$^2$ (n = 9), to basolateral H$_2$O$_2$, respectively (p < 0.01) (Fig. 1, C and D). These bioelectric changes seem likely to include predominantly anion transport because both of them were markedly reduced to 24.0 ± 1.3 (n = 4, p < 0.01), and 22.8 ± 2.3 μA/cm$^2$ (n = 4, p < 0.01) by the presence of NPPB (100 μM), a Cl$^-$ channel blocker. Previous investigations have shown that airway epithelial cells release cyclooxygenase (COX) products in response to ROS-related stimuli (Matyas et al., 2002). Indomethacin, a COX inhibitor, is generally believed to suppress endogenous production of prostaglandins and thus intracellular cAMP (Mall et al., 1998). As shown in Fig. 1, E through G, the H$_2$O$_2$-induced effects were diminished to 27.2 ± 2.2 (n = 4, p < 0.01) and 21.7 ± 1.2 μA/cm$^2$ (n = 4, p < 0.01) by pretreatment with indomethacin (10 μM). Similar results were obtained when we used SC-560 (1 μM, a COX-1 inhibitor) and NS-398 (10 μM, a COX-2 inhibitor), resulting in a suppressed peak $I_{SC}$ in response to the imposed voltage pulses (Haws et al., 1994; Wine et al., 1994). In this basolateral solution, CaCl$_2$ was increased to 4 mM to compensate for the Ca$^{2+}$-chelating capacity of the gluconate (Devor et al., 1999).

CAMP Assay. Confluent Calu-3 cells on the permeable supports were exposed to forskolin (FK) (10 μM) for 15 min in the presence of H$_2$O$_2$ and its absence using a cAMP Biotrack enzyme immunoassay kit (Amersham, Arlington, IL). The concentrations of cAMP (IC$_{50}$) in the samples were determined, according to the manufacturer’s instructions. The cAMP levels were expressed as femtomole/well.

Chemicals. FK, 8-bromo-cAMP (8-Br-cAMP), DNDS, NPPB, bumetanide, indomethacin, nystatin, pyruvate, and cytochalasin D (Cyto-D) were obtained from Sigma-Aldrich Co. NS-398 and SC-560 were purchased from Cayman Chemicals (Ann Arbor, MI). H$_2$O$_2$ and charybdotoxin (ChTx) were products of Wako Chemical (Tokyo, Japan) and Peptide Institute Inc. (Osaka, Japan), respectively. Stock solutions of 8-Br-cAMP, DNDS, pyruvate, and ChTx were prepared by dissolving them in distilled water. All of the other drugs were dissolved in dimethyl sulfoxide. Nystatin stock solution (100 mM) was made and sonicated for 30 s just before use.

Analysis of Results. Numerical data are presented as mean ± S.E.M., where n refers to the number of experiments. Statistical differences were determined by Student’s t test. A value of p < 0.05 was considered statistically significant.
values of the control response to apical (Δ) and basolateral (†) H2O2, /H11569 agents, such as FK (10 μM), an adenylate cyclase activator), with anion secretion that reflects ISC changes (Fig. 2A) (Devor et al., 1999; Ito et al., 2004a). Next, we examined the effects of H2O2 on the subsequent ISC changes in response to FK. Surprisingly, despite the similarity of apical and basolateral H2O2-induced ISC, subsequent FK-elicited responses behaved differently, depending on to which side the oxidative stimuli were applied. Namely, FK-induced responses, which were composed of rapid and subsequent sustained components (Fig. 2A), were attenuated by the apical presence of H2O2 (5 mM, Fig. 2B), whereas they were contrastingly augmented by H2O2 applied from the basolateral side (Fig. 2C). Concretely, the peak values of the ISC caused by FK (71.3 ± 423.9 fmol/well, n = 7–10) were diminished to 41.2 ± 826.1 fmol/well (n = 5, p < 0.01) and potentiated to 101.7 ± 14,464.3 fmol/well (n = 9, p < 0.01) by apical and basolateral oxidant stimuli, respectively. As shown in Fig. 2D, the H2O2-induced down-regulation and up-regulation of the FK-induced responses occurred in a concentration-dependent fashion in opposite directions from each other.

Effects of H2O2 on Intracellular cAMP Production. Based on the data in Fig. 2, we naturally assumed that the ISC responses to FK would be correlated with the changes in [cAMP]. However, this was not so. As shown in Fig. 3, FK (10 μM)-induced [cAMP] elevation, which had increased from 423.9 ± 826.1 fmol/well (n = 8, p < 0.05) compared with the control) 15 min after its application, was inhibited by the oxidative stimuli from the basolateral side [3926.1 ± 582.1 fmol/well (n = 8, p < 0.05) compared with the FK group without H2O2]. However, we here observed the paradoxical situation that the FK-induced [cAMP] elevation was also hindered by the oxidative stimuli from the basolateral side [3926.1 ± 582.1 fmol/well (n = 8, p < 0.05) compared with the FK group without H2O2], inconsistent with the movement of ISC (see Fig. 2C). Neither inhibitory effect of H2O2 seems likely to be caused by leakage of oxidative agents from the basolateral side to the apical side.
enzymatic reaction with \( \text{H}_2\text{O}_2 \), which produces acetate, \( \text{CO}_2 \), and antioxidant effects of pyruvate are produced by a direct non-peroxide dismutase mimetics (Cuzzocrea et al., 2001). The oxidative stress include pyruvate and cell-permeable sulfonamides. Current pharmacological approaches to prevent the burden of oxidative stress are shown in Fig. 2C but consistent with the data in Fig. 3. Paraadoxically, however, the presence of \( \text{H}_2\text{O}_2 \), an antioxidant agent, the inhibitory effects of \( \text{H}_2\text{O}_2 \) on \( \text{ICl} \) were well prevented (D and E). Pyruvate was applied 20 min before commencing exposure to \( \text{H}_2\text{O}_2 \).

**Effects of \( \text{H}_2\text{O}_2 \) on \( \text{FK-induced Apical Cl\textsuperscript{−}} \) Conduction.** Figure 4 shows the apical membrane \( \text{Cl\textsuperscript{−}} \) conductance (\( G_{\text{Cl\textsuperscript{−}}} \)), which was estimated as apical membrane \( \text{Cl\textsuperscript{−}} \) current (\( I_{\text{Cl\textsuperscript{−}}} \)) after establishment of an apical-basolateral \( \text{Cl\textsuperscript{−}} \) gradient and permeabilization of the basolateral membrane with nystatin (100 \( \mu \text{M} \)). As shown in Fig. 4A, application of \( \text{FK} \) (10 \( \mu \text{M} \)) caused development of the inward \( I_{\text{Cl\textsuperscript{−}}} \) (\( \Delta I_{\text{Cl\textsuperscript{−}}} = 61.3 \pm 4.5 \mu \text{A/cm}^2, n = 4 \)). As correlated with the \( I_{\text{SC}} \) data (see Fig. 2), the addition of \( \text{H}_2\text{O}_2 \) from either side of the membrane caused inward \( I_{\text{Cl\textsuperscript{−}}} \) development and decay (Fig. 4, B and C). In the apical presence of \( \text{H}_2\text{O}_2 \), the \( \text{FK-induced I}_{\text{Cl\textsuperscript{−}}} \) changes were markedly suppressed (\( \Delta I_{\text{Cl\textsuperscript{−}}} = 14.0 \pm 3.5 \mu \text{A/cm}^2, n = 5, p < 0.01 \)), consistent with the data in Figs. 2B and 3. Paraadoxically, however, the basolateral presence of \( \text{H}_2\text{O}_2 \), as shown in Fig. 4C, inhibited the \( \text{FK-induced I}_{\text{Cl\textsuperscript{−}}} \) development (\( \Delta I_{\text{Cl\textsuperscript{−}}} = 24.1 \pm 4.4 \mu \text{A/cm}^2, n = 5, p < 0.01 \)), inconsistent with the data in Fig. 2C but consistent with the data in Fig. 3. Current pharmacological approaches to prevent the burden of oxidative stress include pyruvate and cell-permeable superoxide dismutase mimetics (Cuzzocrea et al., 2001). The antioxidant effects of pyruvate are produced by a direct non-enzymatic reaction with \( \text{H}_2\text{O}_2 \), which produces acetate, \( \text{CO}_2 \), and restoration of the balance between reduced and oxidized glutathione (Leon et al., 2004). Figure 4, D and E, shows that preincubation with pyruvate (5 mM) counteracted the inhibition of \( I_{\text{Cl\textsuperscript{−}}} \) development as a result of the apical (\( \Delta I_{\text{Cl\textsuperscript{−}}} = 55.4 \pm 2.8 \mu \text{A/cm}^2, n = 4, p < 0.01 \)) compared with the values in the absence of pyruvate and basolateral presence of \( \text{H}_2\text{O}_2 \) (\( \Delta I_{\text{Cl\textsuperscript{−}}} = 61.4 \pm 4.7 \mu \text{A/cm}^2, n = 4, p < 0.01 \)) compared with the values in the absence of pyruvate. In addition, acute \( I_{\text{Cl\textsuperscript{−}}} \) changes caused by apical and basolateral \( \text{H}_2\text{O}_2 \) seem to be up-regulated by the presence of pyruvate.

**8-Br-cAMP-Induced Responses in the Presence of \( \text{H}_2\text{O}_2 \).** The aspect-specific effects of \( \text{H}_2\text{O}_2 \) were reproduced in \( I_{\text{SC}} \) responses to the cell-permeable cAMP analog 8-Br-cAMP (1 mM) (Fig. 5, A–C). The \( I_{\text{SC}} \) responses to 8-Br-cAMP (59.6 \( \pm \ 4.4 \mu \text{A/cm}^2, n = 12 \)) were down-regulated to 40.8 \( \pm \ 1.5 \mu \text{A/cm}^2 (n = 7, p < 0.01) \) (Fig. 5B) and up-regulated to 84.9 \( \pm \ 3.6 \mu \text{A/cm}^2 (n = 12, p < 0.01) \) (Fig. 5C) by apical and basolateral pretreatment, respectively, with \( \text{H}_2\text{O}_2 \). These observations suggest that cAMP production via adenylate cyclase is not necessarily a primary target of \( \text{H}_2\text{O}_2 \) in the airway epithelium. Based on the apparently paradoxical effects of \( \text{H}_2\text{O}_2 \) on the cAMP-mediated \( I_{\text{SC}} \), we suspected that up-regulation of the basolateral anion entry rate would...
Fig. 6. Representative traces analyzing anionic composition of FK-induced $I_{\text{sc}}$ in the presence and absence of $H_2O_2$. After cells reached a sustained state, bumetanide (Bume) and DNDS were sequentially applied to estimate the blocker-sensitive components (A). In the basolateral presence of $H_2O_2$, the Bume-sensitive component was selectively augmented (B). Summarized data for the Bume-sensitive and DNDS-sensitive $I_{\text{sc}}$ values are displayed in C and D, respectively, compared with the values in each FK group without $H_2O_2$. * ($p < 0.01$) and † ($p < 0.05$), significant increases and decreases, respectively, compared with the values in each FK group without $H_2O_2$ stress.

Mechanisms Underlying Up-Regulated cAMP-Mediated NKCC1-Mediated Anion Transport under Basolateral $H_2O_2$.

The airway epithelial cells secrete Cl$^{-}$ via NKCC1 in response to activation of human intermediate conductance Ca$^{2+}$-activated K$^{+}$ channels (KCNN4), so that anion secretion induced by 1-ethyl-2-benzimidazolinone (a KCNN4 activator) and thapsigargin (a cytosolic Ca$^{2+}$ mobilizing agent) is markedly inhibited by either bumetanide, a NKCC1 inhibitor, or ChTx, a KCNN4 channel blocker (Devor et al., 1997; Ito et al., 2004a,b). NKCC1 is also activated by cAMP/protein kinase A (PKA)-mediated phosphorylation (Kaas and Forbush, 2000; Matthews, 2002); this mechanism fails to involve KCNN4 activation because of the lower sensitivity of ChTx to cAMP-mediated anion secretion (Ito et al., 2004a). This was confirmed by the results shown in Fig. 7A. Although exposure to basolateral $H_2O_2$ further potentiated the bumetanide-sensitive component in FK-induced $I_{\text{sc}}$ from 12.4 ± 1.0 ($n = 7$) to 37.4 ± 1.5 $\mu$A/cm$^2$ ($n = 13$), preincubation with ChTx did not affect the potentiation (Fig. 7B). Namely, bumetanide-sensitive $I_{\text{sc}}$ was augmented from 10.2 ± 0.9 ($n = 7$) to 36.0 ± 2.9 $\mu$A/cm$^2$ ($n = 7$) in the basolateral presence of ChTx and $H_2O_2$ (Fig. 7E).

Previous studies have shown that NKCC1 is functionally linked to the cortical cytoskeleton adjacent to the basolateral membrane (Matthews, 2002). Thus, we hypothesized that the cytoskeletal remodeling induced by oxidative stress around the basolateral membrane could be attributed to the augmentation of cAMP/PKA-dependent NKCC1 activity. To test this hypothesis, the effects of $H_2O_2$ on FK-induced responses were observed in the presence of Cyto-D (10 $\mu$M), which is conventionally used to disrupt microfilament function (Matthews et al., 1997). After the addition of Cyto-D, we observed gradual increases in $\Delta I_{\text{sc}}$ in response to imposed voltage.
with the values in the FK groups pretreated with and without H2O2, H2O2-induced CFTR-mediated anion secretion seems likely. Cyto-D rather offsets the bumetanide-sensitive component in ICl that almost reflect augmentation of CFTR-mediated cm2, respectively.

(barnett et al., 1994) and a COX-1 inhibitor (SC-560, K of COX-1/COX-2 = 9 nM/6.5 μM) (smith et al., 1998). Although the responses to apical and basolateral H2O2 seem likely to be commonly relevant to COX signals, we found that the ISc responses to apical H2O2 were larger than those to basolateral H2O2. These observations allow us to speculate that polarization of the cells produces laterality between the apical and basolateral membrane in the membrane-located COX activity.

Despite the apparent similarity in the responses to the oxidative stimuli from either side of the membrane, the result that FK-induced anion secretion is inhibited by the apical presence of H2O2 but potentiated by its basolateral presence led us to hypothesize that H2O2 induces different signal transduction in each membrane. The effect of apical H2O2 is reasonable because the ISc changes correlated with FK-induced ICl and [cAMP] elevation. In the hindrance of cAMP production, oxidative stress may operate primarily at the level of the plasma membrane on the functioning of adenylate cyclase by altering the state of phosphorylation (see et al., 2001). However, the effect of H2O2 is also observed in the apical ICl in response to 8-Br-cAMP, a cell-permeable cAMP analog, suggesting that oxidative stress to either aspect hinders activation of CFTR by hindrance of the channel gating and cAMP synthesis. Regarding the mechanisms underlying dysfunction of CFTR under oxidative insults, previous studies have shown that redox reagents alter the kinetics of CFTR gating such that reducing conditions speed up gating and increase the open probability, whereas oxidizing conditions slow down CFTR gating, probably through cysteine residues located on the nucleotide-binding domains of CFTR (harrington et al., 1999; Harrington and Kopito, 2002). Alternatively, AMP-activated protein kinase, which is activated by oxidative stress and consequently phosphorylates CFTR to inhibit its conductance, may also be involved in the mechanisms (Walker et al., 2003). Naturally, because FK-induced and 8-Br-cAMP-induced ISc were potentiated by the presence of basolateral H2O2, we first assumed that these cAMP-related parameters, such as cAMP production and cAMP-elicited ICl, were correlated with the up-regulated ISc changes. Unexpectedly, however, we found that these parameters were inhibited by basolateral H2O2, inconsistent with the behavior of ISc. These observations led us to conclude that H2O2 stimulation from either side hindered the cAMP/PKA signal transduction (cAMP synthesis process and CFTR activation) from the cytosolic side and simultaneously allowed us to deduce the presence of a specific pathway, which is localized around the basolateral membrane.

Anion secretion is the end result of coordinated activities of several different anion transporters. This event requires not only the activity of the apical anion channel but also basolateral transporters (devor et al., 1999; Ito et al., 2004b). The apical CFTR, which is well accepted as a common pathway for HCO3 and Cl export (Devor et al., 1999), displays no less than ~60% of maximum conductance at rest, so that the Calu-3 cell is fully capable of anion secretion even under the cAMP-unstimulated state (Moon et al., 1997). Thus, rather than CFTR as an anion exit pathway, anion uptake across the basolateral membrane is thought to be the rate limiter that largely determines the overall secretion capacity, as is the case of other polarized epithelia (Matthews, 2002). In epithelial cells, anion entry across the basolateral membrane...
chiefly depends on the activity of basolateral anion transporters, such as NBC1, NKCC1, and AE2 (Loffing et al., 2000; Liedtke et al., 2002). Thus, a possible explanation for the seemingly paradoxical results is that \( \text{H}_2\text{O}_2 \)-induced up-regulation of anion uptake across the basolateral membrane would compensate for the down-regulation of CFTR activation of anion exits across the apical membrane. Estimation of the anionic composition of the FK-elicited \( \text{I}_{\text{sc}} \) revealed that both bumetanide (an NKCC1 inhibitor)- and DNDS (an NBC1/AE2 inhibitor)-sensitive components are similarly increased in the control, whereas the increase evidently favored the bumetanide-sensitive \( \text{I}_{\text{sc}} \) in the presence of basolateral \( \text{H}_2\text{O}_2 \) (see Fig. 5, A and B); this suggests that cAMP-activated \( \text{I}_{\text{sc}} \) potentiated by the presence of basolateral \( \text{H}_2\text{O}_2 \) mirrored the augmentation of \( \text{Cl}^- \) current mediated through NKCC1, whose sensitivity to PKA may be up-regulated by the basolaterally localized effect of the oxidative stress.

Previous studies (Devor et al., 1999; Ito et al., 2004b) reported that the switch between \( \text{HCO}_3^- \) secretion and \( \text{Cl}^- \) secretion is determined by the basolateral membrane potential regulated by a ChTx-sensitive \( \text{Ca}^{2+} \)-activated \( \text{K}^- \) channel, KCNN4. Namely, when the basolateral membrane is hyperpolarized by KCNN4 activation, the driving force for \( \text{HCO}_3^- \) entry via NBC1 (the \( \text{Na}^+-2\text{HCO}_3^- \) transporter), which carries electrogenically negative charges into the cell, is reduced, whereas the driving force for \( \text{Cl}^- \) entry across the electroneutral NKCC1 (the \( \text{Na}^+-\text{K}^+-2\text{Cl}^- \) cotransporter) is up-regulated. Because the hyperpolarization simultaneously provides a driving force for anion export across the apical CFTR, the activation of the KCNN4 would cause a large \( \text{Cl}^- \) secretion (Moon et al., 1997; Devor et al., 1999). To exclude the possibility of NKCC1 up-regulation by way of KCNN4 activation, we observed the effect of basolateral oxidative stress on the FK-induced responses in the presence of ChTx, but it made no significant difference in the oxidant-induced modulation.

It is now been established that a complex cortical meshwork of cytoskeleton proteins localizes adjacent to the cytosolic faces of the plasma membrane, where it is uniquely placed to interact with a variety of transmembrane proteins such as NKCC1 (Matthews, 2002). For NKCC1 regulation, cAMP may induce the surface recruitment of membrane proteins to form a regulatory complex with NKCC1 (D’Andrea et al., 1996). Furthermore, several lines of evidence have shown that cAMP-dependent signals themselves are transduced to NKCC1, at least in part, by dynamic remodeling of F-actin microfilaments within the cortical submembranous cytoskeleton (Shapiro et al., 1991; Matthews, 2002). It has been shown that exposure to \( \text{H}_2\text{O}_2 \) remodels actin structures that take the form of microfilaments associated with cortical F-actin in Calu-3 cells (Boardman et al., 2004). Thus, it is most conceivable that the oxidant-induced remodeling of the cytoskeleton would help the PKA-induced reorganization of the submembranous cytoskeleton linked to NKCC1, resulting in selective augmentation of bumetanide-sensitive \( \text{I}_{\text{sc}} \). Indeed, the oxidant-induced up-regulation of the NKCC1-mediated \( \text{I}_{\text{sc}} \) in response to FK was markedly suppressed by Cyto-D. Considering the results obtained from the present study, we propose the hypothetical scheme of \( \text{H}_2\text{O}_2 \)-elicited effects on cAMP-dependent anion secretion shown in Fig. 8.

Collectively, we found that basolaterally but not apically applied \( \text{H}_2\text{O}_2 \) potentiates the subsequent cAMP-mediated \( \text{Cl}^- \) secretion via basolateral NKCC1 whose sensitivities to cAMP/PKA are up-regulated, overcoming the negative effects of \( \text{H}_2\text{O}_2 \) on the apical anion conductance via CFTR. The effects of \( \text{H}_2\text{O}_2 \) localized on the basolateral membrane may be relevant not to basolateral \( \text{Ca}^{2+} \)-activated \( \text{K}^- \) channels (KCNN4) but to the basolaterally localized cytoskeleton, which is believed to interact with NKCC1.

Airway epithelial cells are exposed to oxidative stress not only through inhalation of ozone and other environmental oxidants from the apical side but also through intrinsic ROS from the basolateral side because formation of ROS takes places constantly in every cell during the metabolic process (Ricciardolo et al., 2006). Especially in acute and chronic airway inflammations, activated phagocytic cells, such as
neutrophils, eosinophils, monocytes, and macrophages, are recruited in the subepithelial sites of the respiratory tract, and they generate and release large amounts of ROS (Ricciardo et al., 2006). In contrast to the basolateral membrane, the apical membrane is fully protected by antioxidant substances such as vitamin C and glutathione at very high concentrations in the human airway (Kelly et al., 1999; Daulthave et al., 2001). Therefore, the responses shown in our study may serve to compensate for basolateral ROS-induced disturbance of mucociliary clearance to help clear infections before consequent tissue damage can be initiated. The site-dependent effects of H2O2 that we have shown here should provide new insight into a variety of epithelial biology and toxicology in which oxidant stress is implicated.

References

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