Redox Biology 28 (2020) 101346

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Proton channel blockers inhibit Duox activity independent of Hv1 effects

Monica Valencia Gattas[1], Adam Jaffe[2], Juliana Barahona[3], Gregory E. Conner*

Department of Cell Biology and Division of Pediatric Pulmonary Disease, University of Miami Miller School of Medicine, 1600 NW 10th Ave, Miami, FL, USA, 33136

ARTICLE INFO

Keywords:
Duox
Hv1
Zinc
Bronchial epithelium

ABSTRACT

The NADPH oxidase reaction produces protons. In the case of the NADPH oxidase, NOX2, activity depends on secretion of these protons and is inhibited by blockade of the voltage-gated proton channel (Hv1). Duox1 and Duox2 activities similarly produce intracellular protons but synthesize hydrogen peroxide directly instead of superoxide. Hv1 contributes to acid secretion in some epithelia that express Duox. To test the hypothesis that Duox activity is also sensitive to Hv1 channel blockers, Duox was assayed in the presence of either Zn^{2+} or 5-chloro-2-guanidinobenzimidazole (ClGBI). Both compounds inhibited Duox activity in normal human bronchial epithelial cells but with an IC50 over 10-fold higher than that reported for Hv1 (IC50 Zn^{2+} = 0.68 mM; IC50 ClGBI = 0.07–0.14 mM). Homogenized HEK293T cells expressing either Duox1 or Duox2 showed similar IC50 values for ClGBI suggesting these compounds inhibit the enzymes through alternate mechanisms independent of Hv1 proton secretion. Inclusion of superoxide dismutase did not restore Duox hydrogen peroxide synthesis. Addition of nigericin to eliminate any possible transmembrane pH gradients in intracellular membrane-localized Duox did not alter activity in HEK293T homogenates. Extracellular Zn^{2+} blocked intracellular Ca^{2+} increases needed for Duox activity. Together the data suggest that Duox enzyme activities in epithelia are inhibited by compounds that block Hv1 but inhibition occurs through Hv1-independent mechanisms and support the idea that Hv1 is not required for Duox activity.

1. Introduction

The NADPH Oxidases are a seven-member family of ubiquitous enzymes that catalyze formation of H_2O_2, either indirectly via dismutation of superoxide or directly, in the case of Duox and Nox4 [1]. H_2O_2 participates in a variety of cellular functions including formation of antibiotic compounds, thyroid hormone synthesis and redox regulation of cellular proteins. NADPH oxidase activity releases a proton from NADPH during catalysis. Notably, in phagocytes that express a high level of Nox2, high NADPH oxidase activity increases intracellular concentration of protons and depolarizes the membrane by extrusion of superoxide. Both the intracellular acidification and the depolarization tend to inhibit Nox2 activity. However, the depolarization and intracellular acidification increase the probability of opening the voltage-gated proton channel (Hv1) that dissipates the generated H^+ gradient and repolarizes the membrane potential by the extrusion of protons [2]. Inhibition of Hv1 in phagocytes with Zn^{2+} inhibits Nox2 activity presumably by blocking the extrusion of protons, which would allow Nox2 to depolarize the membrane and acidify the cytosol [3]. Duox activity is linked to proton secretion and can contribute to intracellular acidity in bronchial epithelial cells [4–6]. Activated Duox activity in epithelial cells appears to be substantially less compared to Nox2 activity in activated phagocytes. For this reason, Fischer [6,7] suggested that Duox does not generate sufficient intracellular protons to require Hv1 activity. On the other hand Duox could generate locally acidic conditions, albeit difficult to measure, that could activate Hv1. In addition, Fischer and colleagues have shown that Hv1 contributes substantially to apical acid secretion in airway epithelia (e.g. 8).

In this study, we examined the effects of Hv1 channel blockers on Duox activity in primary human epithelia and in a cell-free assay of heterologous expressed Duox. The data showed that Duox activity is sensitive to Hv1 channel blockers but at concentrations significantly above that needed to inhibit Hv1. The data suggested that Duox activity does not rely on movement of protons by the voltage-gated channel Hv1 and is not inhibited by blockade of the major proton secretion mechanisms at work in primary bronchial epithelial cells. Instead, the data

* Corresponding author. Department of Cell Biology, University of Miami Miller School of Medicine, Miami, FL, USA, 33136.
E-mail addresses: MValencia3@med.miami.edu (M.V. Gattas), adam.h.jaffe@gmail.com (A. Jaffe), luz.barahona@unchealth.unc.edu (J. Barahona), gconner@miami.edu (G.E. Conner).
[1] Present address: Department of Human Genetics, University of Miami Miller School of Medicine, 1501 NW 10th Ave, Miami FL 33136.
[2] Present Address: 2463 SW 11th St, Miami, FL 33135.
[3] Present Address: UNC Pulmonology & Sleep Medicine at Lenoir, 706 Rosanne Dr., Kinston, NC 28504.

https://doi.org/10.1016/j.redox.2019.101346
Received 5 August 2019; Received in revised form 1 October 2019; Accepted 16 October 2019
Available online 20 October 2019

Abbreviations

AR	10-Acetyl-3,7-dihydroxyphenoxazine, Amplex Red
ALI	air-liquid interface
ClGBI	5-chloro-2-guanidinobenzimidazole
DPBS	Dulbecco's Phosphate buffered saline
NHBE	normal human bronchial epithelia
pHi	intracellular pH

suggested Hv1 inhibitors reduced Duox activity through alternate mechanisms unrelated to Hv1 activity and point to a role for the H^+/K^+ ATPase in regulating pHi.

2. Materials and methods

2.1. Cell culture

Primary normal human bronchial epithelial (NHBE) cells were isolated from organ donor lungs rejected for transplant and obtained by the Life Alliance Organ Recovery Agency of the University of Miami according to IRB approved protocols. Isolation, air-liquid interface (ALI) culture and redifferentiation of NHBE were previously described [9,10]. All experiments were performed with date, passage and lung matched control cultures. shRNA-mediated knockdown in NHBE culture used MISSION® shRNA in pLKO.1 lentiviruses as described [11]. HEK293T (ATCC, CRL-3216) were cultured as recommended by ATCC. All cultures were discarded after 10 passages. HEK293T cell lines, expressing either Duox1 or Duox2 activity, were constructed using pCDH lentiviral vectors (System Biosciences, Palo Alto CA). See Supplemental methods for details.

2.2. NHBE H_2O_2 synthesis activity

NHBE cell H_2O_2 synthesis was measured using modifications of an Amplex Red assay described previously [12]. For Zn^{2+} inhibition experiments, apical phosphate buffers were replaced by either HEPES Ringers, pH 7.4 or Tris Ringers pH 7.4 [13] to prevent Zn^{2+} precipitation. Zn^{2+} containing buffer was added to apical surface for 30 min prior to assays. For ClGBI inhibition, either PBS or HEPES Ringers were used (see Supplemental methods for composition) and ClGBI was added to the basolateral solution 30 min before assay. Buffer containing 25–50 μM Amplex Red (AR) (10-Acetyl-3,7-dihydroxyphenoxazine, Cayman Chemical, Ann Arbor, MI) and 0.05–0.1 U/ml HRP (SigmaAldrich, P2088) was added to the apical surface of NHBE cultures and recorded continuously in a Biotek SynergyH1 plate reader (530 nm ex/590 nm em). After a 5–10 min baseline, cultures were stimulated by addition of 100 μM ATP. When assays used Fura-2 loaded cells, excitation was at 545 nm. Fluorescence was recorded at 30–40 s intervals and H_2O_2 synthesis rates were calculated from the slope of RFU change over time using a 1–2 min sliding window with a 30–40 s step. Initial rates were normalized to cultures treated with vehicle alone and IC50 values were calculated by nonlinear regression using PRISM 5.0b (GraphPad Software). See Supplemental methods for details on

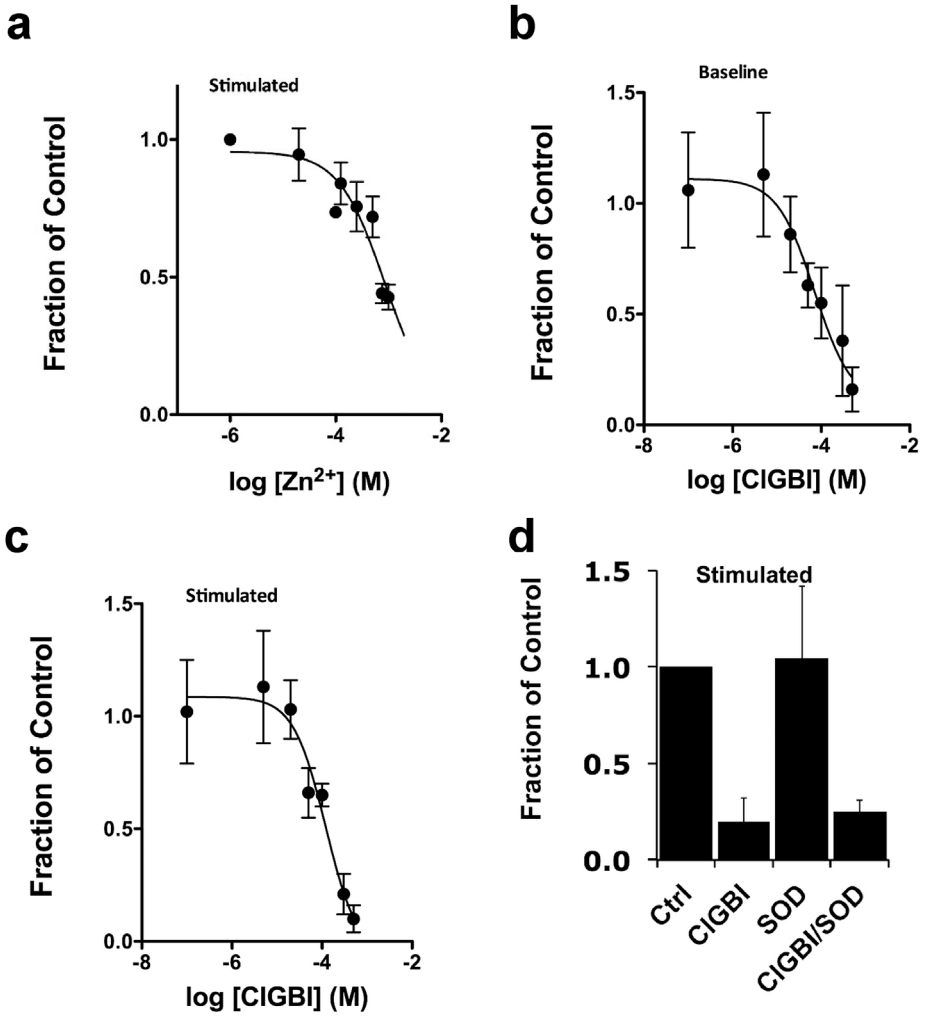

Fig. 1. High concentrations of Hv1 channel blockers inhibit Duox H_2O_2 synthesis. H_2O_2 synthesis by fully differentiated NHBE cells was assayed in the absence and presence of either Zn^{2+} (panel a, Tris-Ringers solution) or ClGBI (panels b–d, PBS). Rates of AR oxidation were normalized to assays in the absence of inhibitors but with vehicle. ATP (100 μM) was used to stimulate Ca^{2+} dependent Duox activity. All values are means ± s.e.m. *Panel a*, Zn^{2+} inhibited ATP stimulated Duox H_2O_2 synthesis (IC50 = 0.68 mM, 3–5 lung donors, triplicate cultures each donor). *Panels b and c*, ClGBI inhibited both baseline Duox activity (IC50 = 0.070 mM, n = 3–6 lung donors, triplicate cultures each donor) and stimulated activity (IC50 = 0.12 mM, n = 3–6 lung donors, triplicate cultures each donor). *Panel d*, Inhibition of stimulated Duox activity by ClGBI (0.3 mM) was not rescued by addition of superoxide dismutase (20 Units/ml), n = 6 cultures, 3 lung donors.

HEK293T homogenization and H_2O_2 synthesis assay.

2.3. Intracellular Ca^{2+} and pH measurement

Differentiated NHBE cultures were loaded with Fura-2AM (10 μM) or BCECF-AM (5 μM) (both from Molecular Probes), washed and fluorescence followed in a microplate reader until fluorescence ratios stabilized. Intracellular Ca^{2+} ($[Ca^{2+}]_i$) was estimated using an intracellular Kd = 0.25 μM. Intracellular pH (pHi) was assessed using high K^+ buffers containing 20 μM nigericin. See Supplemental methods for details.

2.4. PCR, western blotting and data analysis

Quantitative PCR used TaqMan gene expression kits. Western blotting of Duox in HEK293T homogenates used rabbit *anti*-Duox1 (SigmaAldrich, SAB2701393), see Supplemental methods. Statistical analysis was performed using JMP Pro (SAS Institute).

3. Results and discussion

3.1. Hv1 channel blockers inhibit Duox H_2O_2 synthesis

Hv1 channel inhibitor effects on Duox activity were studied in differentiated NHBE cells cultured at the ALI. These cells express Duox1 and Duox2 at levels much higher than other members of the NOX family [11] and express functional Hv1 [4,8]. Previously, NHBE H_2O_2 synthesis was shown to be due to Duox1 and Duox2 activity [12,14,15] and that Duox contributes to intracellular acid production in NHBE [4]. Extracellular Zn^{2+} inhibits Hv1 and decreases H^+ secretion in a variety of cells, including NHBE [4,8]. Thus, cultures were assayed for H_2O_2 synthesis in various apical $[Zn^{2+}]$ with and without ATP stimulation that increases $[Ca^{2+}]_i$ and stimulates Duox activity [12,16,17]. Assays were conducted at room temperature since it was previously shown that Zn^{2+} stimulated thyroid NADPH oxidases (Duox1 and Duox2) at elevated temperature in cell free assays [18]. In NHBE cultures, Zn^{2+} inhibited ATP -stimulated H_2O_2 synthesis with an apparent IC50 = 0.68 mM (Fig. 1a) but failed to affect baseline activity. This IC50 is substantially higher than that expected for Hv1 inhibition [19]. The solubility of free Zn^{2+} at 25° and pH 7.4 in phosphate free buffers was calculated to be 0.48 mM [20] and limits the accurate determination of the IC50.

Since Hv1 gating is strongly temperature dependent [21,22] it was possible that the lower temperature used for Zn^{2+} inhibition might prevent observation of an effect on H_2O_2 synthesis. For this reason, assays were also conducted in 5-chloro-2-guanidinobenzimidazole (ClGBI, SigmaAldrich, CAS# 70590-32-8), a cell permeable Hv1 inhibitor [23]. These assays were conducted at 37°. ClGBI inhibited both

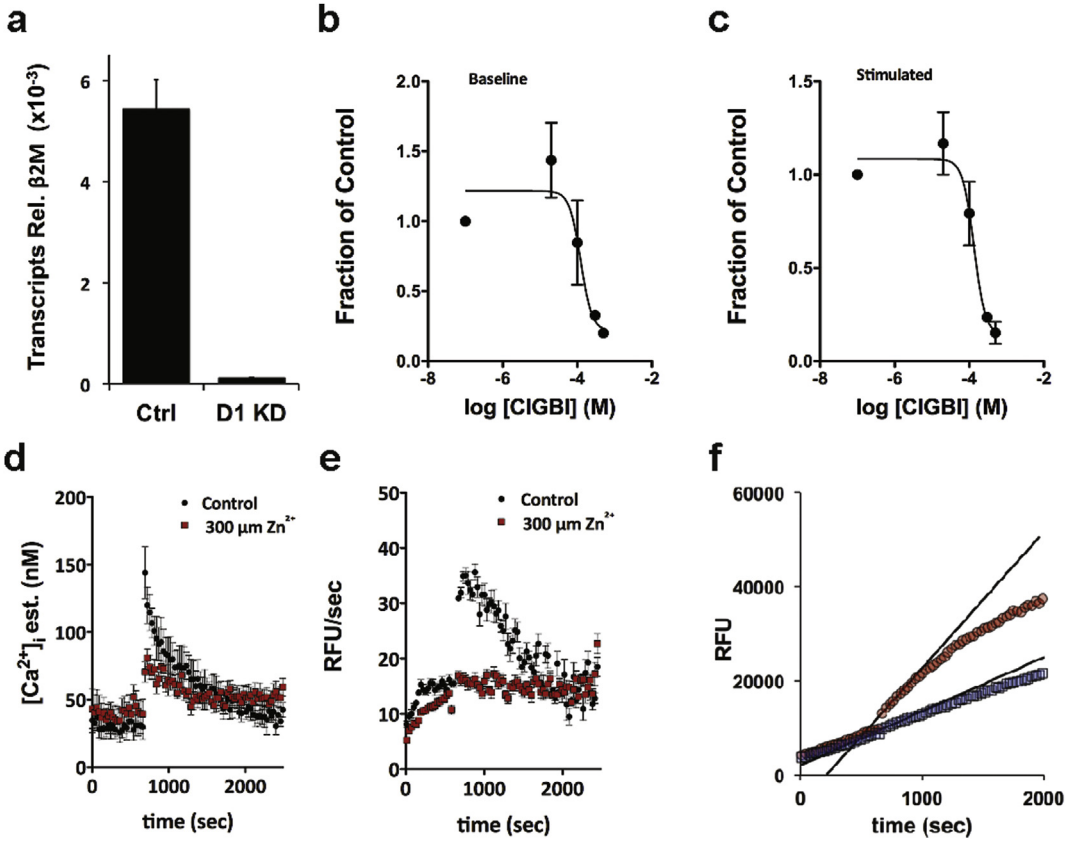

Fig. 2. Both Duox2 and Duox1 are inhibited by ClGBI and Zn^{2+} inhibits intracellular Ca^{2+} increases concurrently with inhibition of Ca^{2+}-stimulated Duox activity. *Panel a*, Duox1 expression was reduced by shRNA expressing lentivirus (see Supplemental methods). Transcripts relative to β_2-microglobulin were reduced > 95%, n = 3 cultures, 1 donor. *Panel b and c*, H_2O_2 synthesis by differentiated NHBE cells with reduced Duox1 activity were assayed in PBS in the absence and presence of ClGBI. Rates of AR oxidation were normalized to assays in vehicle only. ClGBI inhibited both baseline Duox2 (IC50 = 0.12 mM) and stimulated activity (IC50 = 0.13 mM), n = 3 cultures, one lung donor. *Panel d and e* NHBE cultures were loaded with Fura-2 prior to H_2O_2 assay. Changes in Fura-2 fluorescence were recorded simultaneously with changes in Resorufin fluorescence and $[Ca^{2+}]_i$ was estimated from ratiometric recordings (vehicle controls, solid circles; Squares, Zn^{2+}-treated) (see Supplemental methods). *Panel d*, Following ATP stimulation, $[Ca^{2+}]_i$ transiently increased in control cultures but the increase was blunted in the presence of Zn^{2+} (300 μM in HEPES Ringers) (n = 2 lung donors, triplicate cultures of each donor). *Panel e*, Following ATP stimulation, H_2O_2 synthesis rate (Resorufin RFU/sec) increased in control cultures but was inhibited by Zn^{2+} (300 μM in HEPES Ringers). *Panels d and e*, n = 6 cultures from 2 lung donors. RFU/sec was calculated with a 60 s sliding window and 30 s steps. *Panel f,* example curves of a control culture (red circles) and a Zn^{2+} treated cultures used in panels d and e are shown along with tangents to the region used for slope calculations over the first 2 min following ATP stimulation.

baseline and ATP-stimulated H_2O_2 synthesis with an apparent IC50 = 0.07 mM and 0.12 mM respectively (Fig. 1b and c) that is about 10 fold higher than expected for Hv1 inhibition [23]. To rule out the possibility that inhibition of Hv1 resulted in synthesis of superoxide rather than H_2O_2, the normal product of Duox activity, superoxide dismutase was added to the assays and did not increase the measured H_2O_2 (Fig. 1d). Thus, the IC50 of both inhibitors for Duox activity was greater than expected based on Hv1 sensitivity [19,22,23].

Since Duox1 is responsible for the majority of ATP-stimulated H_2O_2 synthesis in NHBE [11], it was possible that Duox2 sensitivity to Zn^{2+} or ClGBI could be obscured by the larger Duox1 activity. NHBE cells, that had shRNA-reduced Duox1 expression (Fig. 2a), were assayed and the baseline and stimulated activity IC50 for ClGBI in these Duox1 knock down cultures was indistinguishable from the control cultures (IC50 = 0.12 and 0.13 mM respectively, Fig. 2b and c).

3.2. Zn^{2+} reduces intracellular Ca^{2+} transients concurrently with Duox inhibition

Zn^{2+} and ClGBI inhibition of H_2O_2 synthesis differed with respect to effects on baseline activity with Zn^{2+} only inhibiting ATP-stimulated activity. Duox1 and Duxo2 both bind Ca^{2+} via an EF hand to stimulate activity. Since Zn^{2+} is known to block $[Ca^{2+}]_i$) transients in epithelial cells [24,25], it was possible Zn^{2+}'s effect on Duox H_2O_2 synthesis was due to reduction of the ATP-stimulated purinergic Ca^{2+}-signaling. To assess the effect of Zn^{2+} on $[Ca^{2+}]_i$, H_2O_2 synthesis and changes in $[Ca^{2+}]_i$ were measured simultaneously. Addition of Zn^{2+} (300 μM) reduced the transient $[Ca^{2+}]_i$ increase following ATP stimulation with concomitant reduction of H_2O_2 synthesis (Fig. 2d and e). It appeared that Zn^{2+} inhibition of H_2O_2 synthesis might be due in part to suppression of Ca^{2+}-induced Duox enzyme activity. The data are confounded by any intracellular Zn^{2+} that would also bind to Fura-2 [26,27], however such binding will shift Fura-2 fluorescence to give an apparent increase in $[Ca^{2+}]_i$ rather than a decrease. Consequently, the Zn^{2+} effect on $[Ca^{2+}]_i$ seen in Fig. 2d is underestimated and a greater reduction of $[Ca^{2+}]_i$ supports that Zn^{2+} reduced the Ca^{2+} transient simultaneously with Duox inhibition.

3.3. Duox in HEK293T cell homogenates is inhibited by ClGBI

Several attempts were made to lower Hv1 expression in NHBE cells via HVCN1-directed shRNA without substantial reduction. As an alternative approach, Duox1 and Duox2 were expressed along with their partners DuoxA1α and DuoxA2 in HEK293T cells (Supplemental Figure) that express barely detectable levels of HVCN1 mRNA (data not shown) and protein (in Supplementary Fig. 2 of [28]). To further support that ClGBI inhibition of Duox is unrelated to blocking Hv1 channels, H_2O_2 synthesis by Duox1 and Duox 2 was assayed in homogenates of these HEK293T cells in the presence and absence of ClGBI. The assays showed that ClGBI inhibited Duox1 and Duox2 in homogenates of HEK293 cells (Fig. 3a and b) with an IC50 similar to that seen in intact NHBE cells (IC50 = 0.14 and 0.11 respectively). Since Duox 1 and 2 are also found in intracellular compartments (e.g. [29]) the data also suggest that intracellular Duox1 and Duox2 were not protected at lower inhibitor concentrations due to cellular location.

3.4. H^+/K^+ ATPase plays a role in controlling NHBE pHi but not Duox activity

In NHBE, Hv1 gating primarily depends on the transmembrane pH gradient, because the membrane voltage is fairly constant about −20 mV in the apical membrane [2,7,30,31]. Measurement of pHi in NHBE in mucosal pH buffers used in the Duox assays (Supplemental Table) suggests that the pH gradient in our assays is insufficient to open Hv1 [31] and, indeed, addition of Hv1 inhibitors failed to lower pHi. These data suggested that effects of Hv1 inhibition on Duox activity might not be detectable because it is inactive under our assay conditions. However, it is possible that intracellular vesicles were providing an opportunity to form transmembrane pH gradients since intracellular organelles can form vesicles that are sealed and any Duox localized in theses vesicles could be subject to ionic transmembrane gradients. To this end, HEK293T homogenates were also assayed in the presence of nigericin to disrupt any possible H^+ gradients. Nigericin requires cholesterol to intercalate into the membrane and although, most cholesterol is found in the plasma membrane, cholesterol is synthesized in the endoplasmic reticulum and traffics both to and from the plasma membrane. Consequently, cholesterol is also found in intracellular membranes although at lower levels than the plasma membrane. Assay of cell homogenates in the presence of nigericin (20 μM) did not alter the ClGBI effects on H_2O_2 synthesis (Fig. 3c), arguing that inhibition of Duox in homogenates is not related to intracellular action of Hv1.

NHBE cells express multiple proteins to control pHi (viz. 7) including apical, Ouabain-sensitive H^+/K^+ exchanger (ATP12A), shown to be highly expressed [32] in bronchial epithelia and to regulate airway surface liquid pH [32–35]. NHBE cells express approximately 10 fold higher levels of ATP12A compared to HVCN1 (Fig. 4a). Ouabain (1 mM) addition to mucosal buffers did not block Duox H_2O_2 synthesis (not shown) but measurement of ASL pH of NHBE cultures at the ALI, in buffer conditions used for ClGBI studies, showed a large apical Ouabain-sensitive H^+ secretion that rapidly reduced the pH (0.01 pH unit/min) of the apical assay buffer (Fig. 4b). Neither addition of Zn^{2+} or ClGBI further reduced the apical acidification suggesting that ATP12A

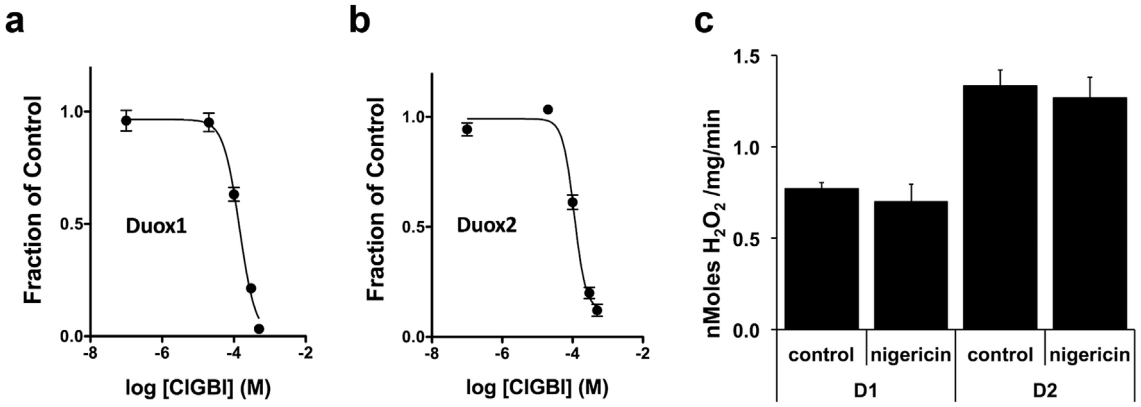

Fig. 3. Duox activity in HEK293T cell homogenates is inhibited by ClGBI. Duox1/DuoxA1α and Duox2/DuoxA2 were expressed in HEK293T cells (see Supplemental methods). *Panels a and b,* Homogenates of expressing cultures were assayed for H_2O_2 synthesis in the presence and absence of ClGBI. Activity was normalized to vehicle controls, n = 3 each point. Duox1 was inhibited with an IC50 = 0.14 mM and Duox2 with an IC50 = 0.11. *Panel c*, Homogenates of expressing cultures were assayed in the presence and absence nigericin, which did not change Duox activity, mean ± s.e.m., n = 3.

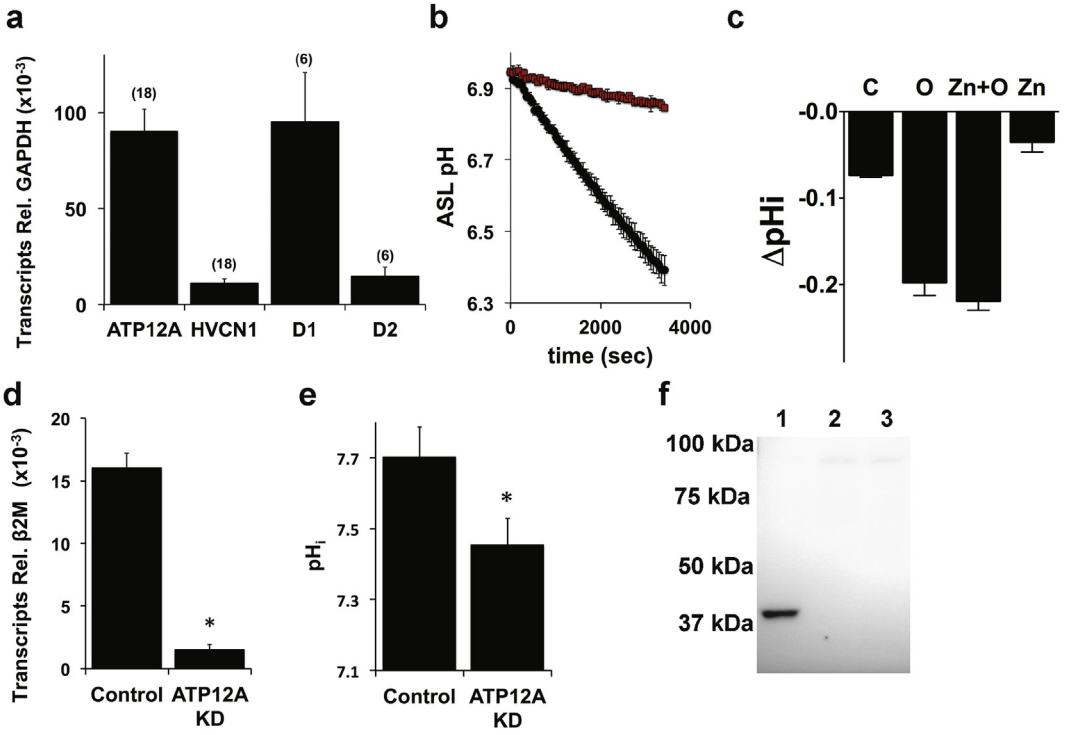

Fig. 4. H^+/K^+ ATPase plays a role in controlling NHBE pHi. *Panel a*, qPCR determination of mRNA levels in freshly isolated NHBE cells showed ATP12A expression greatly exceeded HVCN1 and was equivalent to Duox1 within the limits of efficiency for different TaqMan® kits and RNA preparations. Transcripts values are relative to GAPDH and are means ± S.E.M, n = 18 lung donors for ATP12A and HVCN1, n = 6 lung donors for Duox1 and Duox2. *Panel b*, Airway surface liquid pH of NHBE cultures was measured using BCECF in DPBS (pH 7.1). Fluorescence signal was confirmed to be extracellular by removal and replacement of apical solutions. Rapid acidification of control culture mucosal buffer (closed circles) was blocked by addition of Ouabain (1 mM) (squares), mean ± S.E.M. n = 3 cultures, 1 lung donor. *Panel c,* NHBE cultures were loaded with BCECF-AM and then treated with either vehicle, Ouabain (1 mM), Zn^{2+} (100 μM) or both inhibitors, all in DPBS. Only Ouabain containing treatments showed a significant reduction in pHi compared to control, mean ± S.E.M., n = 3. one lung donor, $p < 0.05$, Tukey-Kramer HSD. *Panel d*, ATP12A expression in NHBE cells was reduced by shRNA expressing lentivirus (see Supplemental methods). Transcripts relative to β_2-microglobulin were reduced > 90% compared to vector controls, n = 5 cultures, 2 lung donors. *Panel e*, NHBE cultures with reduced ATP12A expression were loaded with BCECF-AM and pHi was measured. Cells with reduced ATP12A expression had lower pHi, mean ± S.EM, n = 5 cultures, 2 lung donors, $p < 0.05$, Wilcoxon test. *Panel f,* SDS extract (20 μg) of NHBE (lane 1), Jurkat cells (lane 2) and HEK293T cells (lane 3) were applied to a 10% polyacrylamide SDS gel, transferred and probed with rabbit anti-human Hv1 C-terminal peptide (0.6 μg/ml, ARP35377_P050, Aviva Systems Biology, San Diego CA). A single band with a Mapp of ~37 kDa was visible in lane 1. This band was absent in HEK293 cells (lane 3) and in a duplicate blot using antibody preincubated with blocking peptide (not shown).

was controlling extracellular pH in the H_2O_2 synthesis assays. Measurement of pHi in the presence of Ouabain and/or Zn^{2+} showed that Ouabain, but not Zn^{2+}, decreased pHi (Fig. 4c). Since Ouabain is known to have off-target effects (e.g. CFTR stimulation [36,37]), ATP12A expression was reduced by shRNA (Fig. 4d) and pHi assessed. Knockdown of ATP12A expression in NHBE cells reduced pHi to a similar extent (Fig. 4e).

4. Conclusions

These studies support the idea that Hv1 conductance is not needed to maintain Duox activity in differentiated NHBE cells. The studies also suggest that inhibition of the non-gastric H^+ K^+ ATPase leads to modest changes in pHi, but this change in pHi does not significantly alter Duox activity. In addition, the studies suggest that effects of extracellular Zn^{2+} (300 μM) may be due to Zn^{2+}-mediated changes in $[Ca^{2+}]_i$ and that ClGBI affects other proteins in addition to Hv1. Thus, these inhibitors' target effects can potentially complicate interpretation of data from inhibitor studies of Hv1 and NADPH oxidase.

Funding sources

This work was supported in part by Flight Attendants Medical Research Institute to GEC [(FAMRI CIA) 1233060] and in part by the Department of Cell Biology incentive fund, University of Miami Miller School of Medicine.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Steve Dennis and Murline Gelin for technical assistance in construction of plasmids and cell lines and with Western blots and Dr. H. Peter Larsson for helpful comments and suggestions during the studies and on the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2019.101346.

References

[1] J.D. Lambeth, A.S. Neish, Nox enzymes and new thinking on reactive oxygen: a double-edged sword revisited, Annu. Rev. Pathol. 9 (2014) 119–145 PubMed PMID: 24050626.
[2] T.E. DeCoursey, Voltage-gated proton channels find their dream job managing the respiratory burst in phagocytes, Physiology 25 (1) (2010) 27–40 PubMed PMID: 20134026.
[3] T.E. DeCoursey, D. Morgan, V.V. Cherny, The voltage dependence of NADPH

oxidase reveals why phagocytes need proton channels, Nature 422 (6931) (2003) 531–534 PubMed PMID: 12673252.
[4] C. Schwarzer, T.E. Machen, B. Illek, H. Fischer, NADPH oxidase-dependent acid production in airway epithelial cells, J. Biol. Chem. 279 (35) (2004) 36454–36461 PubMed PMID: 15210697.
[5] H. Fischer, L.K. Gonzales, V. Kolla, C. Schwarzer, F. Miot, B. Illek, P.L. Ballard, Developmental regulation of DUOX1 expression and function in human fetal lung epithelial cells, Am. J. Physiol. Lung Cell Mol. Physiol. 292 (6) (2007) L1506–L1514 PubMed PMID: 17337509.
[6] H. Fischer, Mechanisms and function of DUOX in epithelia of the lung, Antioxidants Redox Signal. 11 (10) (2009) 2453–2465 PubMed PMID: 19358684.
[7] H. Fischer, Function of proton channels in lung epithelia, Wiley Interdiscip. Rev. Membr. Transp. Signal 1 (3) (2012) 247–258 PubMed PMID: 22662311.
[8] D. Iovannisci, B. Illek, H. Fischer, Function of the HVCN1 proton channel in airway epithelia and a naturally occurring mutation, M91T, J. Gen. Physiol. 136 (1) (2010) 35–46 PubMed PMID: 20548053.
[9] M.C. Nlend, R.J. Bookman, G.E. Conner, M. Salathe, Regulator of G-protein signaling protein 2 modulates purinergic calcium and ciliary beat frequency responses in airway epithelia, Am. J. Respir. Cell Mol. Biol. 27 (4) (2002) 436–445 PubMed PMID: 12356577.
[10] M.L. Fulcher, S. Gabriel, K.A. Burns, J.R. Yankaskas, S.H. Randell, Well-differentiated human airway epithelial cell cultures, Methods Mol. Med. 107 (2005) 183–206 PubMed PMID: 15492373.
[11] M.V. Gattas, R. Forteza, M.A. Fragoso, N. Fregien, P. Salas, M. Salathe, G.E. Conner, Oxidative epithelial host defense is regulated by infectious and inflammatory stimuli, Free Radic. Biol. Med. 47 (10) (2009) 1450–1458 PubMed PMID: 19703552.
[12] R. Forteza, M. Salathe, F. Miot, G.E. Conner, Regulated hydrogen peroxide production by Duox in human airway epithelial cells, Am. J. Respir. Cell Mol. Biol. 32 (5) (2005) 462–469 PubMed PMID: 15677770.
[13] E. Bouskela, W. Grampp, Spontaneous vasomotion in hamster cheek pouch arterioles in varying experimental conditions, Am. J. Physiol. 262 (2 Pt 2) (1992) H478–H485 PubMed PMID: 1539706.
[14] M. Geiszt, J. Witta, J. Baffi, K. Lekstrom, T.L. Leto, Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense, FASEB J. 17 (11) (2003) 1502–1504 PubMed PMID: 12824283.
[15] R.W. Harper, C. Xu, J.P. Eiserich, Y. Chen, C.Y. Kao, P. Thai, H. Setiadi, R. Wu, Differential regulation of dual NADPH oxidases/peroxidases, Duox1 and Duox2, by Th1 and Th2 cytokines in respiratory tract epithelium, FEBS Lett. 579 (21) (2005) 4911–4917 PubMed PMID: 16111680.
[16] C. Dupuy, D. Deme, J. Kaniewski, J. Pommier, A. Virion, Ca2+ regulation of thyroid NADPH-dependent H2O2 generation, FEBS Lett. 233 (1) (1988) 74–78 PubMed PMID: 3133245.
[17] R. Ameziane-El-Hassani, S. Morand, J.L. Boucher, Y.M. Frapart, D. Apostolou, D. Agnandji, S. Gnidehou, R. Ohayon, M.S. Noel-Hudson, J. Francon, K. Lalaoui, A. Virion, C. Dupuy, Dual oxidase-2 has an intrinsic Ca2+-dependent H2O2-generating activity, J. Biol. Chem. 280 (34) (2005) 30046–30054 PubMed PMID: 15972824.
[18] C. Dupuy, A. Virion, V. De Sandro, R. Ohayon, J. Kaniewski, J. Pommier, D. Deme, Activation of the NADPH-dependent H2O2-generating system in pig thyroid particulate fraction by limited proteolysis and Zn2+ treatment, Biochem. J. 283 (Pt 2) (1992) 591–595 PubMed PMID: 1315520.
[19] M.P. Mahaut-Smith, The effect of zinc on calcium and hydrogen ion currents in intact snail neurones, J. Exp. Biol. 145 (1989) 455–464 PubMed PMID: 22912993.
[20] R.A. Reichle, K.G. Mccurdy, L.G. Hepler, Zinc hydroxide - solubility product and hydroxy-complex stability-constants from 12.5-75 degreesc, Can. J. Chem. 53 (24) (1975) 3841–3845 PubMed PMID: WOS:A1975BG39900023.
[21] T.E. DeCoursey, V.V. Cherny, Temperature dependence of voltage-gated H+ currents in human neutrophils, rat alveolar epithelial cells, and mammalian phagocytes, J. Gen. Physiol. 112 (4) (1998) 503–522 PubMed PMID: 9758867.
[22] B. Musset, S.M. Smith, S. Rajan, V.V. Cherny, S. Sujai, D. Morgan, T.E. DeCoursey, Zinc inhibition of monomeric and dimeric proton channels suggests cooperative gating, J. Physiol. 588 (Pt 9) (2010) 1435–1449 PubMed PMID: 20231140.
[23] L. Hong, I.H. Kim, F. Tombola, Molecular determinants of Hv1 proton channel inhibition by guanidine derivatives, Proc. Natl. Acad. Sci. U. S. A. 111 (27) (2014) 9971–9976 PubMed PMID: 24912149.
[24] J.K. Foskett, D.C. Wong, [Ca2+]i inhibition of Ca2+ release-activated Ca2+ influx underlies agonist- and thapsigargin-induced [Ca2+]i oscillations in salivary acinar cells, J. Biol. Chem. 269 (50) (1994) 31525–31532 PubMed PMID: 7989320.
[25] C.R. Jan, S.N. Wu, C.J. Tseng, Zn2+ increases resting cytosolic Ca2+ levels and abolishes capacitative Ca2+ entry induced by ATP in MDCK cells, Naunyn Schmiedeberg's Arch. Pharmacol. 360 (3) (1999) 249–255 PubMed PMID: 10543425.
[26] G. Grynkiewicz, M. Poenie, R.Y. Tsien, A new generation of Ca2+ indicators with greatly improved fluorescence properties, J. Biol. Chem. 260 (6) (1985) 3440–3450 PubMed PMID: 3838314.
[27] J.L. Martin, C.J. Stork, Y.V. Li, Determining zinc with commonly used calcium and zinc fluorescent indicators, a question on calcium signals, Cell Calcium 40 (4) (2006) 393–402 PubMed PMID: 16764924.
[28] I.S. Ramsey, M.M. Moran, J.A. Chong, D.E. Clapham, A voltage-gated proton-selective channel lacking the pore domain, Nature 440 (7088) (2006) 1213–1216 PubMed PMID: 16554753.
[29] S. Luxen, D. Noack, M. Frausto, S. Davanture, B.E. Torbett, U.G. Knaus, Heterodimerization controls localization of Duox-DuoxA NADPH oxidases in airway cells, J. Cell Sci. 122 (Pt 8) (2009) 1238–1247 PubMed PMID: 19339556.
[30] N.J. Willumsen, R.C. Boucher, Intracellular pH and its relationship to regulation of ion transport in normal and cystic fibrosis human nasal epithelia, J. Physiol. 455 (1992) 247–269 PubMed PMID: 1336551.
[31] V.V. Cherny, V.S. Markin, T.E. DeCoursey, The voltage-activated hydrogen ion conductance in rat alveolar epithelial cells is determined by the pH gradient, J. Gen. Physiol. 105 (6) (1995) 861–896 PubMed PMID: 7561747.
[32] R.D. Coakley, B.R. Grubb, A.M. Paradiso, J.T. Gatzy, L.G. Johnson, S.M. Kreda, W.K. O'Neal, R.C. Boucher, Abnormal surface liquid pH regulation by cultured cystic fibrosis bronchial epithelium, Proc. Natl. Acad. Sci. U. S. A. 100 (26) (2003) 16083–16088 PubMed PMID: 14668433.
[33] V.S. Shah, D.K. Meyerholz, X.X. Tang, L. Reznikov, M. Abou Alaiwa, S.E. Ernst, P.H. Karp, C.L. Wohlford-Lenane, K.P. Heilmann, M.R. Leidinger, P.D. Allen, J. Zabner, P.B. McCray Jr., L.S. Ostedgaard, D.A. Stoltz, C.O. Randak, M.J. Welsh, Airway acidification initiates host defense abnormalities in cystic fibrosis mice, Science 351 (6272) (2016) 503–507 PubMed PMID: 26823428.
[34] A.T. Lennox, S.L. Coburn, J.A. Leech, E.M. Heidrich, T.R. Kleyman, S.E. Wenzel, J.M. Pilewski, T.E. Corcoran, M.M. Myerburg, ATP12A promotes mucus dysfunction during Type 2 airway inflammation, Sci. Rep. 8 (1) (2018) 2109 PubMed PMID: 29391451.
[35] P. Scudieri, I. Musante, E. Caci, A. Venturini, P. Morelli, C. Walter, D. Tosi, A. Palleschi, P. Martin-Vasallo, I. Sermet-Gaudelus, G. Planelles, G. Crambert, L.J. Galietta, Increased expression of ATP12A proton pump in cystic fibrosis airways, JCI Insight 3 (20) (2018) PubMed PMID: 30333310.
[36] K. Jansson, J. Venugopal, G. Sanchez, B.S. Magenheimer, G.A. Reif, D.P. Wallace, J.P. Calvet, G. Blanco, Ouabain regulates CFTR-mediated anion secretion and Na,K-ATPase transport in ADPKD cells, J. Membr. Biol. 248 (6) (2015) 1145–1157 PubMed PMID: 26289599.
[37] J. Venugopal, G. Blanco, On the many actions of Ouabain: pro-cystogenic effects in autosomal dominant polycystic kidney disease, Molecules 22 (5) (2017) PubMed PMID: 28467389.