Synthetic chloride channel restores glutathione secretion in cystic fibrosis airway epithelia

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Gao, Lin, James R. Broughtman, Takeo Iwamoto, John M. Tomich, Charles J. Venglarik, and Henry Jay Forman. Synthetic chloride channel restores glutathione secretion in cystic fibrosis airway epithelia. Am J Physiol Lung Cell Mol Physiol 281: L24–L30, 2001.—Cystic fibrosis (CF), an inherited disease characterized by defective epithelial Cl− transport, damages lungs via chronic inflammation and oxidative stress. Glutathione, a major antioxidant in the epithelial lining fluid, is decreased in the apical fluid of CF airway epithelium due to reduced glutathione efflux (Gao L, Kim KJ, Yankaskas Jr, and Forman HJ. Am J Physiol Lung Cell Mol Physiol 277: L113–L118, 1999). The present study examined the question of whether restoration of chloride transport would also restore glutathione secretion. We found that a Cl− channel-forming peptide (N-K, M2GlyR) and a K+ channel activator (chlorzoxazone) increased Cl− secretion, measured as bumetanide-sensitive short-circuit current, and glutathione efflux, measured by high-performance liquid chromatography, in a human CF airway epithelial cell line (CPT1). Addition of the peptide alone increased glutathione secretion (181 ± 8% of the control value), whereas chlorzoxazone alone did not significantly affect glutathione efflux; however, chlorzoxazone potentiated the effect of the peptide on glutathione release (359 ± 16% of the control value). These studies demonstrate that glutathione efflux is associated with apical chloride secretion, not with the CF transmembrane conductance regulator per se, and the defect of glutathione efflux in CF can be overcome pharmacologically.

In CF lungs, invading neutrophils produce superoxide and hydrogen peroxide and release myeloperoxidase, which catalyzes formation of hypochlorous acid. This chronic oxidative stress is exacerbated by decreased antioxidant capacity in both the airway lining fluid and plasma of CF patients (3, 10). The fluid covering the lung epithelium normally contains a high concentration of the antioxidant glutathione (GSH) (4, 27). GSH content is decreased in the plasma and bronchoalveolar lavage fluid from CF patients and the apical fluid from CF airway epithelial cells (10, 24). Furthermore, Gao et al. (10) have recently shown that decreased apical GSH content in CF is caused by defective GSH efflux. Because GSH is particularly effective in reducing hypochlorous acid (9), the major protein-damaging agent in CF sputum (29), the defective secretion of GSH has a potentially profound effect on the course of the disease.

Although it is now known that CFTR deficiency causes decreased GSH export (10), little else is known regarding GSH export from airway epithelial cells. In general, the relationship between anion conductance and the GSH transporter system(s) is poorly understood. In isolated rat hepatocytes, GSH transport is membrane potential dependent (8). GSH efflux in the rat liver is also carrier mediated, possibly by the multidrug resistance protein (MRP) (19, 21). More recently, CFTR itself has been suggested to be permeable to GSH by the patch-clamp technique (16). Nonetheless, significant residual GSH secretion is present in CF airway cells, suggesting that CFTR may regulate transport rather than carry GSH.

Although transfection of normal CFTR remains a therapeutic goal, treatment of symptoms and the use of antibiotics are considered the predominant therapeutic strategies in CF. Recently, novel pharmacological approaches have been used to restore Cl− transport in CF, including activation of defective CFTR (25), activation of alternative non-CFTR Cl− channels (20), and incorporation of an artificial Cl− channel-forming pep-
tide into the apical membrane of epithelial cells (22, 32).

Reddy et al. (22) and Wallace et al. (32) have described synthetic peptides that form a Cl⁻-permeable pathway, bypassing the low abundance of CFTR Cl⁻ channels in Madin-Darby canine kidney monolayers. M2GlyR is a synthetic 23-amino acid peptide, PAR-VGLIGTVLMTTQSSSRA, derived from the second transmembrane segment of the α-subunit of the glycine receptor, a glycine-gated Cl⁻ channel. This peptide self-assemblys into a presumed pentamer, forming anion-selective channels in phospholipid bilayers (22). Four lysine residues can be added to either the NH₂ terminus (N-K₄-M2GlyR) or the COOH terminus (C-K₄-M2GlyR) of M2GlyR to increase its solubility and activity (28). These peptides induce transepithelial activity (28). These peptides induce transepithelial passage once in Ham’s F-12 medium without cholera toxin. The cells were cultured in collagen-coated flasks for 3 days and then transferred to 4.2-cm² permeable inserts. Ham’s F-12 medium without cholera toxin was added to the apical (1 ml) and basolateral (2 ml) sides, respectively, and replaced with the 1:1 medium mixture the next day.

For GSH efflux experiments, 5 × 10⁵ cells were seeded on 4.2-cm² permeable inserts. Ham’s F-12 medium without cholera toxin was added to the apical (1 ml) and basolateral (2 ml) sides, respectively, and replaced with the 1:1 medium mixture the next day.

METHODS

Cell lines and cell culture. CFT1 cells are immortalized human tracheal epithelial cells from a homozygous ΔF508 CFTR CF patient (33). CFT1-LCFSN cells are the same CFT1 clone stably transduced with normal CFTR (18). The cells were grown in serum-free Ham’s F-12 medium supplemented with seven additives: 5 μg/ml of insulin, 3.7 μg/ml of endothelial cell growth supplement, 25 ng/ml of epidermal growth factor, 5 mM triiodothyronine, 3 × 10⁻⁶ M triiodothyronine, and 10 ng/ml of cholera toxin. The cells were cultured in collagen-coated flasks for growth and collagen-coated permeable inserts (Becton Dickinson, Bedford, MA) for treatments at 37°C in 5% CO₂. Before being seeded on the permeable membrane, the cells were passaged once in Ham’s F-12 medium without cholera toxin.

For the Ussing chamber experiments, 3.3 × 10⁴ cells were seeded on 0.3-cm² permeable inserts. Ham’s F-12 medium without cholera toxin was added to the inner (apical side; 67 μl) and outer (basolateral side; 50 μl) wells. After 24 h, the cells were exposed to a 1:1 medium mixture of Ham’s F-12 medium without cholera toxin and NIH/3T3 cell-conditioned DMEM containing 2% fetal bovine serum. Transepithelial resistance was measured to monitor the development of tight junctions.

For GSH efflux experiments, 5 × 10⁵ cells were seeded on 4.2-cm² permeable inserts. Ham’s F-12 medium without cholera toxin was added to the apical (1 ml) and basolateral (2 ml) sides, respectively, and replaced with the 1:1 medium mixture the next day.

Short-circuit current measurements. Confluent cell monolayers were mounted in Ussing chambers (Jim’s Instrument, Iowa City, IA) and bathed with symmetrical Ringer solution containing 2.5 mM K₂HPO₄, 2.0 mM CaCl₂, 1.2 mM MgSO₄, 5 mM D-glucose, 5 mM sodium acetate, 6 mM L-alanine, 1 mM sodium citrate, 115 mM NaCl, 4 mM sodium lactate, 0.5 mM n-butyril acid, 20 mM NaHCO₃, and 14.1 mM raffinose (pH 7.4). The temperature was maintained at 37°C, and the solutions in both compartments were equilibrated with 5% CO₂. Short-circuit currents (Isc) were measured by a voltage clamp (Physiologic Instruments, San Diego, CA) as previously described (30). Initially, 10 or 100 μM amiloride was added to the apical solution to inhibit activity of the epithelial sodium channel. Then, different concentrations of the peptide N-K₄-M2GlyR were applied to the apical side followed 50–80 min later by the addition of 500 μM chlorzoxazone to both sides. Chlorzoxazone is a basolateral K⁺ channel activator with a structure and activity similar to 1-ethyl-2-benzimidazoladoline (6, 26). Both drugs increase the driving force for Cl⁻ secretion across the epithelia (6, 26).

Intracellular and extracellular GSH measurements with HPLC. Experiments were performed after the cell monolayers developed a maximum transepithelial resistance (≈7–9 days). To examine the effect of the peptide on GSH secretion, the medium was replaced with 0.5 and 1.5 ml of Ringer solution to the apical and basolateral sides, respectively. Acivicin (0.2 mg/ml) was added to the Ringer solution to inhibit extracellular GSH degradation by γ-glutamyl transpeptidase. The channel-forming peptide was added to the apical solution, and where indicated, 500 μM chlorzoxazone was added to both sides. The cells were incubated for 4 h at 37°C in 5% CO₂.

To determine whether Cl⁻ channel inhibitors affected GSH efflux, 100 μM glibenclamide or 4,4′-difontrostilbene-2,2′-dinitrostilbene (DNDS; Pfaltz & Bauer, Waterbury, CT) was added to both sides of the cell monolayers. Glibenclamide and DNDS block numerous Cl⁻ channels including CFTR. However, DNDS is a poor CFTR channel blocker under physiological conditions because access to its binding site on the cytosolic side of CFTR is limited by its impermeability (15). Glibenclamide was dissolved in dimethyl sulfoxide (DMSO), and DMSO was maintained at 0.1% in both the treatment and control groups. Krebs-Ringer phosphate buffer containing 144 mM NaCl, 5 mM KCl, 8.5 mM Na₂HPO₄, 1.4 mM NaH₂PO₄, 1.3 mM CaCl₂, 1.0 mM MgSO₄, 5 mM glucose, and 10.0 mM HEPES (pH 7.4) plus 0.2 μg/ml of acivicin was used for the inhibitor studies. The cells were treated for 4 h at 37°C without CO₂.

At the end of the incubation, intracellular and extracellular GSH contents were determined. The apical and basolateral fluids (0.5 ml) were collected and centrifuged to spin down any detached cells. The cell monolayers were washed once with ice-cold phosphate-buffered saline and scrapped into 10% (vol/vol) perchloric acid containing 10 μM γ-glutamyl-glutamic acid (GGA; Bachem, Torrance, CA) as an internal standard. Perchloric acid (70%) containing GGA was added
to the collected apical and basolateral fluids to make a final concentration of 10% perchloric acid and 10 μM GGA. The protein was precipitated by centrifugation. The protein concentration was measured with a bicinchoninic acid protein assay kit (Pierce, Rockford, IL). GSH and glutathione disulfide (GSSG) contents in the supernatant were determined by derivatization with 2,4-dinitrofluorobenzene and high-performance liquid chromatography (HPLC) as previously described by Fariss and Reed (7). Total GSH content (GSH + 2GSSG) was calculated and is presented as nanomoles per milligram of cellular protein.

Statistical analysis. Data are expressed as means ± SE and were evaluated by one-way analysis of variance followed by Tukey’s test. \( P < 0.05 \) was considered significant.

RESULTS

Elevation of \( I_{sc} \) in CF cells with a Cl\(^-\) channel-forming peptide and a K\(^+\) channel activator. We investigated whether Cl\(^-\) secretion was improved in CF cells by treatment with a Cl\(^-\) channel-forming peptide (N-K\(_4\)-M2GlyR) (28). CFT1 cells formed resistive monolayers (211 ± 24 Ω·cm\(^2\)) with a basal \( I_{sc} \) of 6.0 ± 0.4 μA/cm\(^2\) in ~7–9 days (\( n = 30 \) experiments). The effects of N-K\(_4\)-M2GlyR and other agents on \( I_{sc} \) are shown in Fig. 1. The addition of amiloride, an inhibitor of the epithelial Na\(^+\) channel, to the apical bathing solution abolished the basal \( I_{sc} \). Subsequent addition of the peptide N-K\(_4\)-M2GlyR caused \( I_{sc} \) to increase slightly over 40–60 min. However, the addition of 500 μM chlorzoxazone, a K\(^+\) channel activator, ~50–80 min after the peptide treatment evoked a rapid elevation in \( I_{sc} \) (\( P \leq 0.001 \)). Chlorzoxazone alone increased \( I_{sc} \) slightly. The marked increase in \( I_{sc} \) caused by N-K\(_4\)-M2GlyR plus chlorzoxazone was abolished by the addition of 300 μM bumetanide, a blocker of the Na-K-2Cl cotransporter, to both sides of the monolayer. This suggests that the \( I_{sc} \) is mediated by Cl\(^-\) secretion. Dose-response data for N-K\(_4\)-M2GlyR plus 500 μM chlorzoxazone are shown in Fig. 2. Another Cl\(^-\) channel-forming peptide, C-K\(_4\)-M2GlyR (0.5 mM), plus chlorzoxazone (0.5 mM) also increased \( I_{sc} \) but was less potent than N-K\(_4\)-M2GlyR plus chlorzoxazone (3.17 ± 0.50 vs. 10.50 ± 1.98 μA/cm\(^2\)). This difference in activity is consistent with previous observations (2). These data indicate that the synthetic channel-forming peptide plus chlorzoxazone elicits a Cl\(^-\)-secretory response across cultured CF airway cells.

Restoration of GSH secretion in CF cells by a Cl\(^-\) channel-forming peptide and a K\(^+\) channel activator. Next, we investigated whether GSH release was related to the addition of agents that restore Cl\(^-\) secretion across CF cells. The rate of efflux was assayed by measuring GSH in the apical solution after a 4-h incubation period (Fig. 3). The addition of 0.5 mM N-K\(_4\)-M2GlyR alone increased GSH secretion (181 ± 8% of the control value; \( P < 0.05 \)), whereas the addition of chlorzoxazone alone did not significantly affect GSH efflux in CFT1 cells. However, chlorzoxazone potentiated the effect of the peptide, causing, for instance, an increase in GSH release of 359 ± 16% of the control value with a concentration of N-K\(_4\)-M2GlyR of 0.5 mM (\( P < 0.001 \)). We tested a second peptide, C-K\(_4\)-M2GlyR, for activity and observed that it alone also enhanced GSH secretion but less potently than N-K\(_4\)-M2GlyR alone (1 mM each peptide; 175 ± 9 vs. 252 ± 10% of control values). Thus C-K\(_4\)-M2GlyR has decreased efficacy compared with N-K\(_4\)-M2GlyR in both \( I_{sc} \) and GSH efflux assays. The addition of 300 μM 1-ethyl-2-benzimidazolinone, another K\(^+\) channel activator, also

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**Fig. 1.** Addition of the Cl\(^-\) channel-forming peptide N-K\(_4\)-M2GlyR plus chlorzoxazone increases short-circuit current (\( I_{sc} \)) across cystic fibrosis airway cells. Monolayers of CFT1 cells were mounted in Ussing chambers bathed with symmetrical Ringer solution and voltage clamped. Amiloride (100 μM) and N-K\(_4\)-M2GlyR (250 μM) were added to the apical bathing solution. Subsequently, chlorzoxazone (500 μM) and bumetanide (300 μM) were added to both baths. All compounds were present continuously after addition. Results are from a representative experiment (\( n = 5 \)).

**Fig. 2.** Concentration-response relations for \( I_{sc} \) after addition of the peptide N-K\(_4\)-M2GlyR (●) without (−) and with (+) chlorzoxazone (500 μM; ○) to CFT1 monolayers. These experiments were designed and performed as illustrated by the representative trace in Fig. 1. Amiloride was added to abolish the \( I_{sc} \) due to Na\(^+\) absorption. N-K\(_4\)-M2GlyR was then added to the apical side. Paired \( I_{sc} \) measurements were obtained both before and after addition of chlorzoxazone to both sides of the monolayers. *Significant difference compared with control group without peptide and chlorzoxazone treatment, \( P < 0.05 \).
amplified the effect of N-K4-M2GlyR (0.5 mM) on GSH efflux (286 ± 1 vs. 181 ± 8% of control values). As shown in Fig. 4, intracellular GSH content was reduced as the concentration of N-K4-M2GlyR increased with the addition of chlorzoxazone. Therefore, our data suggest that treatment with N-K4-M2GlyR in combination with chlorzoxazone corrects the defect of GSH efflux in CFT1 cells by stimulation of Cl− secretion.

To ascertain whether the peptide itself might allow GSH efflux, the peptide N-K4-M2GlyR (0-500 μM) was added to a 1:2 dilution of liposomes (~5 mM; 22.5 wt% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 10 wt% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine, and 67.5 wt% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine) containing 2 mM GSH in 100 mM NaCl, 100 mM sucrose, and 10 mM HEPES, pH 7.2, and incubated for 1 h at 25°C. The peptide appeared to be incapable of transporting GSH, which could, however, be released from the liposomes by detergent treatment.

**Blockade of GSH efflux in CF and non-CF cells by Cl− channel inhibitors.** To further determine the relationship between GSH efflux and Cl− transport, CFTR-sufficient cells (CFT1-LCFSN) and CF cells (CFT1) were treated with the Cl− channel inhibitors glibenclamide and DNDS, and GSH efflux was measured (Table 1). Glibenclamide significantly reduced GSH efflux in CFT1-LCFSN cells (58 ± 3% of the control value; P < 0.001), whereas DNDS had no effect. In contrast, neither glibenclamide nor DNDS altered GSH efflux from CFT1 cells. The ability of glibenclamide to selectively reduce GSH efflux from cells expressing wild-type CFTR provides further evidence that GSH and Cl− secretion are closely linked. These data also indicate that airway epithelial cells possess both Cl− secretion-dependent (i.e., glibenclamide-sensitive) and independent (glibenclamide-insensitive) GSH efflux pathways.

**DISCUSSION**

CF is characterized by oxidative stress and chronic inflammation in the respiratory tract. Decreased levels of GSH in the apical fluid from CF airway cells (10) and CF patients (24) may increase the susceptibility of the airway to oxidative damage during chronic inflammation. Therefore, the possibility of restoring the apical GSH content to protect the integrity of the lung epithelium in CF patients is of great interest. We explored whether administration of pharmacological agents causing an increase in Cl− secretion would also enhance GSH secretion.

**Table 1.** Comparison of Cl− channel inhibitors on glutathione efflux from CFT1 and CFT1-LCFSN cells

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<tr>
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<th>Total Apical Glutathione</th>
<th>Control</th>
<th>Glibenclamide</th>
<th>DNDS</th>
</tr>
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<tbody>
<tr>
<td>CFT1-LCFSN</td>
<td>100.0 ± 0.0</td>
<td>58.4 ± 3.0*</td>
<td>109.0 ± 7.2</td>
<td></td>
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<tr>
<td>CFT1</td>
<td>30.5 ± 0.0</td>
<td>29.6 ± 1.4</td>
<td>28.9 ± 4.3</td>
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Values are means ± SE expressed as percentage of control value for CFT1 cells transected with the normal cystic fibrosis transmembrane conductance regular gene (CFT1-LCFSN). Cells on filters were treated with 100 μM glibenclamide or 4,4'-dinitrostilbene-2,2'-disulfonic acid (DNDS) added to the both apical and basolateral sides for 4 h. Antioxidant glutathione (GSH) and glutathione disulfide (GSSG) were assayed by HPLC as described previously (7). Total apical glutathione was 7.99 ± 1.12 nmol/mg cellular protein in the control group of CFT1-LCFSN cells. *Significant difference compared with the control group of CFT1-LCFSN cells, P < 0.05.
One approach to stimulate Cl\(^-\) secretion is the use of artificial Cl\(^-\) channels (28, 32). In the present study, the N-K\(_4\)-M2GlyR peptide enhanced Cl\(^-\) secretion when added to CFT1 cells together with chlorzoxazone. This is in agreement with earlier findings by Broughman et al. (2). Chlorzoxazone itself had a small effect on I\(_{sc}\) across CFT1 cells. An earlier study (26) showed that chlorzoxazone stimulates transepithelial Cl\(^-\) transport by activation of basolateral K\(^+\) channels in normal airway epithelium but not in the nasal epithelium of CF patients, suggesting that apical Cl\(^-\)-permeable pathways are necessary for the chlorzoxazone effect. Amiloride was used to confirm that the peptide-mediated current was carried by Cl\(^-\) instead of Na\(^+\). Although it is demonstrated here that N-K\(_4\)-M2GlyR peptide formed anion channels permeable to Cl\(^-\), it does not rule out the possibility of the permeability of other anions, including HCO\(_3\)\(^-\), through the N-K\(_4\)-M2GlyR peptide. The lack of effect of peptide alone on Cl\(^-\) permeability suggests that the basolateral membrane is the rate-limiting factor for Cl\(^-\) secretion across CFT1 cells. This is supported by an earlier study (6) that showed that activation of basolateral K\(^+\) channels is necessary for the Cl\(^-\)-secretory response in T-84 cells.

N-K\(_4\)-M2GlyR alone increased GSH efflux in CFT1 cells, which was further enhanced by the addition of chlorzoxazone (Fig. 3). The effect of the peptide on GSH release is dose dependent. The dose-response curves of apical GSH content and I\(_{sc}\) follow similar patterns but do not correlate perfectly. N-K\(_4\)-M2GlyR alone did not significantly affect I\(_{sc}\) but caused GSH release. Because I\(_{sc}\) indicates transepithelial ion transport, whereas GSH is released from inside the cells to the apical space, perhaps the peptide alone may slightly increase the Cl\(^-\) permeability across the apical membrane. However, because the basolateral membrane is the rate-limiting factor, changes in I\(_{sc}\) are not significant without the addition of chlorzoxazone to increase K\(^+\) transport across the basolateral membrane. On the other hand, a small increase in Cl\(^-\) permeability across the apical membrane might be sufficient to cause some elevation in GSH secretion. When chlorzoxazone was added, it provided a driving force, causing more Cl\(^-\) to be secreted across the apical membrane and a significantly enhanced GSH release. N-K\(_4\)-M2GlyR (0.5 mM) plus chlorzoxazone increased GSH efflux ~3.6-fold, which is comparable to the apical GSH content in CFTR-sufficient cells (CFT1-LCFSN). This indicates that by increasing Cl\(^-\) secretion, the defect of GSH secretion could be overcome in CF cells. As the concentration of the peptide increased, more intracellular GSH was released to the extracellular space (Fig. 4).

Although the identity of the GSH transporter is not presently known, the studies here provide some insight into the relationship between Cl\(^-\) transport and GSH efflux. The inability of N-K\(_4\)-M2GlyR to release GSH in liposomes suggests that N-K\(_4\)-M2GlyR itself does not function as a GSH transporter. GSH has a net negative charge, and transport was shown to be dependent on the membrane potential in hepatocytes and renal plasma membranes (8, 11, 14). However, our results presented here cannot be explained solely by changes in potential difference. Chlorzoxazone increases the basolateral K\(^+\) permeability in CFT1 cells (data not shown), which causes the membrane potential to hyperpolarize. On the other hand, Cl\(^-\) is initially accumulated in CFT1 cells via the bumetanide-sensitive Na-K-2Cl cotransporter in the absence of CFTR (Fig. 1). The addition of N-K\(_4\)-M2GlyR allows more Cl\(^-\) to exit, thereby depolarizing the membrane potential. However, chlorzoxazone amplified the effect of N-K\(_4\)-M2GlyR on GSH efflux. Because chlorzoxazone and N-K\(_4\)-M2GlyR have opposing effects, it is unlikely that GSH releases solely by changing the membrane potential. Rather, our data suggest that GSH efflux is generally related to Cl\(^-\) efflux. Specifically, apical anion permeability is essential (i.e., N-K\(_4\)-M2GlyR or CFTR), and the membrane potential plays an important although secondary role. Changes in intracellular potential alone are not sufficient to affect GSH efflux because chlorzoxazone itself did not affect GSH release. However, in the presence of an apical Cl\(^-\)-secretory pathway (N-K\(_4\)-M2GlyR), changes in membrane potential (chlorzoxazone) altered the GSH release as shown in Fig. 3. Although the intracellular potential may be quickly restored after perturbation, it is possible that N-K\(_4\)-M2GlyR in combination with chlorzoxazone may have changed the intracellular potential substantially and maintained it at that level under our experimental conditions.

An earlier study (16) suggested that CFTR conducts GSH based on an increased current as measured with the patch-clamp technique with the addition of GSH; however, no definitive evidence has shown that the CFTR protein itself transports GSH. The activity of CFTR itself is believed to mediate the activity of other ion channels and enzymes (1, 12) and to influence fetal development (5, 13) as well; however, as with the
transport of GSH, the underlying mechanisms of these regulatory events are a relatively new but intense area of investigation. With the use of the Cl⁻ channel-forming peptide N-K₄-M2GlyR, the CFTR-dependent Cl⁻-permeable pathway was bypassed in the present study. Therefore, our results suggest that GSH release is associated with Cl⁻ permeability rather than with the CFTR protein per se. Further studies are necessary to characterize the specific protein(s) that transport GSH.

Glibenclamide, an inhibitor of CFTR activity, inhibited GSH efflux in CFTR-sufficient cells (CFT1-LCFSN) but not in CFTR-deficient cells (CFT1). CFT1-LCFSN cells treated with glibenclamide are similar to CFT1 cells because they are CFTR defective. This is in agreement with the earlier findings by Gao et al. (10) that GSH release is decreased in CFTR-defective cells. This provides further evidence that GSH and Cl⁻ secretion are closely linked. DNDS is a Cl⁻ channel inhibitor that is a very poor CFTR blocker under physiological conditions. The lack of effect of DNDS on GSH release in CFT1-LCFSN cells suggests that CFTR is the major Cl⁻ channel in these cells and that the non-CFTR-dependent Cl⁻ transport in this cell type is absent or very small. The lack of effect of glibenclamide in CFT1 cells suggests that the residual GSH secretion in these cells is CFTR independent. Because DNDS did not make any difference in GSH release from CFT1 cells, endogenous non-CFTR-dependent Cl⁻ transport appeared insufficient to affect GSH transport in CF cells. Taken together, these results indicate that there are Cl⁻ transport-dependent and -independent pathways for GSH efflux in airway epithelial cells.

A diagram of predicted GSH efflux pathways based on the results from the present study is shown in Fig. 5. In normal airway epithelial cells, CFTR provides the major Cl⁻-secretory pathway. Both Cl⁻-dependent and -independent GSH transports are functioning. MRP is a potential candidate for GSH transporter (21, 34), although independent GSH transports are functioning. MRP is a potential candidate for GSH transport. In the present study, the release in CFT1-LCFSN cells suggests that CFTR is a major Cl⁻-secretory pathway. This restores the function of the Cl⁻-transport-dependent GSH transport system. When the Cl⁻ channel-forming peptide N-K₄-M2GlyR is applied to the apical side, it forms another Cl⁻-secretory pathway. This restores the transport of GSH, the underlying mechanisms of these regulatory events are a relatively new but intense area of investigation. With the use of the Cl⁻ channel-forming peptide N-K₄-M2GlyR, the CFTR-dependent Cl⁻-permeable pathway was bypassed in the present study. Therefore, our results suggest that GSH release is associated with Cl⁻ permeability rather than with the CFTR protein per se. Further studies are necessary to characterize the specific protein(s) that transport GSH. Glibenclamide, an inhibitor of CFTR activity, inhibited GSH efflux in CFTR-sufficient cells (CFT1-LCFSN) but not in CFTR-deficient cells (CFT1). CFT1-LCFSN cells treated with glibenclamide are similar to CFT1 cells because they are CFTR defective. This is in agreement with the earlier findings by Gao et al. (10) that GSH release is decreased in CFTR-defective cells. This provides further evidence that GSH and Cl⁻ secretion are closely linked. DNDS is a Cl⁻ channel inhibitor that is a very poor CFTR blocker under physiological conditions. The lack of effect of DNDS on GSH release in CFT1-LCFSN cells suggests that CFTR is the major Cl⁻ channel in these cells and that the non-CFTR-dependent Cl⁻ transport in this cell type is absent or very small. The lack of effect of glibenclamide in CFT1 cells suggests that the residual GSH secretion in these cells is CFTR independent. Because DNDS did not make any difference in GSH release from CFT1 cells, endogenous non-CFTR-dependent Cl⁻ transport appeared insufficient to affect GSH transport in CF cells. Taken together, these results indicate that there are Cl⁻ transport-dependent and -independent pathways for GSH efflux in airway epithelial cells.

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In CF airway epithelial cells, CFTR-dependent Cl⁻ transport pathway is blocked. This inhibited Cl⁻ transport-dependent GSH transport because CFTR is the major pathway for Cl⁻ transport. GSH is released to the apical fluid only by a Cl⁻-transport-independent system. Apical GSH concentration is decreased. Although the GSH concentration gradient force favors more GSH to be released to the apical fluid from CF cells compared with normal cells, GSH cannot be secreted to the apical fluid due to the defect in the Cl⁻ transport-dependent GSH transport system.

REFERENCES


