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JH Poulsen, H Fischer, B Illek, and TE Machen

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Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator

JØRGEN HEDEMARK POULSEN*, HORST FISCHER, BEATE ILLEK, AND TERRY E. MACHEN†

Department of Molecular and Cell Biology, Division of Cell and Developmental Biology, 231 Life Science Addition, University of California, Berkeley, CA 94720

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ABSTRACT **The cystic fibrosis transmembrane conductance regulator (CFTR) is an epithelial Cl^- channel regulated by protein kinase A. The most common mutation in cystic fibrosis (CF), deletion of Phe-508 (ΔF508-CFTR), reduces Cl^- secretion, but the fatal consequences of CF have been difficult to rationalize solely in terms of this defect. The aim of this study was to determine the role of CFTR in HCO_3^- transport across cell membranes. HCO_3^- permeability was assessed from measurements of intracellular pH [pH_i; from spectrofluorimetry of the pH-sensitive dye 2',7'-bis(2-carboxyethyl)-5-(and -6)carboxyfluorescein] and of channel activity (patch clamp; cell attached and isolated, inside-out patches) on NIH 3T3 fibroblasts and C127 mammary epithelial cells transfected with wild-type CFTR (WT-CFTR) or ΔF508-CFTR, and also on mock-transfected cells. When WT-CFTR-transfected cells were acidified (pulsed with NH_4Cl) and incubated in Na^+-free (*N*-methyl-D-glucamine substitution) solutions (to block Na^+-dependent pH_i regulatory mechanisms), pH_i remained acidic (pH ≈ 6.5) until the cells were treated with 20 μM forskolin (increases cellular [cAMP]); pH_i then increased toward (but not completely to) control level (pH_i 7.2) at a rate of 0.055 pH unit/min. Forskolin had no effect on rate of pH_i recovery in ΔF508 and mock-transfected cells. This Na^+-independent, forskolin-dependent pH_i recovery was not observed in HCO_3^-/CO_2-free medium. Forskolin-treated WT-CFTR-transfected (but not ΔF508-CFTR or mock-transfected) cells in Cl^--containing, HCO_3^--free solutions showed Cl^- channels with a linear *I/V* relationship and a conductance of 10.4 ± 0.5 pS in symmetrical 150 mM Cl^-. When channels were incubated with different [Cl^-] and [HCO_3^-] on the inside and outside, the Cl^-/HCO_3^- permeability ratio (determined from reversal potentials of *I/V* curves) was 3.8 ± 1.0 (mean ± SEM; *n* = 9); the ratio of conductances was 3.9 ± 0.5 (at 150 mM Cl^- and 127 mM HCO_3^-. We conclude that in acidified cells the WT-CFTR functions as a base loader by allowing a cAMP-dependent influx of HCO_3^- through channels that conduct HCO_3^- about one-quarter as efficiently as it conducts Cl^-. Under physiological conditions, the electrochemical gradients for both Cl^- and HCO_3^- are directed outward, so CFTR likely contributes to the epithelial secretion of both ions. HCO_3^- secretion may be important for controlling pH of the luminal, but probably not the cytoplasmic, fluid in CFTR-containing epithelia. In CF, a decreased secretion of HCO_3^- may lead to decreased pH of the luminal fluid.**

The cystic fibrosis transmembrane conductance regulator (CFTR) is an epithelial Cl^- channel regulated by cAMP-dependent protein kinase A (1–3). The most common mutation in CF is deletion of Phe-508 of the CFTR (ΔF508-CFTR), which reduces the ability of cells to secrete Cl^- (4). However, the fatal consequences in CF have been difficult to rationalize solely as a defect in Cl^- secretion (5, 6). If the CFTR were also conductive to HCO_3^-, then movements of HCO_3^- might affect the pH of the luminal solution of some epithelia. For example, in the lungs, where the apical fluid layer is small and CO_2 levels are kept low by intermittent exposure to atmospheric air, the amount of HCO_3^- secreted across the airway epithelia could have important effects on the pH of this fluid. It might further be expected that in CF, when the ΔF508-CFTR seems to be prevented from reaching its proper apical membrane location (7) thereby reducing Cl^- permeability (4), HCO_3^- secretion would also be reduced, and pH of the luminal fluid might be lower than normal.

Previous work has given indications that the CFTR might be permeable to HCO_3^-. Smith and Welsh (8) showed that human and canine airway epithelial cells incubated in Cl^--free, HCO_3^--containing solutions exhibited cAMP-regulated ionic currents that were inhibited by acetazolamide and diphenylamine carboxylate and were absent in cells isolated from CF individuals. Smith and Welsh (9) also found recently that cultured human airway epithelia secrete acid; cAMP causes a reduction in this acid secretion, and the "luminal" solution becomes more alkaline. Gray, Argent and coworkers (10) found that the small, linear anion channel present in the apical membranes of rat pancreatic ducts (presumably the CFTR) was permeant to both Cl^- and HCO_3^- in the ratio HCO_3^-/Cl^- = 0.13:1. Also, the outwardly rectifying, depolarization-induced Cl^- channel, which has been found to be regulated abnormally in CF cells (11–13) as well as in airway cells isolated from CF "knockout" mice (14), has a HCO_3^- conductance that is ≈1/2 that of Cl^- (15, 16).

We report here that expression of wild-type (WT) CFTR in NIH 3T3 fibroblasts and C127 mammary cells gives rise to a regulator of intracellular pH (pH_i) that is forskolin regulated, Na independent, and HCO_3^- dependent. These WT-CFTR-transfected cells also exhibit a low conductance, cAMP- and protein kinase A-dependent Cl^- channel that has a significant HCO_3^- permeability, with a HCO_3^-/Cl^- permeability ratio of 1:4. The pH_i regulatory capability and the Cl^-- and HCO_3^--permeable channels were not observed in ΔF508 or mock-transfected cells.

MATERIALS AND METHODS

Cell Culture. NIH 3T3 fibroblasts and C127 mammary cells expressing WT-CFTR or ΔF508-CFTR or mock-transfected cells were obtained from William Reenstra (Childrens Hospital, Oakland, CA) and Michael Welsh (University of Iowa, Iowa City), respectively. 3T3 cells were grown using standard cell culturing techniques in Dulbecco's minimal essential (DME) H-21 medium (University of California, San

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Abbreviations: CF, cystic fibrosis; CFTR, CF transmembrane conductance regulator; WT, wild type; pH_i, intracellular pH; NMG, *N*-methyl-D-glucamine.

*Present address: Department of Medical Physiology, University of Copenhagen, The Panum Institute, DK2200 Copenhagen, Denmark.
†To whom reprint requests should be addressed.

Francisco, Cell Culture Facility) supplemented with 10% newborn calf serum and were passaged every 5 days in a 1:10 fashion. C127 cells were grown in a 50:50 mixture of DME H-16 and F-12 supplemented with 10% fetal calf serum. Both media contained 100 units of penicillin per ml and 0.1 mg of streptomycin per ml. Cells were used 1–3 days after seeding. As judged by immunoprecipitation with anti-CFTR antibodies on NIH 3T3 cells, WT-CFTR-transfected cells expressed the characteristic "fuzzy" band at 170 kDa (7), and ΔF508-CFTR-transfected cells expressed a less glycosylated protein with a slightly lower apparent molecular mass, similar to what has been reported previously (7). The expression of the CFTR in WT-CFTR-transfected cells was stable over a wide range of passages, but the ΔF508-CFTR-transfected cells lost the transfection after 14 passages, since there was no detectable protein after this time. No immunoprecipitable protein was detected in the mock-transfected cells.

Measurements of pH_i. The fluorescent pH indicator 2',7'-bis(2-carboxyethyl)-5-(and -6)carboxyfluorescein (BCECF) (Molecular Probes) was used to measure pH_i as described in detail previously (17). Briefly, cells grown on cover glasses were incubated with 2–4 μM BCECF acetoxymethyl ester in cultured medium for 20–30 min at room temperature. Cells were then washed and allowed to recover for 30–45 min before mounting in a heated (37°C) flow-through chamber (turnover time, ≈6 s) that was placed on the stage of an inverted microscope. pH_i was then measured with a digital imaging microscope or a photomultiplier-based system. In each case, dye-loaded cells were excited alternately (once every s) with 440-nm and 490-nm light using a filter wheel. Emitted fluorescent light (>520 nm) from single cells was collected by the photomultiplier or the television camera and image processor.

The NaCl/HCO_3^- Ringer's solution used for the pH_i measurements contained 120 mM NaCl, 25 mM $NaHCO_3$, 2.5 mM K_2HPO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 10 mM Hepes, 5.5 mM glucose (pH 7.4) equilibrated with 5% CO_2/95% O_2. In Na^+-free (Na^+-free/HCO_3^-) solutions, Na^+ was replaced with *N*-methyl-D-glucamine (NMG). In HCO_3^--free solutions, $NaHCO_3$ was replaced with NaCl (in NaCl/Hepes solution) or with NMGCl (in Na^+-free/Hepes solution); these solutions were equilibrated with air. In experiments in which cells were acidified, 30 mM NH_4Cl replaced an equal amount of NMGCl.

The ratio of the BCECF fluorescence emitted from dye-loaded cells was calibrated in terms of pH_i by incubating the cells in a high [K^+] solution (KCl replaced NaCl in the NaCl/Hepes solution) and then permeabilizing the cells with 10 μM nigericin to equilibrate pH_i and pH_o (extracellular pH). Then the pH of the bathing solution was stepped between pH 6.3 and 7.4. The 490/440 ratio was linear over this pH range.

Relative rates of HCO_3^- permeation in the WT-CFTR-, ΔF508-CFTR-, and mock-transfected cells were assessed from the relative rates of pH_i recovery from acid loads. Cells were first perfused with either the NaCl/HCO_3^- or NaCl/Hepes Ringer's in the chamber for 15 min to allow pH_i to stabilize. Then the cells were switched to Na^+-free Ringer's (either Na^+-free/HCO_3^- or Na^+-free/Hepes, depending on the experiment) for 3–5 min to remove Na^+ from the cells and prevent any contribution of Na^+-dependent pH_i regulatory mechanisms (18–22). The cells were then treated with the NH_4-containing solution for 5 min, and when the NH_4-containing solution was removed, cells were acidified to pH_i 6.4–6.8. Rates of pH_i recovery after treatment with forskolin were calculated by linear regression analysis between pH_i 6.4 and 6.8.

Analysis of Single-Channel Conductance and *I/V* Characteristics. Single-channel currents were recorded as described (23, 24) from NIH 3T3 fibroblast cells transfected with the WT-CFTR. Measurements were performed in a thermostated (37°C), continuously perfused chamber on the stage of a microscope. Patch pipettes were sealed onto unstimulated cells and then the bath was perfused with 10 μM forskolin to verify the cAMP sensitivity of the channels. After excision of the patched membrane, catalytic subunit of protein kinase A (75 nM) and MgATP (3 mM; both from Sigma) were present in the respective bath solutions (i.e., the intracellular face of the CFTR). For the patch clamp experiments, NaCl Ringer's solution contained 141 mM NaCl, 4 mM KCl, 1.7 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM KH_2PO_4, 10 mM Hepes, 25 mM glucose (pH 7.4). Cl^- solution contained: 147 mM NMGCl, 1.7 mM $CaCl_2$, 10 mM Hepes, 25 mM glucose (pH 7.4), nominally HCO_3^--free; air equilibrated solutions contained 0.158 mM HCO_3^- as calculated from the partial pressure of CO_2 in air (0.03%), its solubility coefficient (0.03 mM/mmHg), and its dissociation constant (pK_a 6.1). HCO_3^- solution contained 127 mM $NaHCO_3$, 20 mM NaCl, 35 mM glucose, continuously gassed with 30% CO_2/70% air, pH 7.4. This HCO_3^- solution contained 20 mM Cl^- so that the electrode potential remained stable. When HCO_3^- solution was used in the bath, the perfusion chamber was kept under a 30% CO_2/70% air atmosphere to maintain pH and HCO_3^- concentration; when HCO_3^- solution was used in the pipette, the solution was gassed with 30% CO_2/70% air until it was used for filling. Potentials are reported as negative pipette potentials. Electrode potentials occurring with $NaHCO_3$ in the pipette of, on average, −27.5 ± 0.7 mV were appropriately zeroed. No other corrections were applied. Liquid junction potentials for nonsymmetrical conditions can be calculated to be +11 mV for $NaHCO_3$ solution in the pipette and NMGCl solution in the bath (25) but are likely smaller, since we did not observe a significant difference of the absolute measured reversal potentials with inverse solutions (see Fig. 4*d*). For display, recordings were filtered at 25 Hz (see Fig. 4*a*) or 50 Hz (see Fig. 4*b*). Single-channel current–voltage relations recorded with Cl^- solution on one side and HCO_3^- solution on the other side of the patched membrane were analyzed by fitting the sum of Goldman equations (26, 27) for Cl^- and for HCO_3^- of the form

$$i_x = P_x(F^2/RT)V\frac{X_c\exp(-FV/RT) - X_p}{\exp(-FV/RT) - 1} \quad [1]$$

to the data (where x is Cl^- or HCO_3^-) in order to estimate the permeability ratio P_{Cl}/P_{HCO_3}. *i* is the single channel current; *F*, *R*, and *T* have their usual meanings; *V* is the applied potential (referring to the cell interior with respect to the pipette); and X_c and X_p are the cytosolic and pipette concentrations of Cl^- or HCO_3^-. The respective conductances of the channel for Cl^- and HCO_3^- (g_x) were calculated as the slope of the fitted Goldman equations in the upper and lower linear range (i.e., at +100 and −100 mV).

RESULTS AND DISCUSSION

Typical experiments in which the pH_i regulatory capability of the CFTR was investigated are shown in Fig. 1. When WT-CFTR-transfected cells were bathed in NaCl/HCO_3^- solution, pH_i was 7.21 ± 0.04 (n = 20) (mean ± SEM). To test for the ability of HCO_3^- to permeate the CFTR, cells were first treated with Na^+-free HCO_3^- solution to block the action of Na^+-dependent pH_i regulatory mechanisms. As shown in Fig. 1*a*, this usually had only a small effect on pH_i. The cells were then acidified with an NH_4 prepulse, still in Na^+-free HCO_3^- solution, and no pH_i recovery occurred until NaCl/HCO_3^- solution was returned to the bath.

In contrast, if the acidified WT-CFTR-transfected cells were treated with 20 μM forskolin (to increase cellular [cAMP]), still in Na^+-free HCO_3^- solution, pH_i began to

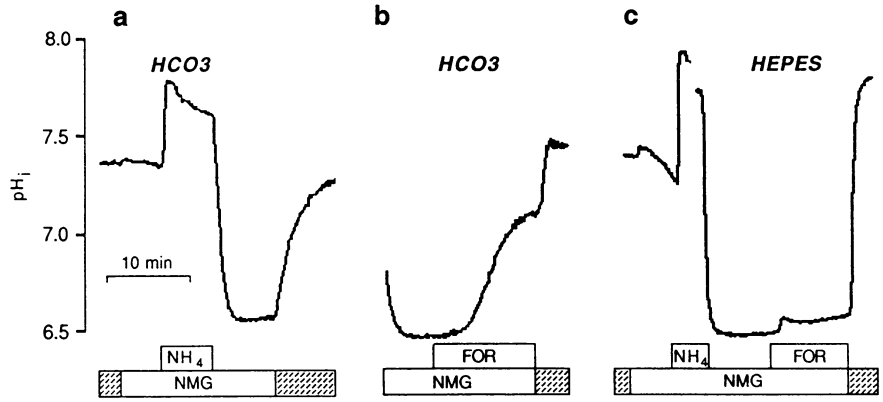

FIG. 1. Forskolin-induced pH_i regulation of NIH 3T3 cells expressing WT-CFTR. (*a*) Treatment of a cell with 30 mM NH_4 in Na^+-free/HCO_3^- solution (NMG) caused pH_i first to increase (due to the entry of the weak base NH_3) and then to decrease when the NH_4 was washed from the bath. The cell remained acidic in the Na^+-free/HCO_3^-, but pH_i recovered quickly when cells were returned to NaCl/HCO_3^- Ringer's solution (hatched bars), likely due to the operation of Na^+/H^+ exchange and other Na^+- and HCO_3^--dependent pH_i regulators. (*b*) When forskolin (FOR) (20 μM) was added to cells that were acidified as in *a* (data not shown) in Na^+-free/HCO_3^- (NMG) solution, pH_i recovered toward the control level, but full recovery occurred only after adding back NaCl/HCO_3^- solution. (*c*) In Hepes-buffered solutions, forskolin had only a very small effect on pH_i in the acidified cells. The break in the record occurred when the excitation light was turned off to reduce exposure and resulting oxidative damage. Traces were measured on single cells and are typical of 3–13 experiments.

recover after a short lag period, and pH_i recovery continued up to a plateau value of $\approx$7.0 (Fig. 1*b*). When these cells were finally returned to NaCl/HCO_3^- solution, pH_i returned to the control pH_i. To test whether the forskolin-activated, Na^+-independent recovery of pH_i was also HCO_3^--dependent, we repeated these experiments in HCO_3^--free, Hepes-buffered solutions. As shown in Fig. 1*c*, under these HCO_3^--free conditions the acidified cells responded to forskolin with a very slight increase in pH_i, and a sustained pH_i recovery occurred when Na^+ was present in the Na^+/Hepes solution. These findings are consistent with a forskolin-regulated HCO_3^- influx in the presence of extracellular HCO_3^-. In all cases, readdition of extracellular Na^+ allowed further pH_i recovery, probably due to the activity of the Na^+/H^+ exchanger in Hepes-containing solutions and a combination of Na^+/H^+ exchange and Na^+- and HCO_3^--dependent transporters in HCO_3^--containing solutions.

Similar experiments were also performed on ΔF508-CFTR- and mock-transfected cells, and typical results are shown in Fig. 2. When incubated in NaCl/HCO_3^- solutions, pH_i was 7.15 ± 0.07 (17) in ΔF508-CFTR and 6.98 ± 0.06 (2) in mock-transfected cells. When these cells were treated first with Na^+-free/HCO_3^- and then pulsed with Na^+-free/HCO_3^- and NH_4, they acidified and remained acidic even after forskolin treatment. Recovery of pH_i to control level occurred only after the return of NaCl/HCO_3^- solution to the bath. Average rates of pH_i recovery in Na^+-free/HCO_3^- solutions in the presence and absence of forskolin are shown in Fig. 3 for all three cell types. Although the absolute rates of pH_i recovery were lower in the C127 cells (a low-expression system) compared to the NIH 3T3 cells (a high-expression system), qualitatively similar findings were observed; forskolin-regulated pH_i recovery was observed only in WT-CFTR expressing cells, while the ΔF508-CFTR- and mock-transfected cells showed very low rates of recovery, which were insensitive to forskolin treatment.

From this it appears obvious that in intact fibroblasts the WT-CFTR is permeable to HCO_3^- after being activated by cAMP. Cells expressing the ΔF508-CFTR show no comparable pH_i regulation, consistent with the notion that ΔF508-CFTR is not functional in the plasma membrane (7) and/or is regulated abnormally by forskolin (28). A corollary of these results is that HCO_3^- transport through other Na^+-independent pathways seems to be small or nonexistent in all

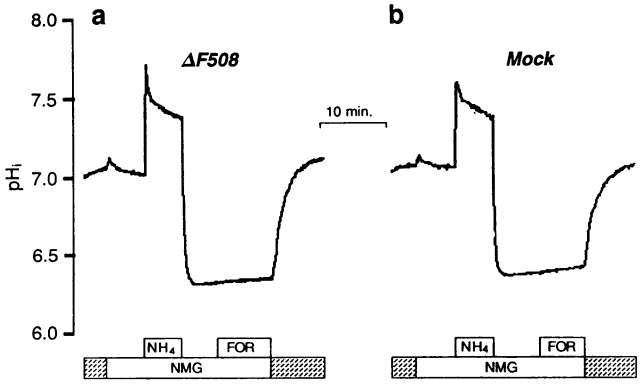

FIG. 2. Lack of effect of forskolin-treatment (FOR) on pH_i regulation in ΔF508-CFTR- (*a*) and mock-transfected (*b*) NIH 3T3 cells and after acidification with an NH_4 prepulse (NH_4). In both cases, cells were incubated first in NaCl/HCO_3^- (hatched bars) and then in Na^+-free/HCO_3^- (NMG) solution and Na^+-free/HCO_3^- (NMG) plus 30 mM NH_4 to acidify the cell. When 20 μM forskolin was added to the acidified cells in Na^+-free/HCO_3^- (NMG) solution, there was no effect on pH_i. Traces were measured on single cells and are typical of five (*a*) and two (*b*) other experiments.

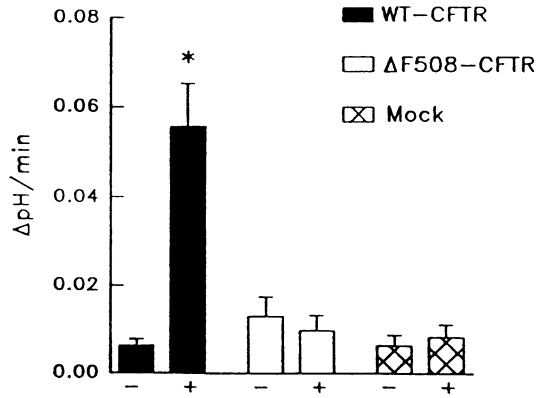

FIG. 3. Average rates of pH_i recovery in Na^+-free/HCO_3^- (NMG) solutions under basal (−) and forskolin-stimulated (+) conditions. Basal rates of pH_i recovery were independent of the expression of the CFTR. Stimulation of WT-CFTR-expressing cells with 20 μM forskolin increased the rates of alkalinization $\approx$9-fold ($P < 0.001$; $n = 14$; paired t test) compared to all other conditions. Forskolin had no effect on rates of pH_i recovery in either ΔF508-CFTR-transfected cells ($n = 6$) or in mock-transfected cells ($n = 3$).

these cells—WT-CFTR, ΔF508-CFTR, and mock transfected. It was noted that the forskolin-activated pH_i recovery was only incomplete (i.e., not back to the baseline), and this likely reflects the fact that HCO_3^- equilibrates across the membrane driven by its electrochemical gradient, and there is a negative cellular membrane potential that opposes complete pH_i recovery. Since pH_i recovered to a point where $pH_i - pH_o \approx 0.4$ pH unit, the membrane potential may have been about −25 mV in the Na^+-free solutions. Although we did not measure membrane potentials here, a previous study (29) has shown that CFTR expression depolarizes cells from −56 (vector transfected) to −24 mV (high CFTR-expressing cells). After forskolin stimulation, the membrane potential likely depolarizes further. These findings are consistent with the data presented here. It is also possible that the cells lose K^+ due to inactivation of the Na^+/K^+-ATPase by the Na^+-free solutions.

These results indicated that the forskolin-treated WT-CFTR is permeable to HCO_3^-, and we wished to determine the relative permeability compared to Cl^-. Preliminary experiments utilizing the Cl^--sensitive dye SPQ (30) indicated that for NIH 3T3 cells the forskolin-stimulated increase in Cl^- permeability was at least 5 times larger than the HCO_3^- permeability. We used the patch clamp technique to obtain more precise estimates of the permeabilities and conductances of the WT-CFTR. Treatment with forskolin stimulated the WT-CFTR in 14 of 20 trials. In similar experiments on mock-transfected cells, forskolin had no effect on channel activity in four trials. A typical recording from a cell-attached patch on a WT-CFTR-transfected NIH 3T3 cell is shown in Fig. 4*a*. This cell was bathed with NaCl/Hepes solution, the pipette contained HCO_3^- Ringer's, and a +100-mV potential was applied. In the unstimulated cells there were no active channels (data not shown), but three or four channels became active in this patch ≈30 s after addition of 10 μM forskolin to the bath. Since the major anion in the pipette was HCO_3^-, a HCO_3^- conductivity of this forskolin-stimulated CFTR appeared likely.

To quantify the conductivity of the WT-CFTR for HCO_3^-, we performed experiments on excised patches with different combinations of Cl^- or HCO_3^- as the principal anion in the bath or pipette. Current–voltage (*I/V*) relations were recorded for the different conditions to obtain relative Cl^-/HCO_3^- permeabilities. Symmetrical ion concentrations on both sides of the excised membranes gave linear *I/V* relations, while nonsymmetric solutions revealed Goldman rectification (26), which allowed estimation of the permeability ratios for Cl^-/HCO_3^-. For these experiments, cell-attached patches were stimulated with forskolin to activate channels and to verify their cAMP dependence; then the patches were pulled off the cell into solutions containing 3 mM ATP and 75 nM catalytic subunit of protein kinase A to ensure the continued activity of the WT-CFTR. Fig. 4*b* (left traces) shows channel activity for patches incubated with symmetrical Cl^- solution in the patch and in the bath and voltage clamped to either −100 or +100 mV so that Cl^- moves in the direction bath to pipette (i.e., the normal secretory direction), and pipette to bath, respectively (as depicted by the arrows in the *Insets*). The *I/V* relation was linear under these conditions (Fig. 4*c*), and the single-channel conductance was

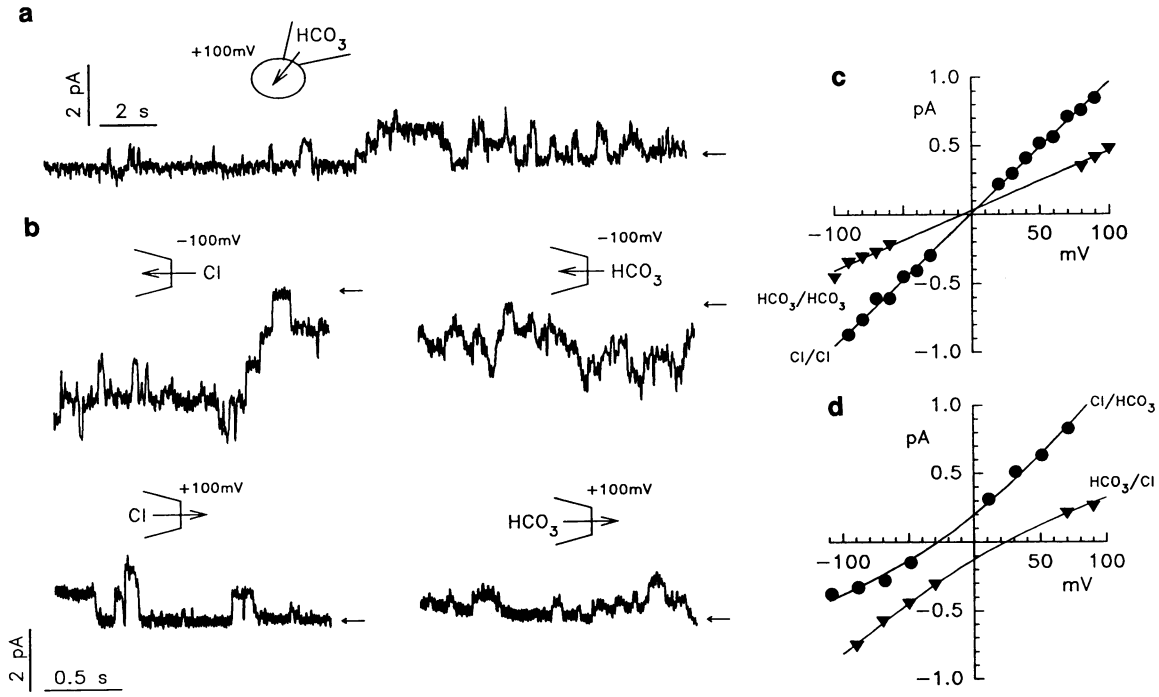

FIG. 4. HCO_3^- conductance of CFTR. (*a*) Activation of single-channel currents by forskolin (10 μM) from a previously silent patch in cell-attached mode. Forskolin was added 30 s before the start of this record. The pipette was filled with HCO_3^- solution and was bathed in Cl^- solution; applied potential was 100 mV. (*Inset*) Arrow indicates direction of anion flow during channel openings. (*b*) Four recordings of the WT-CFTR in excised, inside-out patches. (*Insets*) Direction of HCO_3^- or Cl^- movement at given potential is shown. Cl^- solution was present in the pipette and the bath for the records on the left (upper and lower). For the record on the upper right, Cl^- solution was present in the pipette and HCO_3^- solution was present in the pipette; for the record on the lower right, Cl^- solution was present in the bath and HCO_3^- solution was present in the pipette. Applied potential caused Cl^- and HCO_3^- movements as depicted in *Insets*. All membrane patches contained multiple CFTRs; arrows next to current traces represent the closed, nonconducting state. (*c*) Current–voltage (*I/V*) relations recorded from excised, inside-out patches. With either Cl^- solutions (●) or HCO_3^- solutions (▼) in both the pipette and bath, the *I/V* plots were linear. The channel conductance was 9.6 pS in Cl^- solutions and 4.3 pS in HCO_3^- solutions for the two *I/V* relations shown. (*d*) *I/V* relations recorded from excised patches with asymmetrical solutions. ●, Pipette contained Cl^- solution, and bath contained HCO_3^- solution; ▼, pipette contained HCO_3^- solution, and bath contained Cl^- solution. Predicted reversal potential for Cl^- was −53 or +53 mV, respectively. Lines show the fitted sum of Goldman equations for Cl^- and HCO_3^- to the data.

10.4 ± 0.5 pS (n = 7) (mean ± SE). These data on the protein kinase A-activated WT-CFTR agree with those of previous investigators (1–3).

Since the HCO_3^- solution contained 20 mM Cl^- (so that the $Ag^+/AgCl$ electrodes were stable), Cl^- contributes to the I/V relation obtained with symmetrical HCO_3^- solutions (i.e., in both the pipette and the bath; Fig. 4*c*). To better separate the Cl^- from the HCO_3^- permeability, we measured I/V characteristics of CFTR in excised patches that were incubated with nonsymmetrical solutions (either Cl^- solution in bath and HCO_3^- solution in pipette or vice versa) and then calculated the HCO_3^-/Cl^- permeability ratio from the I/V plots. Examples of Goldman rectification of the I/V relation under these conditions are shown in Fig. 4*d*. From the Goldman fits of the data, the Cl^-/HCO_3^- permeability ratio of the CFTR was calculated to be 3.8 ± 1.0 (n = 9). The respective conductances of the CFTR were 8.1 ± 1.2 pS in the Cl^- solution and 2.2 ± 0.28 pS in the HCO_3^- solution containing 127 mM HCO_3^- and 20 mM Cl^-, giving a ratio for the conductances of 3.9 ± 0.5. Comparable values have been found for the small conductance Cl^- channel (presumably the CFTR) in rat pancreatic duct cells, where the permeability ratio varied between 4.0 and 7.7 (10). It therefore seems clear that the cAMP-activated WT-CFTR conducts both Cl^- and HCO_3^-. Our data indicate that the relative permeability is Cl^-/HCO_3^- = 4:1.

There are a number of important implications of these findings. In the intact animal, CFTR-containing epithelial cells will likely secrete significant amounts of HCO_3^- across their apical membranes (where the CFTR is usually located) into the lumen. It is possible to estimate the relative rates of Cl^- and HCO_3^- secretion across the CFTR in the apical membranes of epithelial cells under physiological conditions. With the following concentrations of Cl^- and HCO_3^- inside (i) and outside (o) the cell, Cl_i^- = 30 mM, Cl_o^- = 114 mM, $HCO_{3,i}^-$ = 12.5 mM, $HCO_{3,o}^-$ = 25 mM, and the apical membrane potential = −50 mV (inside negative), HCO_3^- secretion will comprise 35% and Cl^- secretion will comprise 65% of the total transport of anions. This level of HCO_3^- secretion may explain why there is a current across isolated tracheal epithelia that could not be explained by the secretion of Cl^- or the absorption of Na^+ (31). The "residual" current is likely to have been due to the secretion of HCO_3^-. A similar residual current that was assumed to be due to HCO_3^- secretion has also been observed in the intestine (32).

Because most epithelial cells have multiple mechanisms for regulating pH_i, it is unlikely that the HCO_3^- permeability of the CFTR will contribute much to their pH_i regulatory abilities. However, this will depend on the relative levels of expression of the CFTR and other pH regulators. The secretion of HCO_3^- could, though, have a significant effect on the pH of the luminal solution in some epithelia. In the trachea, where the apical fluid layer is small and CO_2 concentrations fluctuate, even a relatively small amount of HCO_3^- secretion could have large effects on pH of this periciliary fluid. In CF caused by the ΔF508 mutation, secretion of both HCO_3^- and Cl^- across the CFTR will be reduced in concert. Whether the loss of the CFTR has an impact on overall epithelial HCO_3^- secretion rates will depend on the relative ability of other anion channels (e.g., Ca^{2+}-activated, volume-activated, or depolarization-induced outward rectifier) to conduct HCO_3^-. It is expected, though, that the amounts of HCO_3^- will be reduced, and pH will be lower in the apical fluid of ΔF508-CFTR epithelia compared to WT-CFTR. These effects may explain some of the pathology (e.g., bacterial accumulation, altered mucus) that cannot be easily understood simply in terms of a lack of Cl^- (and the accompanying H_2O) secretion. Consistent with this idea, a recent study by Smith and Welsh (9) on cultured human airway epithelia containing WT-CFTR showed that cAMP (activates CFTR) did not affect net transepithelial H_2O transport, but the pH of the luminal fluid alkalinized from 6.4 to 6.8. Also, CF has some of its most severe pathological consequences in the pancreas, intestine, and prostate, epithelia in which the secretion of HCO_3^- is important to their overall function. It will be interesting to measure the HCO_3^- permeability and pH_i regulatory capabilities of the CFTR in these (and other) epithelia. Since the CFTR seems also to be active in endosomes (33), and perhaps in other intracellular membranous compartments (6), the role of HCO_3^- in the control of the pH of these internal organelles should also be investigated.

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