Role of HCO3- in regulation of cytoplasmic pH in ciliary epithelial cells

H. Helbig, C. Korbmacher, F. Stumpff, M. Coca-Prados and M. Wiederholt

Am J Physiol Cell Physiol 257:C696-C705, 1989. ;

You might find this additional info useful...

This article has been cited by 1 other HighWire-hosted articles:
http://ajpcell.physiology.org/content/257/4/C696#cited-by

Updated information and services including high resolution figures, can be found at:
http://ajpcell.physiology.org/content/257/4/C696.full

Additional material and information about ***American Journal of Physiology - Cell Physiology*** can be found at:
http://www.the-aps.org/publications/ajpcell

This information is current as of March 18, 2013.

American Journal of Physiology - Cell Physiology is dedicated to innovative approaches to the study of cell and molecular physiology. It is published 12 times a year (monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991. Copyright © 1989 the American Physiological Society. ISSN: 0363-6143, ESSN: 1522-1563. Visit our website at http://www.the-aps.org/.

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

Role of HCO_3^- in regulation of cytoplasmic pH in ciliary epithelial cells

HORST HELBIG, CHRISTOPH KORBMACHER, FRIEDERIKE STUMPFF, MIGUEL COCA-PRADOS, AND MICHAEL WIEDERHOLT
Institut für Klinische Physiologie, Klinikum Steglitz der Freien Universität Berlin, D-1000 Berlin 45, Federal Republic of Germany; and Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Connecticut 06510

HELBIG, HORST, CHRISTOPH KORBMACHER, FRIEDERIKE STUMPFF, MIGUEL COCA-PRADOS, AND MICHAEL WIEDERHOLT. *Role of HCO_3^- in regulation of cytoplasmic pH in ciliary epithelial cells.* Am. J. Physiol. 257 (Cell Physiol. 26): C696–C705, 1989.—Cytoplasmic pH (pH_i) was monitored using the pH-sensitive absorbance of 5(6)carboxy-4′,5′-dimethylfluorescein in monolayers of a cell clone derived from bovine pigmented ciliary epithelium (PE) transformed with the simian virus 40. *1*) Changing extracellular media from a nominally HCO_3^--free solution to a solution containing 28 mM HCO_3^--5% CO_2 at constant extracellular pH (7.4) resulted in a delayed alkalinization of pH_i, which was 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) sensitive and was inhibited in Na^+-free medium and in Cl^--depleted cells. *2*) DIDS pretreatment acidified pH_i in HCO_3^--containing media. *3*) Replacing extracellular Cl^- resulted in a DIDS-sensitive, HCO_3^--dependent, and Na^+-independent alkalinization. *4*) Replacing extracellular Na^+ in HCO_3^--containing media led to a partly DIDS-sensitive intracellular acidification. *5*) Recovery of pH_i after an alkali load (acetate prepulse) had a HCO_3^--dependent and DIDS-sensitive component. *6*) Two Na^+-dependent components participated in pH_i regulation after an acid load (NH_4^+ prepulse) in HCO_3^--containing solution. One was amiloride sensitive, the other was DIDS sensitive and was inhibited in HCO_3^--free media and after Cl^- depletion. We conclude that in cultured PE, in addition to Na^+-H^+ exchange, two HCO_3^- transporters participate in pH_i regulation. Cl^--dependent Na^+-HCO_3^- symport regulates pH_i during steady state and after an acid load, and Na^+-independent Cl^--HCO_3^- exchange is involved in pH_i recovery after an alkali load.

ciliary epithelium; tissue culture; intracellular pH; chloride-bicarbonate exchange; sodium-bicarbonate cotransport

THE REGULATION of cytoplasmic pH (pH_i) is an essential feature of cell homeostasis. Most biochemical and biophysical processes are sensitive to change in pH_i. Production of acid equivalents derived from metabolic activities or H^+ leaking downhill into the cell must be continuously extruded out of the cell to keep pH_i constant. In many cells Na^+-H^+ exchange is one of the mechanisms responsible for regulation of pH_i. In addition, some cells possess HCO_3^--dependent transport systems that participate in pH_i regulation (28).

In some epithelia, transporters for HCO_3^- or H^+ have a dual role. In addition to their function in pH_i regulation they are involved in transepithelial transport of acid equivalents (28), Na^+, or Cl^- (27). Similar mechanisms may also be present in the ciliary epithelium. This double-layered epithelium, with the pigmented layer (PE) on the stromal side and the nonpigmented layer facing the aqueous humor, is responsible for the formation of aqueous humor and the maintenance of intraocular pressure mainly by active cellular electrolyte secretion (23). Inhibitors of the carbonic anhydrase are widely used clinically to reduce aqueous humor formation rate and thereby lower intraocular pressure (3), although their exact mode of action is unknown. In Ussing-chamber experiments transepithelial current across the ciliary epithelium (21) and transepithelial net Cl^- fluxes (20) were found to be HCO_3^- dependent. Thus HCO_3^- seems to be involved in the basic mechanisms of aqueous humor secretion.

Recently we have shown that in cultured bovine PE Na^+-H^+ exchange is responsible for regulation of pH_i during steady-state conditions and after an acid load in HCO_3^--free media (14, 16). The aim of the present study was to investigate the role of HCO_3^--transport systems in pH_i regulation. pH_i was monitored using the pH-sensitive absorbance of 5(6)carboxy-4′,5′-dimethylfluorescein (DMCF) in cultured pigmented ciliary epithelial cells transformed with the wild-type simian virus 40 (SV-40). Our data suggest that PE cells possess two HCO_3^--transporting systems (in addition to Na^+-H^+ exchange): Na^+-independent Cl^--HCO_3^- exchange and a Cl^--dependent cotransport for Na^+ and HCO_3^-.

MATERIALS AND METHODS

Tissue culture. Experiments were performed on the same cell clone as previously described (16). In brief, bovine ciliary processes were enzymatically disaggregated (trypsin/EDTA, 0.05/0.02%) and the resulting ciliary epithelial cells infected with SV-40. A transformed clone was isolated essentially as previously described for a human cell clone (12). The cell clone retained properties of PE cells (16). Cultures were grown on Nunclon plastic tissue culture flasks (NUNC, Roskilde, Denmark). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in a 5% CO_2 atmosphere at 37°C. The medium was changed twice a week. Cells were passaged weekly at a

0363-6143/89 $1.50 Copyright © 1989 the American Physiological Society

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

split ratio of 1:2 or 1:3. Experiments were performed with monolayers grown on plastic cover slips in Leighton tubes (Costar, Cambridge, MA) 3–5 days after reaching confluency, between passage numbers 9 and 29.

Determination of intracellular pH. A detailed description of the experimental setup has been previously reported (19). In brief, the absorbance of DMCF is pH sensitive at 509 nm, while being nearly pH insensitive at 470 nm. Thus the ratio of absorbance at 509 and 470 nm gives an estimate of pH. For pH_i experiments a cell-covered cover slip was cut in half. One-half (indicator cells) was incubated for 40–50 min with DMCF diacetate (100 μM). Intracellular esterases cleave the acetate moiety. The resulting DMCF cannot leave the cell and accumulates intracellularly. The second half of the cover slip (control cells) was incubated in an identical solution that contained no dye. Indicator and control cells were placed in a cuvette in which they could be superfused with different solutions. These test solutions were temperature controlled and appropriately gassed. Transmittance was monitored continuously using a dual-beam (control and indicator) dual-wavelength (509 and 470 nm) photometer. Data are presented as the transmittance ratio at 509 and 470 nm corrected for the non-pH_i related absorbance changes in the control cells. In some experiments the dual-beam photometer was used to investigate the effect of 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS) preincubation on pH_i regulation. In these experiments one-half of the cover slip was incubated for 30–45 min in HCO_3^--free Ringer solution containing 1 mM DIDS at 37°C, whereas the other half of the cover slip was incubated without DIDS. Subsequently both halves of the cover slip were loaded with DMCF as described above, and the pH_i changes in cells with and without DIDS pretreatment were recorded in parallel in the same experiment. At the end of the recording each experiment was individually calibrated, making pH_i equal to extracellular pH (pH_o) by using the K^+-H^+ ionophore nigericin. The results of each calibration step are indicated on the *Y*-axis of Figs. 1–10.

Solutions and sources of chemicals. Standard HCO_3^- saline solution contained the following ionic concentrations (in mM): 123 NaCl, 28 $NaHCO_3$, 4 KCl, 1.7 $CaCl_2$, 1 KH_2PO_4, 0.9 $MgSO_4$, and 5 glucose. HCO_3^--containing solutions were gassed with 5% CO_2 resulting in a pH of 7.4. HCO_3^--free solutions were buffered with 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) to pH 7.4 (if not indicated otherwise) and not gassed. In these solutions 28 mM $NaHCO_3$ was replaced by 28 mM NaCl. In solutions containing a lower Na^+ or Cl^- concentration, Na^+ was replaced by *N*-methyl-D-glucamine (NMDG) and Cl^- by equimolar amounts of cyclamate. In some experiments in Cl^--free media, Ca^{2+} concentration was raised to 6 mM to compensate for formation of Ca^{2+} complexes with large anions. We obtained identical results with solutions containing either 1.7 or 6 mM Ca^{2+}, indicating that changes in extracellular Ca^{2+} activity were not responsible for the pH_i changes observed in Cl^--free media. In solutions designed to change pH_i by nonionic diffusion, 20 mM NaCl was replaced by 20 mM NH_4Cl [or 10 mM $(NH_4)_2SO_4$ in Cl^--free media], or 40 mM NaCl by 40 mM sodium acetate

TABLE 1. *Intracellular pH at different extracellular pH in HCO_3^--free media*

Extracellular pH	Intracellular pH	*n*
7.0	6.89±0.05	8
7.5	7.08±0.03	19
8.0	7.26±0.02	11

Values are means ± SE for pH units; *n*, no. of experiments.

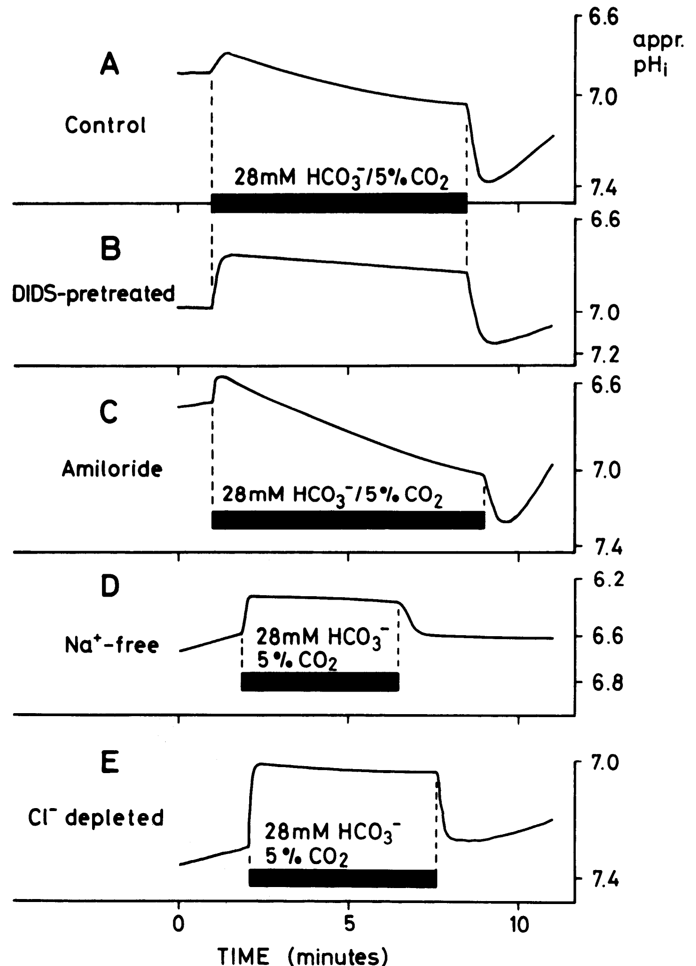

FIG. 1. *A*: effect of addition of 28 mM HCO_3^--5% CO_2 at constant extracellular pH (pH_o) 7.4 on intracellular pH (pH_i) under control conditions (12 experiments with similar results). *B*: same experiments as in *A*, but cells were preincubated for 35 min at 37°C in HCO_3^--free Ringer solution containing 1 mM DIDS, before they were loaded with dye, whereas in *A*, cells were preincubated without DIDS (4 experiments with similar results). *C*: effect of amiloride on pH_i changes induced by addition of HCO_3^--CO_2. Before HCO_3^--CO_2 was added, cells had been superfused for 15 min with a solution containing 1 mM amiloride, until pH_i approached a new steady-state value (7 experiments with similar results). *D*: monolayer had been superfused for 10 min with a HCO_3^- and Na^+-free saline solution, (9 experiments with similar results). *E*: effect of addition of HCO_3^--CO_2 to Cl^--depleted cells. Monolayer had been loaded with dye for 50 min in a Cl^- and HCO_3^--free (isosmotically replaced by cyclamate) saline solution (4 experiments with similar results).

or sodium propionate. Osmolality was adjusted with mannitol as required. All tissue culture materials and supplements were obtained from Biochrom KG, Berlin, FRG. DMCF diacetate was purchased from Molecular

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

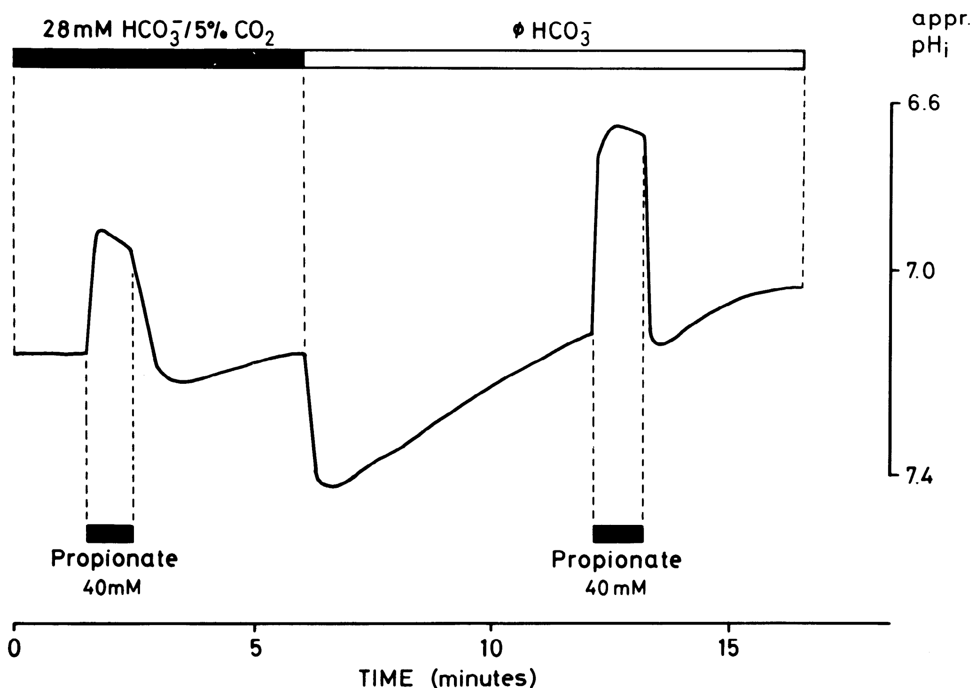

FIG. 2. Effect of addition of 40 mM propionate on pH_i in HCO_3^--CO_2-containing and HCO_3^--CO_2-free (ϕHCO_3^-) media (8 experiments with similar results). For calculations of intracellular buffering capacity pH_i change induced by addition of acid in absence of regulatory acid extrusion must be determined. We corrected observed maximum acidification by estimating regulatory acid extrusion from slope of recovery after acid load.

Probes, Eugene, OR. Nigericin, DIDS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS), and amiloride were purchased from Sigma Chemical, St. Louis, MO.

RESULTS

Steady-state pH_i. In standard HCO_3^- Ringer solution (28 mM HCO_3^- gassed with 5% CO_2, pH 7.4), pH_i in SV-40-transformed bovine PE averaged 7.13 ± 0.01 (n = 77). In HCO_3^--free solution buffered with 10 mM HEPES to pH 7.4, steady-state pH_i in the same cell clone was 6.98 ± 0.01 (n = 57) (16). Thus at constant pH_o the steady-state pH_i was 0.15 pH units more alkaline in a HCO_3^--containing solution than in HCO_3^--free medium. Steady-state pH_i in HCO_3^--CO_2 containing media was 0.09 ± 0.03 (n = 12) pH units more acidic after DIDS pretreatment than in control cells ($P < 0.01$, paired Student's t test).

In another set of experiments we tested the effect on pH_i of changing pH_o in HCO_3^--free solutions. When pH_o was changed from 7.5 to 7.0 or 8.0, pH_i changed very slowly and reached a new steady-state value within ~10–15 min. Exact values are given in Table 1. pH_o changes of 0.5 pH units result in changes of pH_i of only ~0.2 pH units.

Changing extracellular HCO_3^--CO_2 concentration. To further investigate the difference in steady-state pH_i between HCO_3^--containing and HCO_3^--free media, experiments such as that shown in Fig. 1 were performed. pH_i was monitored when the superfusing solution was changed from a HCO_3^--free HEPES-buffered solution to a solution containing 28 mM HCO_3^- gassed with 5% CO_2 at a constant pH_o of 7.4. On addition of HCO_3^--CO_2, pH_i initially dropped, probably due to nonionic diffusion of CO_2. Subsequently we observed an alkalinization of pH_i toward a new steady-state value, which was 0.13 ± 0.01 (n = 2) pH units more alkaline than the steady-state pH_i in HCO_3^--free solution. If HCO_3^--CO_2 was removed inverted pH_i changes occurred. Because the Cl^- concentration in HCO_3^--free media was 28 mM higher than in HCO_3^--containing solutions, we performed experiments such as that shown in Fig. 1*A*, in which 28 mM cyclamate replaced HCO_3^-. We observed identical results as shown in Fig. 1*A* (n = 4).

In DIDS-pretreated cells (n = 4, Fig. 1*B*) we observed the initial acidification after addition of HCO_3^--CO_2. The subsequent alkalinization was nearly totally blocked. In the presence of 1 mM amiloride the pH_i changes on addition of HCO_3^--CO_2 were similar to the changes in control experiments, but the difference in steady-state pH_i was increased (0.33 ± 0.04 pH units; n = 7; Fig. 1*C*). However, in Na^+-free media (n = 9; Fig. 1*D*) only the initial (probably CO_2 related) fall in pH_i was observed. The subsequent alkalinization was markedly reduced. A similar inhibition of the delayed alkalinization was observed if the experiment was performed in Cl^--free medium with Cl^--depleted cells (incubation with the dye for 50 min in Cl^- and HCO_3^--free medium; n = 4; Fig. 1*E*).

Intracellular buffering capacity. Intracellular buffering capacity was evaluated using the weak acid propionic acid. Addition of 40 mM propionic acid to the superfusing solution resulted in a sharp fall in pH_i due to nonionic diffusion of uncharged propionic acid and intracellular cleavage into H^+ and anionic propionate. In the experiment shown in Fig. 2, 40 mM propionic acid was added for a brief period in HCO_3^--free and HCO_3^--containing media. It is obvious that in HCO_3^--free solution propionic acid resulted in a much larger pH_i change (−0.45 ± 0.02 pH units; n = 8) than in HCO_3^- Ringer solution (−0.25 ± 0.02 pH units; n = 8). From the amplitude of the pH_i changes, the intracellular buffering capacity (β) can be estimated[1]. The data revealed values of 35.6 mM H^+/pH

[1] $\beta = \Delta[(CH_3)_2COO^-]_i \cdot (\Delta pH_i)^{-1}$; see Ref. 28.

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

TABLE 2. *Effect of Cl^- replacement by cyclamate on pH_i*

Modified Ringer Solution	Initial Rate of Alkalinization on Cl^- Replacement	n
28 mM HCO_3^- gassed with 5% CO_2		
Control	0.18±0.02	12
DIDS preincubated	0.01±0.01	3
DIDS acute	0.01±0.01	3
Na^+ free	0.11±0.02	7
HCO_3^--CO_2-free media	0.01±0.01	3

Values are means ± SE for pH units/min; *n*, no. of experiments.

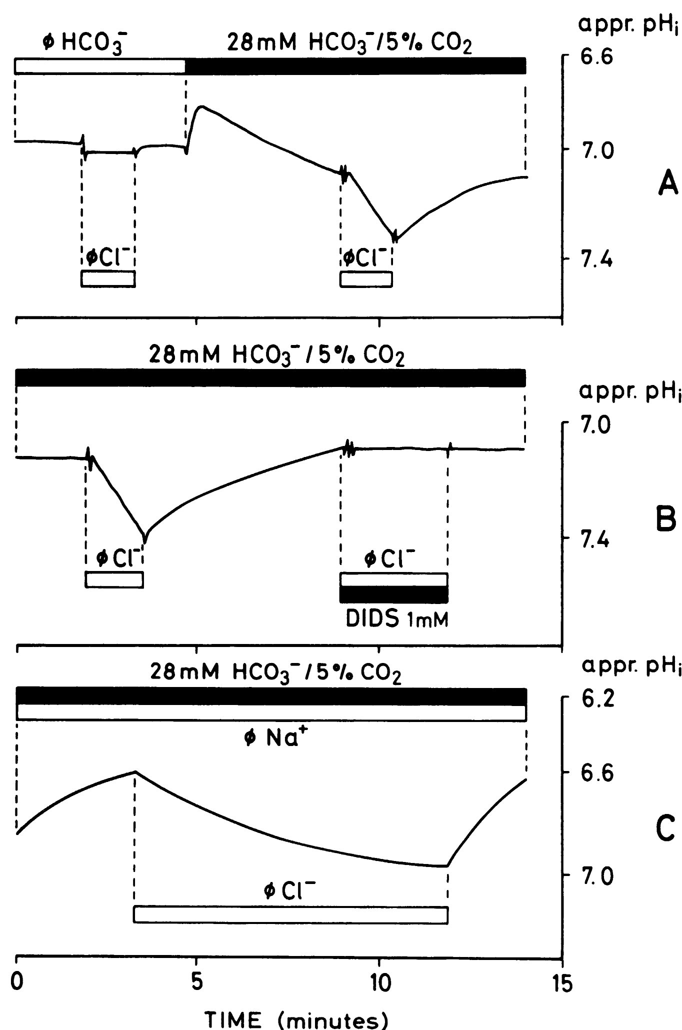

FIG. 3. *A*: original recording illustrating effect of Cl^- replacement by cyclamate on cytoplasmic pH in HCO_3^--CO_2-containing and HCO_3^--CO_2-free (ϕHCO_3^-) media (3 experiments with similar results). *B*: effect of Cl^- replacement on pH_i in absence and in presence of 1 mM DIDS (3 experiments with similar results). *C*: effect of Cl^- replacement by cyclamate on pH_i in Na^+-free (ϕNa^+) media [Na^+ isosmotically replaced by *N*-methyl-D-glucamine (NMDG)]. Cells had been superfused for 10 min with Na^+-free medium before extracellular Cl^- was removed (7 experiments with similar results). ϕCl^-, Cl^- free.

unit for β in HCO_3^--containing media and 12.5 mM H^+/pH unit for β in HCO_3^--free solutions.

Removing extracellular chloride. A summary of the effects on pH_i of replacing extracellular Cl^- is given in Table 2. The effect on pH_i of removing extracellular Cl^- (by isosmotic replacement with cyclamate) was HCO_3^- dependent. In HCO_3^--free media no significant change in pH_i was observed on Cl^- replacement (n = 3; Fig. 3*A*). On the other hand, in HCO_3^- Ringer solution pH_i increased by 0.18 ± 0.02 pH units/min (n = 12, Fig. 3*A*) on Cl^- removal. In the presence of 1 mM DIDS this response was blocked (n = 3; Fig. 3*B*). To test for the Na^+ dependence of this pH_i response, cells were Na^+ depleted until a new (more acidic) steady-state pH_i was reached. Subsequently, Cl^- was replaced in a Na^+-free HCO_3^--containing solution (Fig. 3*C*). This maneuver resulted in an increase of pH_i (0.11 ± 0.02 pH units/min; n = 7), which was significantly slower than in Na^+-containing media ($P < 0.05$, unpaired Student's *t* test).

To evaluate the kinetic parameters of the effect of Cl^- on pH_i, experiments such as that shown in Fig. 4 were performed. Cells were Cl^- depleted in a HCO_3^--containing solution for 10 min. Subsequently different concentrations of Cl^- were added. The initial rate of acidification on addition of Cl^- exhibited saturation with increasing Cl^- concentration (Fig. 5*A*). Linear transformation of the data according to Lineweaver-Burk (Fig. 5*B*) revealed an apparent K_m of 55 mM and a V_{max} of −0.23 pH units/min.

Removing extracellular Na^+. Table 3 summarizes the effect of Na^+ removal on intracellular pH. Na^+ replacement in HCO_3^- Ringer solution decreased pH_i (−0.08 ± 0.01 pH units/min, n = 12) and addition of 1 mM amiloride during steady state in HCO_3^--containing media did not significantly change pH_i (n = 12; Fig. 6*A*). Although amiloride (1 mM) slightly inhibited the acidification induced by removing external Na^+ in HCO_3^- Ringer solution, there was still significant acidification when Na^+ was removed in the presence of amiloride (−0.06 ± 0.02 pH units/min; n = 5; Fig. 6*A*). On the other hand in the presence of 1 mM DIDS the acidification induced by replacing external Na^+ was markedly reduced (−0.04 ± 0.01 pH units/min; n = 7; Table 3). Different results were obtained in nominally HCO_3^--free solutions. Replacing extracellular Na^+ by NMDG in HCO_3^--free media acidified pH_i by −0.10 ± 0.01 pH units/min (n = 25; Fig. 6*B*). Addition of amiloride (1 mM) to HCO_3^--free media resulted in a slight acidification (−0.03 ± 0.01 pH units/min; n = 9; Fig. 6*B*). When Na^+ was removed in the presence of amiloride, there was no significant additive acidification on Na^+ removal in HCO_3^--free media (−0.04 ± 0.01 pH units/min; n = 13; Fig. 6*B*).

Regulation of pH_i after an alkali load. An alkali load was imposed using the acetate prepulse technique. Cells were incubated for 10 min in Ringer solution containing 40 mM acetate. Subsequently acetate was removed. pH_i increased because of the exit of uncharged acetic acid by nonionic diffusion followed by a recovery of pH_i toward a normal steady-state value. The initial recovery rates after acetate prepulse under different conditions are summarized in Table 4. In HCO_3^--containing Ringer solution the recovery rate after acetate prepulse averaged −0.08 ± 0.01 pH units/min (n = 9) whereas in HCO_3^--free solution the rate of recovery was only −0.05 ± 0.01 pH units/min (n = 11). After preincubation with 1 mM DIDS the recovery was markedly reduced in HCO_3^-

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

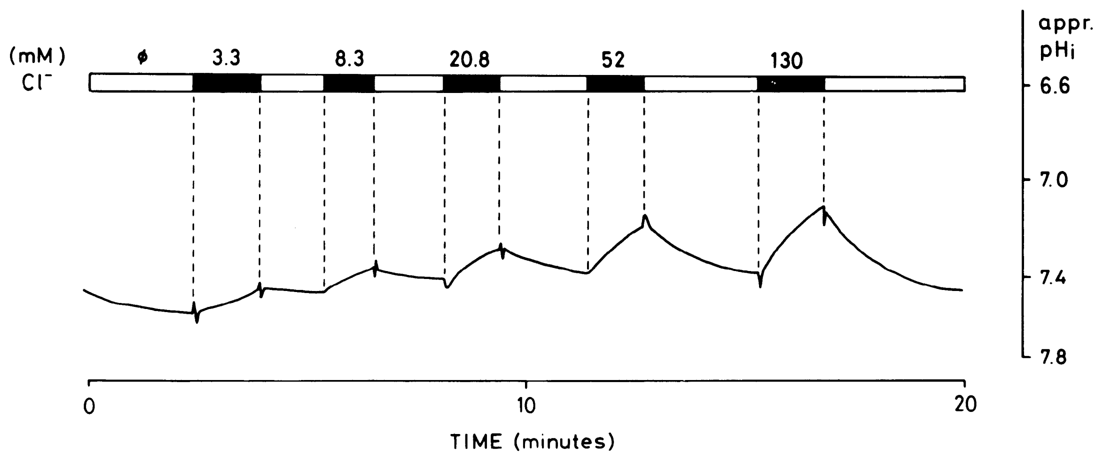

FIG. 4. After dye loading in standard (Cl^--containing) solution cells were superfused for 10 min with Cl^--free HCO_3^--containing media, which led to an alkalinization of pH_i. Subsequently, different Cl^- concentrations were added for a brief time period (5 experiments with similar results). ϕ, Cl^- free.

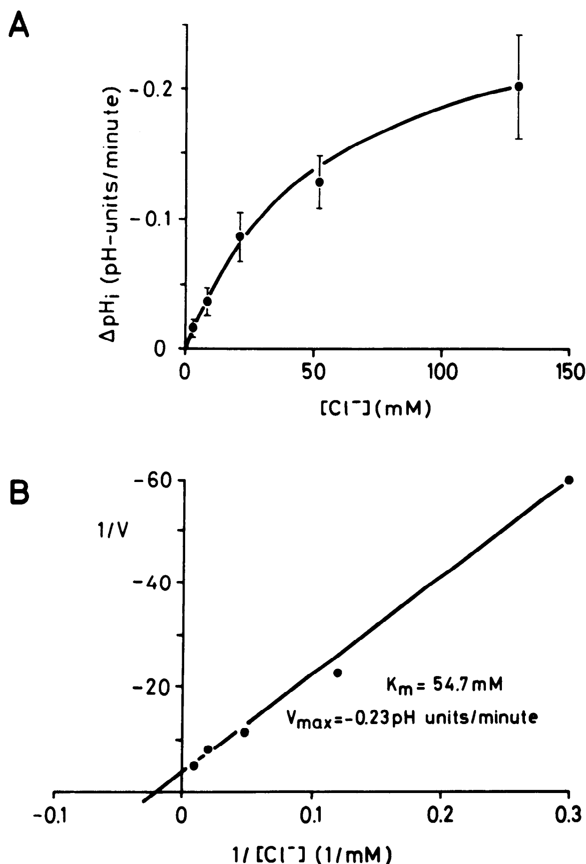

FIG. 5. *A*: data from 5 experiments such as that shown in Fig. 4 (means ± SE) were plotted as rate of acidification of pH_i induced by addition of Cl^- vs. respective Cl^- concentration. No attempt was made to compensate for changes in base line, which occurred before and after addition of some of test solutions (see Fig. 4). Therefore rate of change in pH should be interpreted with caution. *B*: linear transformation of data from *A* according to Lineweaver-Burk. Regression line (least square method) reveals an apparent K_m of 54.7 mM, a V_{max} of −0.23 pH units/min, and r^2 of 0.99.

TABLE 3. *Effect of Na^+ replacement and amiloride on pH_i*

Modified Ringer Solution	Initial Rate of pH_i Change	*n*
28 mM HCO_3^- gassed with 5% CO_2		
Na^+ replacement	−0.08±0.01	12
1 mM amiloride	−0.004±0.003	12
Na^+ replacement + 1 mM amiloride	−0.06±0.02	5
Na^+ replacement + 1 mM DIDS	−0.04±0.01	7
HCO_3^--CO_2-free media		
Na^+ replacement	−0.10±0.01	25
1 mM amiloride	−0.03±0.01	9
Na^+ replacement + 1 mM amiloride	−0.04±0.01	13

Values are means ± SE in pH units/min. *n*, no. of experiments.

Ringer (−0.02 ± 0.02 pH units/min; n = 7; Fig. 7). Recovery after an alkali load induced by addition of NH_4^+ was also partly inhibited after DIDS preincubation (data not shown). In HCO_3^--free solution replacement of Cl^- had no significant effect on recovery after acetate prepulse (−0.05 ± 0.01 pH units/min; n = 4), whereas in HCO_3^--containing solution removing Cl^- during this recovery reversed pH_i recovery (0.14 ± 0.03 pH units/min; n = 5).

Regulation of pH_i after an acid load. Intracellular acid loading was achieved by the NH_4^+ prepulse technique (8). Recovery of pH_i was almost linear during the first few minutes. Initial recovery rates after NH_4^+ prepulse (after incubation for 3–5 min with 20 mM NH_4^+) are summarized in Table 5. Recovery in HCO_3^--free and HCO_3^--containing media is shown in Fig. 8. Initial pH_i recovery rate after NH_4^+ prepulse was 0.15 ± 0.02 pH units/min (n = 17) in HCO_3^--free solution and 0.19 ± 0.02 pH units/min (n = 22) in HCO_3^--containing Ringer solution. Addition of 1 mM amiloride almost completely abolished pH_i recovery in HCO_3^--free solution, whereas in HCO_3^--containing Ringer solution only ~50% of pH_i recovery was inhibited by addition of 1 mM amiloride (Fig. 8). Recovery was Na^+ dependent in HCO_3^--containing as well as in HCO_3^--free solutions. Under both conditions Na^+ replacement reversed recovery.

Acute addition of 1 mM DIDS (n = 6) or SITS (n = 6) during pH_i recovery after an acid load inhibited up to 50% of the recovery rate in HCO_3^- Ringer solution (Fig. 9). In HCO_3^--free media no significant effect of 1 mM DIDS on pH_i regulation after NH_4^+ prepulse was observed

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

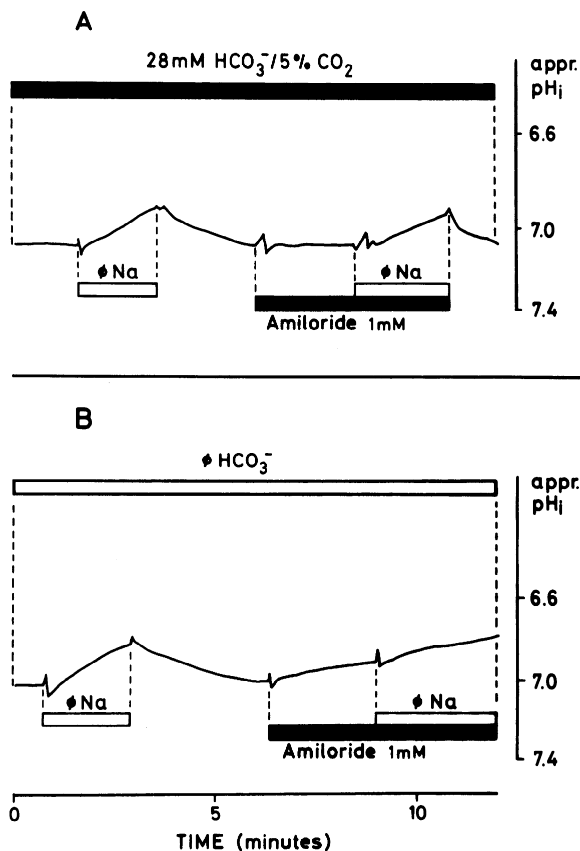

FIG. 6. Effect of Na^+ replacement by NMDG in absence and presence of 1 mM amiloride in HCO_3^--CO_2-containing solution (5 experiments with similar results) (*A*) and in HCO_3^--CO_2 free (ϕHCO_3^-) media (*B*) (4 experiments with similar results). ϕNa^+, Na^+ free.

(n = 6). Cells pretreated for 30 min (or longer) with 1 mM DIDS also showed a slower pH_i recovery than control cells (DIDS pretreated: 0.08 ± 0.02 pH units/min; n = 5). Addition of 1 mM amiloride to DIDS-pretreated cells almost completely abolished residual pH_i recovery (0.02 ± 0.003, n = 5). In Cl^--depleted cells (15 min in Cl^--free HCO_3^--containing solution) pH_i recovery after

TABLE 4. *Recovery rate of pH_i after acetate prepulse*

Modified Ringer Solution	Initial Rate of pH_i Recovery	n
28 mM HCO_3^- gassed with 5% CO_2		
Control	−0.08±0.01	9
DIDS preincubated	−0.02±0.02	7
Cl^- free	+0.14±0.03	5
HCO_3^--CO_2-free media		
Control	−0.05±0.01	11
Cl^- free	−0.05±0.01	4

Values are means ± SE in pH units/min. n, no. of experiments.

NH_4^+ prepulse was 0.06 ± 0.01 pH units/min (n = 6; Fig. 10) in HCO_3^- Ringer solution. This recovery was almost completely abolished by addition of 1 mM amiloride (n = 6; Fig. 10).

DISCUSSION

In this study we have observed marked differences in the regulation of pH_i in the presence and absence of HCO_3^-. These differences can be explained by two HCO_3^--transporting systems operating in cultured PE: a Cl^--HCO_3^- exchange and a Cl^--dependent Na^+-HCO_3^- cotransport. Before the evidence for these transporters is discussed, it should be pointed out that β has to be taken into account if transport of acid equivalents is measured as change in pH_i. We calculated a β of 12.5 mM H^+/pH unit for cultured PE in HCO_3^--free media (intrinsic buffering capacity, β_i). This value is rather low compared with other animal cells (28). However, most cultured cells seem to have a relatively low β_i (19). In HCO_3^--containing media, β in PE was 35.6 mM H^+/pH unit. This value is in reasonable agreement with the theoretically calculated additional buffering power for the CO_2-HCO_3^- system (β_{CO_2}) of ~25 mM H^+/pH unit at pH_i 7.0 and 5% CO_2 (28). Thus, in HCO_3^--CO_2-containing media, β is more than twice as large as β_i. The same pH_i change therefore represents more than twice the rate of H^+ (or OH^- or HCO_3^-) transport in HCO_3^- Ringer solution

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

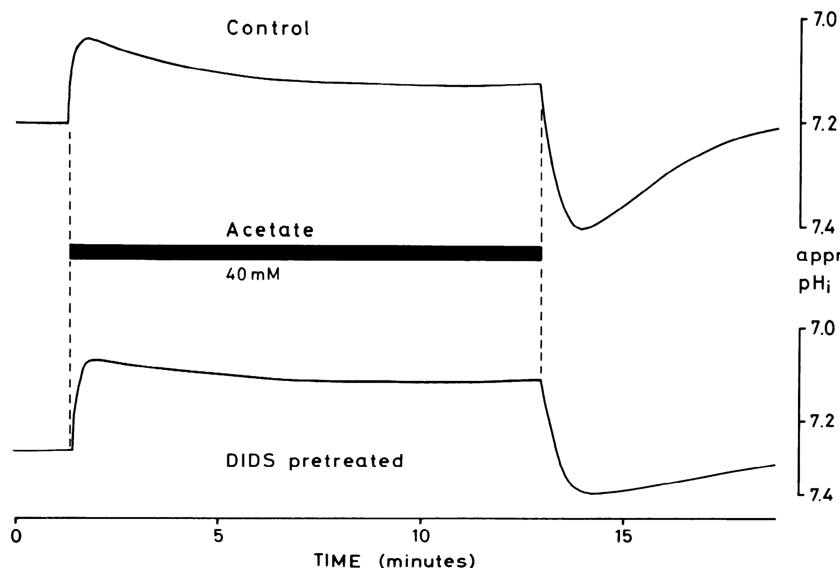

FIG. 7. Cells from same cover slip were incubated in HCO_3^--free media at 37°C for 45 min either in absence (control) or in presence of 1 mM DIDS (DIDS pretreated). Subsequently both monolayers were dye loaded. Cells were superfused for 10 min with acetate (40 mM). Removal of acetate led to an alkalinization of pH_i followed by a pH_i recovery toward normal steady-state pH_i, which was markedly slower in DIDS-pretreated cells (5 experiments with similar results).

compared with HCO_3^--free medium. However, the values for β are only valid for the pH_i range at which they were obtained. β_{CO_2} strongly depends on pH_i, and β_i may also vary considerably at different pH_i values (28). Thus measurements of pH_i changes allow only an approximate estimation for transport of acid and base equivalents.

Cl^--HCO_3^- exchange. Replacement of extracellular Cl^- by cyclamate resulted in a HCO_3^--dependent and DIDS-sensitive alkalinization of pH_i (Table 2; Fig. 3) that can be explained by an anion transporter exchanging extracellular HCO_3^- for intracellular Cl^-. Cl^--HCO_3^- exchange has been described in a variety of different cell types (24) including PE cells by means of ^{36}Cl-uptake studies (15). Two types of Cl^--HCO_3^- exchangers have been described: sodium-dependent and sodium-independent anion exchange (22, 23, 25, 32). We therefore tested the alkalinization induced by Cl^- replacement for Na^+ dependence. We still found significant alkalinization, when Cl^- was replaced in Na^+-free medium (Fig. 3*C*). However, the rate of pH_i change on Cl^- replacement was reduced in

TABLE 5. *Recovery of pH_i after NH_4^+ prepulse*

Modified Ringer Solution	Initial Rate of pH_i Recovery	n
28 mM HCO_3^- gassed with 5% CO_2		
Control	0.19±0.02	22
1 mM amiloride	0.10±0.01	19
DIDS preincubated	0.08±0.02	5
DIDS preincubated + 1 mM amiloride	0.02±0.003	5
Cl^- depleted	0.06±0.01	6
Cl^- depleted + 1 mM amiloride	0.01±0.004	6
Na^+ free	−0.02±0.01	3
HCO_3^--CO_2-free media		
Control	0.15±0.02	17
1 mM amiloride	0.02±0.01	14
Na^+ free	−0.03±0.02	8

Values are means ± SE for pH units/min; *n*, no. of experiments.

Na^+-free solution. This reduction cannot be taken as evidence for Na^+ dependence of part of the alkalinization induced by Cl^- removal. Anion exchange has been shown to be pH_i dependent: intracellular acidification inhibited and intracellular alkalinization stimulated anion exchange (26). pH_i in Na^+-free solution was more acidic, which could explain the reduced effect of Cl^- removal on pH_i in Na^+-free solutions. Nevertheless we have presented evidence that at least part of Cl^--HCO_3^- exchange is Na^+ independent.

Recovery of pH_i after an alkali load in PE cells was reversed by replacement of Cl^- and was partly DIDS sensitive (Fig. 7). If corrected for the different intracellular buffering capacities, the alkali extrusion rate was markedly faster in HCO_3^--containing compared with HCO_3^--free media (Table 4). These results are similar to those in Vero cells (32) and indicate involvement of Cl^--HCO_3^- exchange in recovery after an alkali load. Participation of anion exchange in regulatory pH_i decrease is not a general finding (28). In PE cells Cl^--HCO_3^- exchange seems to be involved but is not the only mechanism for recovery after alkali load. There seem to be additional mechanisms, because significant recovery was observed in HCO_3^--free medium. Involvement of Cl^--OH^- exchange was excluded, since Cl^- replacement had no effect on regulatory pH_i increase in HCO_3^--free solutions (Table 4). Passive ionic conductances (H^+, OH^-) or accumulation of metabolic acid equivalents may account for the pH_i regulation observed in HCO_3^--free media (28).

We also tried to evaluate kinetic parameters for the effect of Cl^- on pH_i. Different concentrations of Cl^- were added to Cl^--depleted cells, and the rate of acidification induced by Cl^- readdition was plotted against Cl^- concentration (Figs. 4, 5*A*). Kinetic analysis revealed an apparent K_m of 55 mM for Cl^- (Fig. 5*B*). Experiments

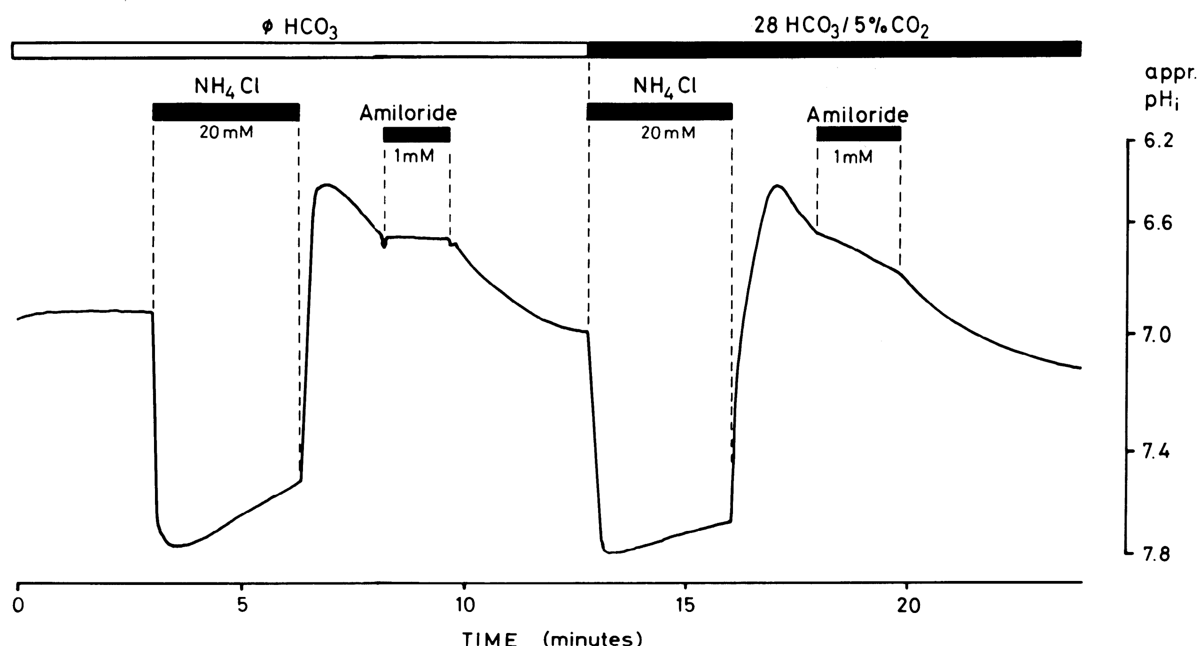

FIG. 8. Effect of amiloride (1 mM) on pH_i recovery after acid load induced by NH_4^+ prepulse in presence and in absence (ϕHCO_3^-) of extracellular HCO_3^- (5 experiments with similar results).

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

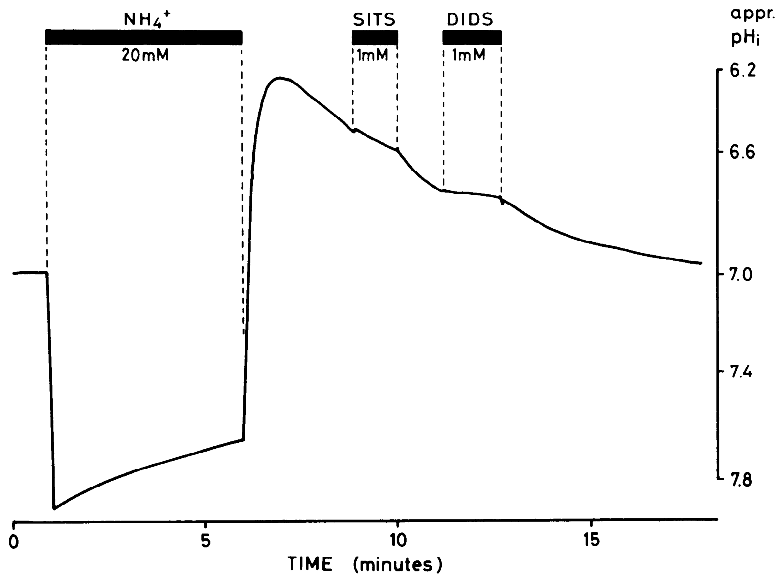

FIG. 9. Effect of DIDS and SITS (1 mM) on pH_i recovery after intracellular acid loading induced by NH_4^+ prepulse in presence of extracellular HCO_3^--CO_2 (5 experiments with similar results).

were performed in the presence of 28 mM extracellular HCO_3^-. Extracellular HCO_3^- is known to compete with Cl^- for a common extracellular binding site. In $^{36}Cl^-$-uptake studies we found a K_m for Cl^- of 8 mM in HCO_3^--free medium (15). From these data we can estimate the affinity of HCO_3^- for the extracellular binding site of the anion exchanger[2]. Thus the K_i for extracellular HCO_3^- in PE cells is ~5 mM, in good agreement with affinities found in other epithelia (26).

Na^+-coupled HCO_3^- transport. Recovery of pH_i after an acid load (NH_4^+ preload) was almost totally blocked by 1 mM amiloride in the absence of HCO_3^- (Fig. 8). Thus, in HCO_3^--free solutions, amiloride-sensitive Na^+-H^+ exchange seemed to be the main mechanism responsible for pH_i regulation after an acid load (16). In HCO_3^- Ringer solution (after correction for the higher buffering capacity in HCO_3^- Ringer) a marked increase in acid extrusion (or alkali accumulation) rate was observed (Table 5). In HCO_3^- Ringer solution we found a second mechanism involved in pH_i recovery after an acid load, being DIDS sensitive and Na^+ dependent (Table 5). Two different cotransport systems for Na^+ and HCO_3^- have been described, both sensitive to stilbenes and both involved in regulation of pH_i after an acid load. An electrogenic Na^+-2 (or 3) HCO_3^- cotransport has been found in amphibian (7) and mammalian (18) kidney cells and in the corneal endothelium (19, 33). In barnacle muscle fibers (10), squid axon (5), and snail neuron (31) an electroneutral (Na^+-HCO_3^-)-(Cl^--H^+) transport was involved in pH_i regulation. This system may be identical to the Na^+-dependent Cl^--HCO_3^- exchanger found in fibroblasts (23) and epidermoid cells (29) or the $NaCO_3^-$-Cl^- exchange present in blood cells (4). To differentiate between Cl^--dependent and Cl^--independent Na^+-HCO_3^- cotransport in PE cells we investigated the properties of pH_i regulation in Cl^--depleted PE cells. For these experiments cells were kept either for 50 min (during the dye incubation) in HCO_3^- and Cl^--free solutions (Fig. 1*E*) or for 15 min in HCO_3^--containing, Cl^--free media (Fig. 10). Although we did not measure intracellular Cl^- activities in PE, we can assume from ^{36}Cl-efflux experiments in PE (15) that both procedures reduce intracellular Cl^- activity by at least 80%. In a previous study in corneal endothelium (19) similar Cl^- depletion did not inhibit Na^+-HCO_3^- symport. However,

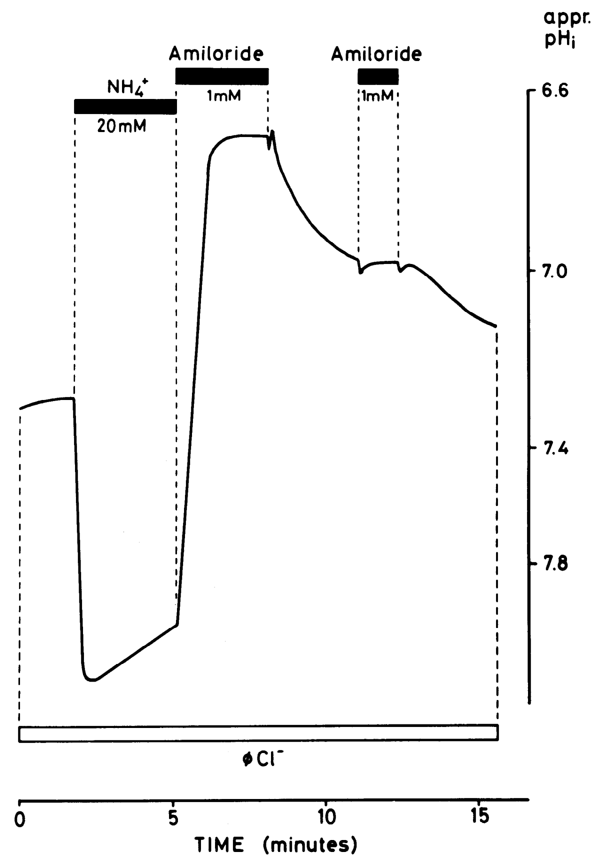

FIG. 10. After dye loading cells were superfused for 15 min with Cl^--free (ϕCl^-) (replaced by cyclamate) HCO_3^--containing solution. Subsequently, effect of 1 mM amiloride on recovery of pH_i after intracellular acid loading induced by NH_4^+ prepulse technique was tested (6 experiments with similar results).

[2] $K_{m\,app} = K_m \cdot [I] \cdot K_i^{-1} + K_m$.

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

in Cl^--depleted PE cells the Na^+-HCO_3^- symport was not operating any more (Figs. 1*E* and 10), suggesting that the Cl^- depletion was sufficient to inhibit Na^+-dependent HCO_3^- influx in PE. Another difference from the Na^+-HCO_3^- symport in the corneal endothelium was found in measurements of membrane potentials in PE. While the symport in the corneal endothelium was clearly electrogenic (33), in PE we could not detect the typical electrical membrane responses of electrogenic Na^+-HCO_3^- symport (Helbig, unpublished observations). Thus there are marked differences between the electrogenic, Cl^--independent Na^+-2 (or 3) HCO_3^- symport in corneal endothelium and the Cl^--dependent Na^+-HCO_3^- symport in the ciliary epithelium.

If the Cl^--dependent cotransport for Na^+ and HCO_3^- is passive, it should be possible to reverse the direction of transport, as has been shown for similar transporters in snail neurone (13) and barnacle muscle (30). Lowering extracellular Na^+ concentration in PE should cause coupled efflux of Na^+ and HCO_3^- and should acidify pH_i. In HCO_3^--free media Na^+ replacement did not lead to a significant additional acidification in the presence of amiloride (Fig. 6*B*). In contrast, in HCO_3^- Ringer solution we found partly amiloride-insensitive and DIDS-sensitive acidification induced by Na^+ replacement (Fig. 6*A*, Table 3). We conclude that DIDS-sensitive Na^+-HCO_3^- transport in PE can be reversed.

Thus PE cells possess two transport systems for HCO_3^-: a Na^+-independent Cl^--HCO_3^- exchanger and a Cl^--dependent Na^+-HCO_3^- transporter. The latter has been shown in squid axon to conform to the kinetics of a $NaCO_3^-$-Cl^- exchanger (5) but in barnacle muscle this ion-pair model was excluded (10). $NaCO_3^-$-Cl^- exchange would require only one binding site on the transport protein on each side of the membrane. Both Cl^--$NaCO_3^-$ and Cl^--HCO_3^- exchange are sensitive to stilbene derivatives and could be mediated by the same transport protein binding either HCO_3^- or $NaCO_3^-$ (4) but would transport alkali equivalents in opposite directions (25). Na^+-dependent Cl^--HCO_3^- exchange has been shown to be activated by intracellular acidification (11), whereas Na^+-independent Cl^--HCO_3^- exchange was activated by increasing pH_i (25, 26). The activation of Na^+-dependent and Na^+-independent Cl^--HCO_3^- exchange cannot be solely explained by pH_i-dependent changes of the intracellular HCO_3^- and $NaCO_3^-$ concentrations. In squid axon ATP-dependent phosphorylation of the transport protein was probably the mechanism of activation of the pH_i-regulating system by intracellular acidification (9). The stimulation of Na^+-dependent and Na^+-independent Cl^--HCO_3^- exchange by opposite pH_i changes suggests either different transport proteins or two different regulatory states of the same protein. However, it is not clear which ion species were transported in PE. Whether Cl^--$NaCO_3^-$ exchange, Na^+-dependent Cl^--HCO_3^- exchange, Cl^--dependent Na^+-HCO_3^- cotransport or (Na^+-HCO_3^-)-(Cl^--H^+) exchange all describe the same transporter has also not been elucidated.

Regulation of steady-state pH_i. Steady-state pH_i in PE cells is more alkaline than would be predicted if protons are passively distributed across the cell membrane at a membrane potential of −46 mV (17). Furthermore, changes in pH_o result in pH_i changes of less than half the amplitude of changes in pH_o (Table 1). Thus PE cells possess mechanisms for regulation of steady-state pH_i. The acidifying action of amiloride during steady state in HCO_3^--free solution (Fig. 6*B*) suggests that Na^+-H^+ exchange in HCO_3^--free medium may be an important (if not the only) regulatory mechanism for steady-state pH_i in HCO_3^--free solution. The Na^+ gradient could extrude protons to even more alkaline pH_i values than 6.98. Thus there should be a regulatory site that "switches Na^+-H^+ exchange on or off" depending on pH_i. Such a regulatory site for internal protons has been described (2). When extracellular media were changed from HCO_3^--free to HCO_3^--CO_2 containing solutions, pH_i approached a new steady-state value that was about 0.15 pH units more alkaline (Fig. 1*A*). Similar differences of steady-state pH_i in HCO_3^--CO_2-free and -containing media have been described by others (1). In HCO_3^--CO_2-containing media amiloride had no effect on steady-state pH_i (Fig. 6*A*), suggesting that Na^+-H^+ exchange was not necessary for regulation of steady-state pH_i in HCO_3^--containing solution. We have shown that the delayed alkalinization on HCO_3^--CO_2 addition was not inhibited by amiloride, but was nearly abolished by DIDS in Cl^--depleted cells and in Na^+-free media (Fig. 1). These are the same characteristics as described above for the HCO_3^- transporter involved in pH_i recovery after NH_4^+ prepulse. HCO_3^- accumulation by Cl^--dependent Na^+-HCO_3^- cotransport seems to be the major process responsible for maintenance of steady-state pH_i in HCO_3^- Ringer solution. The set point for the HCO_3^- transporter and for the Na^+-H^+ exchange, both denoted as "smart" transporters because of their pH_i sensitivity (6), in PE cells is different. Na^+-H^+ exchange will be only activated when pH_i falls to ~0.15 pH units below steady-state pH_i in HCO_3^- Ringer solution, which is maintained by the Na^+-HCO_3^- transport.

The PE cells used in the present study were obtained after transformation with SV-40. This may have changed their physiological properties. However, our results from isotope-uptake experiments provided evidence for Na^+-H^+ exchange (14), Cl^--HCO_3^- exchange (15) and Cl^--dependent Na^+-HCO_3^- symport (unpublished observations) in primary cultured PE. Thus the transporters described in the present study in virus-transformed PE are also present in nontransformed PE.

In conclusion, PE cells possess two HCO_3^- transport systems in addition to Na^+-H^+ exchange: *1*) Na^+-independent Cl^--HCO_3^- exchange involved in pH_i recovery after alkali load, and *2*) Cl^--dependent Na^+-HCO_3^- cotransport responsible for maintenance of steady-state pH_i and regulation of pH_i after an acid load. In addition, these transporters may be involved in transepithelial transport across the ciliary epithelium. Coupled transport of Na^+ and HCO_3^- could be an important mechanism for aqueous humor formation.

The expert technical assistance of M. Koch in making the drawings and A. Krolik in preparing solutions is gratefully acknowledged. We thank D. Sorgenfrei for his invaluable help with electronic problems and Dr. M. T. Coroneo for proofreading the manuscript.

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013

The present work was supported by the Deutsche Forschungsgemeinschaft (Wi 328/11) and by National Eye Institute Grant EY-04873. Additional support was obtained from the Alcon Research Institute.

Address for reprint requests: M. Wiederholt, Institute für Klinische Physiologie, Klinikum Steglitz der Freien Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45, FRG.

Received 30 August 1988; accepted in final form 23 May 1989.

REFERENCES

1. AICKEN, C. C. Direct measurement of intracellular pH and buffering power in smooth muscle cells of guinea pig vas deferens. *J. Physiol. Lond.* 349: 571–585, 1984.
2. ARONSON, P. S., J. NEE, AND M. A. SUHM. Modifier role of internal H^+ in activating the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Nature Lond.* 299: 161–163, 1982.
3. BECKER, B. Carbonic anhydrase and the formation of aqueous humor. *Am. J. Ophthalmol.* 47: 342–361, 1959.
4. BECKER, B. F., AND J. DUHM. Evidence for anionic cation transport of lithium, sodium, and potassium across the human erythrocyte membrane induced by divalent anions. *J. Physiol. Lond.* 282: 149–168, 1978.
5. BORON, W. F. Intracellular pH-regulating mechanism of the squid axon. Relation between the external Na^+ and HCO_3^- dependence. *J. Gen. Physiol.* 85: 325–345, 1985.
6. BORON, W. F. Intracellular pH regulation in epithelial cells. *Annu. Rev. Physiol.* 48: 377–388, 1986.
7. BORON, W. F., AND E. L. BOULPAEP. Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral HCO_3^- transport. *J. Gen. Physiol.* 81: 53–94, 1983.
8. BORON, W. F., AND P. DE WEER. Intracellular pH transients in squid axons caused by CO_2, NH_3, and metabolic inhibitors. *J. Gen. Physiol.* 67: 91–112, 1976.
9. BORON, W. F., E. HOGAN, AND J. M. RUSSEL. pH-sensitive activation of the intracellular-pH regulating system in squid axon by ATP-γ-S. *Nature Lond.* 332: 262–265, 1988.
10. BORON, W. F., W. C. MCCORMICK, AND A. ROOS. pH regulation in barnacle muscle fibers: dependence on extracellular sodium and bicarbonate. *Am. J. Physiol.* 240 (*Cell Physiol.* 9): C80–C89, 1981.
11. BORON, W. F., J. M. RUSSEL, M. S. BRODWICK, D. W. KEIFER, AND A. ROOS. Influence of cyclic AMP on intracellular pH regulation and chloride fluxes in barnacle muscle fibres. *Nature Lond.* 276: 511–513, 1978.
12. COCA-PRADOS, M., AND M. B. WAX. Transformation of human ciliary epithelial cells by simian virus 40: Induction of cell proliferation and retention of β_2-adrenergic receptors. *Proc. Natl. Acad. Sci. USA* 83: 8754–8758, 1986.
13. EVANS, M. G., AND R. C. THOMAS. Acid influx into snail neurones caused by reversal of the normal pH_i regulating system. *J. Physiol. Lond.* 346: 143–154, 1984.
14. HELBIG, H., C. KORBMACHER, S. BERWECK, D. KÜHNER, AND M. WIEDERHOLT. Kinetic properties of Na^+/H^+ exchange in cultured bovine pigmented ciliary epithelial cells. *Pfluegers Arch.* 412: 80–85, 1988.
15. HELBIG, H., C. KORBMACHER, D. KÜHNER, S. BERWECK, AND M. WIEDERHOLT. Characterization of Cl^-/HCO_3^--exchange in cultured pigmented ciliary epithelium. *Exp. Eye Res.* 47: 515–523, 1988.
16. HELBIG, H., C. KORBMACHER, F. STUMPFF, M. COCA-PRADOS, AND M. WIEDERHOLT. Na^+/H^+ regulates intracellular pH in a cell clone derived from bovine pigmented ciliary epithelium. *J. Cell. Physiol.* 137: 384–389, 1988.
17. HELBIG, H., C. KORBMACHER, AND M. WIEDERHOLT. K^+-conductance and electrogenic Na^+/K^+-transport of cultured bovine pigmented ciliary epithelium. *J. Membr. Biol.* 99: 173–186, 1987.
18. JENTSCH, T. J., I. JANICKE, D. SORGENFREI, S. K. KELLER, AND M. WIEDERHOLT. The regulation of intracellular pH in monkey kidney epithelial cells (BSC-1). *J. Biol. Chem.* 261: 12120–12127, 1986.
19. JENTSCH, T. J., C. KORBMACHER, I. JANICKE, D. G. FISCHER, F. STAHL, H. HELBIG, E. J. CRAGOE, JR., S. K. KELLER, AND M. WIEDERHOLT. The regulation of cytoplasmatic pH of cultured bovine corneal endothelial cells in the absence and presence of bicarbonate. *J. Membr. Biol.* 103: 29–40, 1988.
20. KISHIDA, K., T. SASABE, S. IIZUKA, R. MANABE, AND T. OTORI. Sodium and chloride transport across the isolated rabbit ciliary body. *Curr. Eye Res.* 2: 149–152, 1982.
21. KRUPIN, T., P. S. REINACH, O. A. CANDIA, AND S. M. PODOS. Transepithelial electrical measurements on the isolated rabbit iris-ciliary body. *Exp. Eye Res.* 38: 115–123, 1984.
22. KURTZ, I., AND K. GOLCHINI. Na^+-independent Cl^--HCO_3^- exchange in Madin-Darby Canine Kidney cells. *J. Biol. Chem.* 262: 4516–4520, 1987.
23. L'ALLEMAIN, G., S. PARIS, AND J. POUYSSÉGUR. Role of a Na^+-dependent Cl^-/HCO_3^- exchange in regulation of intracellular pH in fibroblasts. *J. Biol. Chem.* 260: 4877–4883, 1985.
24. LOWE, A. G., AND A. LAMBERT. Chloride-bicarbonate exchange and related transport processes. *Biochim. Biophys. Acta* 694: 353–374, 1983.
25. OLSNES, S., J. LUDT, T. I. TØNNESSEN, AND K. SANDVIG. Bicarbonate/chloride antiport in Vero cells: II. Mechanism for bicarbonate-dependent regulation of intracellular pH. *J. Cell. Physiol.* 132: 192–202, 1987.
26. OLSNES, S., T. I. TØNNESSEN, AND K. SANDVIG. pH regulated anion antiport in nucleated mammalian cells. *J. Cell Biol.* 102: 967–971, 1986.
27. REUSS, L., AND J. S. STODDARD. Role of H^+ and HCO_3^- in salt transport in gallbladder epithelium. *Annu. Rev. Physiol.* 49: 35–49, 1987.
28. ROOS, A., AND W. F. BORON. Intracellular pH. *Physiol. Rev.* 61: 296–434, 1981.
29. ROTHENBERG, P., L. GLASER, P. SCHLESINGER, AND D. CASSEL. Activation of Na^+/H^+ exchange by epidermal growth factor elevates intracellular pH in A431 cells. *J. Biol. Chem.* 258: 12644–12653, 1983.
30. RUSSEL, J. M., W. F. BORON, AND M. S. BRODWICK. Intracellular pH and Na fluxes in barnacle muscle with evidence for reversal of the ionic mechanism of intracellular pH regulation. *J. Gen. Physiol.* 82: 47–78, 1983.
31. THOMAS, R. C. The role of bicarbonate, chloride and sodium ions in the regulation of intracellular pH in snail neurones. *J. Physiol. Lond.* 273: 317–338, 1977.
32. TØNNESSEN, T. I., J. LUDT, K. SANDVIG, AND S. OLSNES. Bicarbonate/chloride antiport in Vero cells: I. Evidence for both sodium-linked and sodium-independent exchange. *J. Cell. Physiol.* 132: 183–191, 1987.
33. WIEDERHOLT, M., T. J. JENTSCH, AND S. K. KELLER. Electrogenic sodium-bicarbonate symport in cultured corneal endothelial cells. *Pfluegers Arch.* 405, *Suppl.* I: S167–S171, 1985.
34. ZADUNAISKY, J. A. Transport in eye epithelia: ciliary body and retina pigment epithelium. In: *Membrane Transport in Biology. Transport across Multi-Membrane Systems*, edited by G. Giebisch, D. C. Tosteson, and H. H. Ussing. Berlin: Springer Verlag, 1978, vol. III, p. 337–354.

Downloaded from http://ajpcell.physiology.org/ at DFG on March 18, 2013