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Control of Cell pH in the T84 Colon Cell Line

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Abstract. Cell pH regulation was investigated in the T84 cell line derived from epithelial colon cancer. Cell pH was measured by ratiometric fluorescence microscopy using the fluorescent probe BCECF. Basal pH was 7.17 ± 0.023 ($n = 48$) in HEPES Ringer. After acidification by an ammonium pulse, cell pH recovered toward normal at a rate of 0.13 ± 0.011 pH units/min in the presence of Na^+, but in the absence of this ion or after treatment with 0.1 mM hexamethylene amiloride (HMA) no significant recovery was observed, indicating absence of Na^+ independent H^+ transport mechanisms in HEPES Ringer. In CO_2/HCO_3^- Ringer, basal cell pH was 7.21 ± 0.020 ($n = 35$). Changing to HEPES Ringer, a marked alkalinization was observed due to loss of CO_2, followed by return to the initial pH at a rate of −0.14 ± 0.012 ($n = 8$) pH/min; this return was retarded or abolished in the absence of Cl^- or after addition of 0.2 mM DIDS, suggesting extrusion of bicarbonate by Cl^-/HCO_3^- exchange. This exchange was not Na^+ dependent. When Na^+ was added to cells incubated in 0 Na^+ Ringer while blocking Na^+/H^+ exchange by HMA, cell alkalinization by 0.19 ± 0.04 ($n = 11$) pH units was observed, suggesting the presence of Na^+/HCO_3^- cotransport carrying HCO_3^- into these cells, which was abolished by DIDS. These experiments, thus, show that Na^+/H^+ and Cl^-/HCO_3^- exchange and Na^+/HCO_3^- cotransport participate in cell pH regulation in T84 cells.

Key words: T84 colon cells — Cell pH — Na^+/H^+ exchange — Cl^-/HCO_3^- exchange — Na^+/HCO_3^- cotransport

Introduction

The mammalian colon is an important site of water and electrolyte absorption. The mechanisms of this transport have been detailed in a number of investigations (Rajendran, Geibel & Binder, 1995; Singh et al., 1995*a;* Greger et al., 1997). Several colon cell lines, such as Caco2 and T84, have been used to study such mechanisms "in vitro." It has been shown that these cells present several properties of epithelial tissue such as polarity and intercellular connections (Dharmsathaphorn et al., 1984; Osypiw et al., 1994; Tai et al., 1996; Chanson, White & Garber, 1996). Studies on several ion channels present in these cells, as well as on aspects of volume regulation and action of peptide hormones and neurotransmitters on their ion transport properties, are available (Worrel et al., 1989; Santos & Reenstra, 1994; Dagher et al., 1994; Devor & Frizzell, 1998).

These findings show that the cell lines discussed above present several properties common to cells studied "in vivo," indicating that they may be used as cell models for intestinal epithelium. However, cells in culture frequently lose some of the properties seen in the tissues from which they originate. Thus, some cultured fibroblast lines lack the Na^+/H^+ exchanger that is found in the "in vivo" tissue, a property that was found to be valuable for the expression of exogenous cloned exchangers, which has contributed much to the study of the molecular biology of these transporters (Wakabayashi, Shigekawa & Pouyssegur, 1997). On the other hand, MDCK cells, originated from dog renal tissue, present most acid-base transporters found in intercalated cells from the mammalian collecting duct, such as Na^+/H^+ exchanger isoforms, vacuolar H^+-ATPase and H^+-K^+ ATPase (Vilella et al., 1992; Fernández & Malnic, 1998).

A number of studies on transport of components of the acid-base system in Caco-2 cells have been reported. These cells contain Na^+/H^+ and HCO_3^- transporters and a H^+-K^+ ATPase in their cell membrane (Abrahamse, Bindels & Van Os, 1992; Osypiw et al., 1994). However, for T84 cells little is known about the mechanisms regulating cell pH and transcellular H^+ and HCO_3^- transport (Arreola, Melvin & Begenisich, 1995).

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In the present work, we will use the T84 cell line, derived from human colon cancer, in order to investigate the most important mechanisms involved in the regulation of its cell pH as well as in transepithelial H^+ and HCO_3^- transport, both in media containing bicarbonate/CO_2 and in nominally bicarbonate/CO_2-free media.

Materials and Methods

Cell Culture

T84 cells, derived from colon carcinoma, were obtained from American Type Culture Collection and kept in Dulbecco's Modified Eagle's Medium, DMEM/F12 (Gibco, Grand Island, NY), supplemented with 14.5 mM $NaHCO_3$, 15 mM HEPES, 5% fetal bovine serum (Cultilab, Campinas, SP), 50.000 IU/l penicillin G and 100 mg/l streptomycin, adjusted to pH 7.4 (Dharmsathaphorn et al., 1984). The cultivated cells were kept in a CO_2 incubator at 5% CO_2 and 37°C. The cells were grown in plastic flasks of 25 cm^2 area until reaching a confluence of 75–90%, which occurred after 5 to 7 days of culture. Culture medium was changed 3 times per week, and cell growth was followed on an inverted microscope. After confluence was reached, the monolayer was treated with 0.05% trypsin, 0.53 mM EDTA and phosphate buffer (PBS) to ressuspend the cells and to transfer them to new flasks for further multiplication or to cultivate them on small glass cover slides (1.3 × 2.4 cm) for utilization in the experiments. Cells of passage 68–78 were used.

Measurement of Cell pH

The glass slides containing the monolayers of T84 cells were mounted in a thermoregulated chamber (Warner, Hamden, CT) on an inverted fluorescence microscope (Nikon Diaphot mod. TMD). The cells were incubated for 30 min at 37°C with the fluorescent pH probe 2,7-bicarboxyethyl-5,6-carboxyfluorescein acetoxy-methyl ester (BCECF-AM) (Molecular Probes, Eugene, OR), 10 μM (Kurtz & Golchini, 1987). They were then superfused by gravity at 3 ml/min and 37°C with the experimental solutions using an electromechanic switching system (Heater and Valve Controller, Yale University Electronics Shop, New Haven, CT). Cell pH was calculated from fluorescence ratios measured at excitation of 495/440 nm and emission at 530 nm using a Georgia Instruments PMT-400 photomultiplier system, as described previously (Fernández & Malnic, 1998). An area of 260 μm diameter was read, including a number of approximately 200–300 cells. Measurements were performed at 2.5 sec intervals for a period of 300 mS per measurement. Cell pH was calibrated by the nigericin method (Thomas et al., 1979) using 10 μM nigericin in a solution containing (in mM): 130 KCl, 20 NaCl, 1 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES, adjusted to pH in a range between 6 and 8. When calibrating cell pH in bicarbonate/CO_2 containing solutions, an equimolar amount of NaCl was substituted by $NaHCO_3$ at 5% CO_2, pH being adjusted by modifying $NaHCO_3$ concentrations. Rates of pH change ($\Delta pH/\Delta t$) were obtained from the linear slope between 0 and 2 min after solution change.

Solutions

The composition of the different solutions used in the present experiments is given on Table 1. Nominally CO_2/HCO_3^- free solutions were buffered by HEPES. Bicarbonate containing solutions were equilibrated with 5% CO_2/95% O_2. Osmolality was kept between 290 and 310 mOsm, and temperature at 37°C.

Reagents

The culture medium, DMEM/F12, l-glutamine, penicillin, streptomycin, EDTA-trypsin and PBS (phosphate-buffered saline) were obtained from Gibco, Grand Island, NY. Fetal bovine serum was obtained from Cultilab, Campinas, SP. BCECF-AM was obtained from Molecular Probes, Eugene, OR. 4-4′-Diisothiocyanatostilbene 2-2′-disulfonic acid (DIDS), hexa-methylene amiloride (HMA) and the remaining reagents were obtained from Sigma (St. Louis, MO).

Statistics

The data are presented as mean ± SE. Comparisons between two means were performed by Student's *t* test; when more than two means were compared, analysis of variance was used, followed by the contrast test of Tukey-Kramer, using the Prism/Instat software (Graphpad, San Diego, CA). Differences were considered significant if $P < 0.05$.

Results

Basal pH and Recovery from an Ammonium Pulse

In Krebs Ringer buffered by Hepes (solution 1, see Table 1) at pH 7.4, without CO_2/HCO_3^-, basal pH was 7.17 ± 0.023 (n = 48), and in bicarbonate buffer at pH 7.4, solution 4, cell pH was 7.21 ± 0.020 ($n = 35$), not significantly different from the value obtained in the absence of CO_2/HCO_3^-. In Ringer Hepes, the ammonium pulse caused an alkalinization (*see* Fig. 1), and when the solution was returned to normal Ringer, a marked acidification was observed, which reached a mean value of 6.57 ± 0.058 ($n = 17$). After this pulse, the cells started to recover toward their normal pH at a rate of 0.13 ± 0.011 ($n = 17$) pH units per min. (*see* Fig. 1), reaching a pH of 7.04 ± 0.083 ($n = 17$), that is, 0.47 ± 0.040 ($n = 17$) units above the most acid level after the ammonium pulse. This behavior is similar to that observed in other tissues (Singh et al., 1995*b;* Fernández & Malnic, 1998).

Role of Na^+ in pH_i Recovery

The Na^+/H^+ exchanger is one of the most ubiquitous and important mechanisms responsible for the recovery of cell pH after an acid pulse (Chen & Boron, 1995). The role of this exchanger was investigated by substituting the ammonium Ringer (solution 2) by a Ringer solution in which Na^+ was substituted by n-methyl-d-glucamine (NMDG), solution 3. As observed in Fig. 1, under

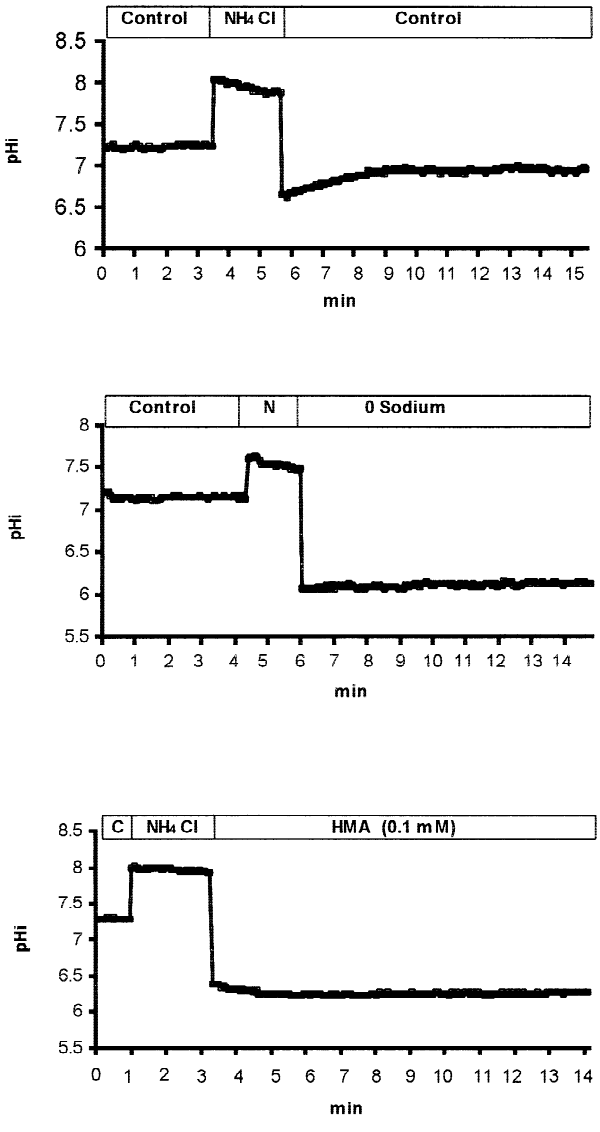

Fig. 1. *Upper graph:* representative example of the effect of ammonium pulse (solution 2, Table 1) on cell pH in T84 cells in HEPES Ringer (solution 1), and recovery of pH in the presence of Na^+. *Middle graph:* role of Na^+/H^+ exchange in cell pH recovery after acid (ammonium) pulse in the absence of Na^+. N, NH_4^+ Ringer (solution 2). (0 Sodium, superfusion with 0 Na^+ Ringer (sol. 3). *Lower graph:* effect of 0.1 mM hexamethylene amiloride (HMA) on pH recovery after ammonium pulse.

these conditions the recovery of cell pH was entirely abolished in HEPES 0 Na^+ Ringer (sol. 3), the pH remaining at 6.15 ± 0.075 ($n = 7$), with a change of −0.00188 ± 0.0067 pH units/min during the following minutes (Fig. 1). This finding was confirmed by showing that Ringer solution containing 0.1 mM hexamethylene amiloride (HMA), a specific inhibitor for Na^+/H^+ exchange (Kleyman & Cragoe, 1988), abolished cell pH recovery after an acid pulse in the same way as the 0 Na^+ solution (*see* Fig. 1). Here, the acid level of

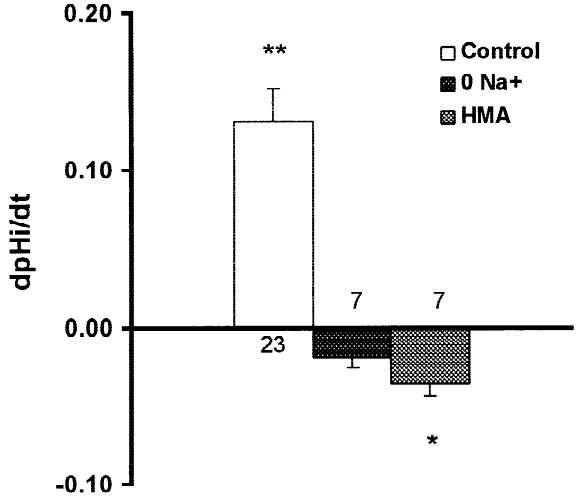

Fig. 2. Mean values of rates of cell pH recovery (dpH/dt) during superfusion with HEPES Ringer with and without Na^+ (substituted by NMDG) and after administration of 0.1 mM HMA in NaCl-HEPES Ringer.

6.18 ± 0.15 ($n = 6$) was kept for a prolonged time, cell pH changing at a rate of −0.036 ± 0.004 ($n = 6$) in the first minutes following the acid pulse. Although the pH after the acid pulse was lower than in controls in these two groups, a situation that stimulates the rate of pH recovery in control medium, recovery was entirely abolished. This change in pH level was probably due to inhibition of Na^+/H^+ exchange. These data are summarized in Fig. 2. The lack of pH recovery shows that no evidence for the presence of other H^+ extrusion mechanisms was found in these experiments.

Regulation of Cell pH in Presence of CO_2/HCO_3^-

In the following, the presence and role of bicarbonate transporters when the T84 cells were bathed in CO_2/HCO_3^- Ringer solution (solution 4, Table 1) was investigated. After 20 min in this solution, it was substituted by Hepes Ringer without CO_2/HCO_3^-, which caused marked alkalinization of cell pH, which reached a mean value of 7.78 ± 0.09 ($n = 8$), that is, 0.60 ± 0.04 pH units higher than the basal value of 7.18 ± 0.06 ($n = 8$). This rise in pH may be due to loss of CO_2 and maintenance of part of the HCO_3^- present in CO_2/HCO_3^- medium, within the cells. After this increase, pH started to fall progressively, as shown in Fig. 3, at a rate of −0.14 ± 0.012 ($n = 8$) pH units/min, which may be due to bicarbonate efflux from the cells (Kurtz et al., 1987) (*see* Table 2). When a similar experiment is performed, but using a Cl^--free Hepes Ringer (solution 5), or in the presence of Cl^- but adding 0.2 mM DIDS, the rate of pH fall during superfusion with the bicarbonate-free solution showed a marked reduction, indicating that under these conditions

Table 1. Solutions used in experiments with T84 cells

	Sol. 1 Control	Sol. 2 NH_4Cl	Sol. 3 0 Na^+	Sol. 4 HCO_3^-	Sol. 5 0 Cl^-	Sol. 6 0 Cl^-	Sol. 7 0 Na^+
NaCl	141.0	121.0	—	120.0	—	—	—
KCl	5.4	5.4	5.4	5.4	—	—	—
$CaCl_2$	1.0	1.0	1.0	1.0	—	—	1.0
KH_2PO_4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
$MgCl_2$	0.5	0.5	0.5	0.5	—	—	0.5
$MgSO_4$	0.4	0.4	0.4	0.4	1.0	1.0	0.4
Na_2HPO_4	0.3	0.3	—	0.3	0.3	0.3	—
HEPES	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Glucose	0.6	0.6	0.6	0.6	0.6	0.6	0.6
NH_4Cl	—	20.0	—	—	—	—	—
NMDG	—	—	141.3	—	—	—	120.0
$NaHCO_3$	—	—	—	20.0	—	20.0	—
Na^+ gluconate	—	—	—	—	141.0	120.0	—
Ca^{2+} gluconate	—	—	—	—	5.0	5.0	—
K^+ gluconate	—	—	—	—	5.4	5.4	—
$KHCO_3$	—	—	—	—	—	—	20.0
pH	7.4	7.4	7.4	7.4	7.4	7.4	7.4

Concentrations in mM.

the efflux of bicarbonate was impaired (Fig. 3 and Table 2). These data are compatible with the presence of a Cl^-/base or, more specifically, a Cl^-/HCO_3^- exchanger on the plasma membrane of the T84 cells. Figure 4 shows an additional experiment supporting the presence of Cl^-/HCO_3^- exchange: the cells were superfused by CO_2/HCO_3^- Ringer (solution 4), an ammonium pulse was applied, then returned to solution 4 but with HMA, in which no recovery was found. When superfusing with 0 Cl^- Ringer, the cells alkalinized, probably due to exchange of Cl^- leaving the cell (along its concentration gradient) against HCO_3^- moving into the cell. When Cl^- is added again to the external medium, pH returns toward the previous level.

To evaluate the Na^+ dependence of this exchange mechanism, similar experiments were performed in CO_2/HCO_3^- Ringer to which 0.1 mmol/l HMA was added. Changing to HEPES Na^+ Ringer + HMA, after alkalinization the rate of return to basal pH was -0.077 ± 0.010 ($n = 12$) pH units/min, while when 0 Na^+ Ringer was used, pH change was -0.057 ± 0.012 ($n = 11$) pH/min. Although the absolute magnitude of these values was smaller when compared to the series without HMA, probably due to the cell acidification found under these conditions, these values were not significantly different, indicating no Na^+ dependence of the Cl^-/HCO_3^- exchanger.

The described results, although compatible with Cl^-/HCO_3^- exchange, might also be due to the exchange of Cl^-/OH^-, which has been described in rat colon crypts (Rajendran & Binder, 1999). To investigate this possibility, four cell monolayers were initially superfused with a HEPES-buffered, nominally CO_2/HCO_3^- free Cl^- containing solution (solution 1, Table 1), in which the control cell pH was 7.20 ± 0.015. Subsequently the cells were superfused by 0.1 mM HMA containing solutions in order to inhibit Na^+/H^+ exchange, in the presence or absence of Cl^- (Cl^- substituted by gluconate). In the presence of Cl^-, cell pH was 6.42 ± 0.14 ($n = 4$), and in the absence of Cl^- cell pH was 6.11 ± 0.22 ($n = 4$). If Cl^-/OH^- exchange were present, cell pH would be expected to increase in Cl^- free medium due to OH^- penetration into cells in exchange for exit of internal Cl^-. Note that in Fig. 4 pH started to rise markedly starting from a similar pH level when Cl^- was removed in the presence of bicarbonate. These data provide no evidence for the presence of Cl^-/OH^- exchange in this preparation, indicating that base efflux from the cells as shown in Fig. 3 is due to Cl^-/HCO_3^- exchange.

To investigate the presence of a possible Na^+/HCO_3^- cotransporter, the cells were superfused with bicarbonate Ringer containing 0.1 mM HMA to eliminate the function of the Na^+/H^+ exchanger. During the first 20 min, the cells were superfused with such a solution with 0 Na^+ (solution 7), which led to an acid basal value of 6.69 ± 0.018 ($n = 11$) (*see* Fig. 5). Then, the solution was switched to a similar one but containing Na^+ (solution 4), which caused a significant alkalinization (*see* Table 3), probably due to influx of HCO_3^- via the Na^+/HCO_3^- cotransporter. Finally, returning to solution 7, cell pH returned toward the initial value, probably by efflux of HCO_3^- in exchange for Cl^-. Here, the observed alkalinization was not caused by loss of CO_2, since CO_2 plus HCO_3^- were kept in the bath along the whole experiment. It was caused by the addition of Na^+ in the presence of external HCO_3^- and during the continued inhibition of Na^+/H^+ exchange, which suggests the presence of a Na^+/HCO_3^- cotransporter carrying HCO_3^- into the cell.

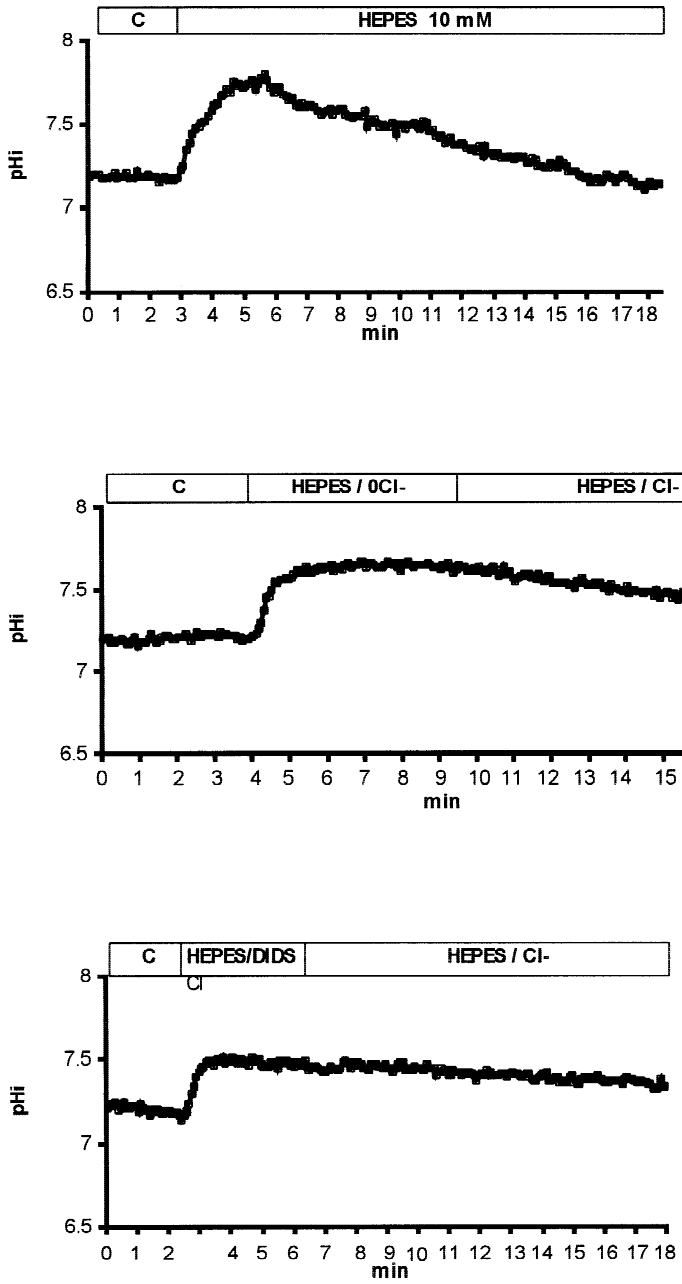

Fig. 3. Role of bicarbonate transport in cell pH regulation in T84 cells. *Upper graph:* control period (C): HCO_3^-, HCO_3^-/CO_2 Ringer (solution 4). HEPES 10 mM, Hepes NaCl Ringer (solution 1). *Middle graph:* after superfusion with HCO_3^-/CO_2 Ringer (C, sol. 4) and change to Hepes Ringer, no pH recovery was observed in the absence of Cl^- (solution 5), but recovery starts after addition of Cl^--Ringer (HEPES/Cl^-). *Lower graph:* the addition of 0.2 mM DIDS to HEPES Ringer also reduced pH recovery, which was only slowly reversible when returning to control Hepes-Ringer.

Discussion

The T84 cell line, derived from a colon cancer, has been used quite extensively as a model for colon electrolyte transport and its regulation; extensive information about these processes is available (Dharmsathaphorn et al., 1984; Worrel et al., 1989; Dagher et al., 1994; Devor & Frizzell., 1998). However, little is known about the regulation of cell pH in this cell line. Considering the role of epithelial colon cells in contributing to the maintenance of the pH of extracellular fluid as well as the importance of the maintenance of cytoplasmic pH of these cells, we decided to investigate these aspects using fluorescence microscopy and ratiometric cell pH determination in order to obtain information about the main acid-base transporters present in their plasma membrane.

Our results concerning stationary cell pH and recovery from an ammonium pulse are comparable to data obtained with other intestinal cell lines, such as IEC-6 (Wenzl et al., 1989), CACO-2 (Watson et al., 1991) and HT29 cells (Koettgen et al., 1994). An interesting feature of the T84 cells when kept in nominally CO_2/HCO_3^- free medium, is the apparent dependence of H^+ extrusion only on Na^+/H^+ exchange, since in Na^+ free medium or after treatment with HMA, an amiloride analogue with high specificity for this exchanger, no recovery of cell

Table 2. Cell pH in T84 cells in HCO_3^-/CO_2 medium, and after alkalinization by superfusion with CO_2/HCO_3^- free, HEPES buffered Ringer; role of Cl^- and DIDS in pH recovery

Condition	Control pH_i HCO_3^-/CO_2	pH_i HEPES	Δ pH_i	dpH/dt	*n*
HEPES + Cl^-	7.18 ± 0.06	7.78 ± 0.09	0.60 ± 0.04	−0.14 ± 0.012	8
HEPES + 0 Cl^-	7.22 ± 0.04	7.54 ± 0.03*	0.32 ± 0.06*	−0.04 ± 0.016*	6
HEPES + DIDS	7.18 ± 0.03	7.56 ± 0.06*	0.38 ± 0.06*	0.017 ± 0.019*	7

Means ± SE; *n*, number of experiments. ΔpHi = pH_i HEPES − pH_i HCO_3^-/CO_2. dpH/min, rate of acidification or alkalinization (−) during superfusion with HEPES Ringer, in the presence or absence of Cl^-, and with 0.2 mM DIDS.
*$P < 0.05$ *vs.* HEPES + Cl^-.

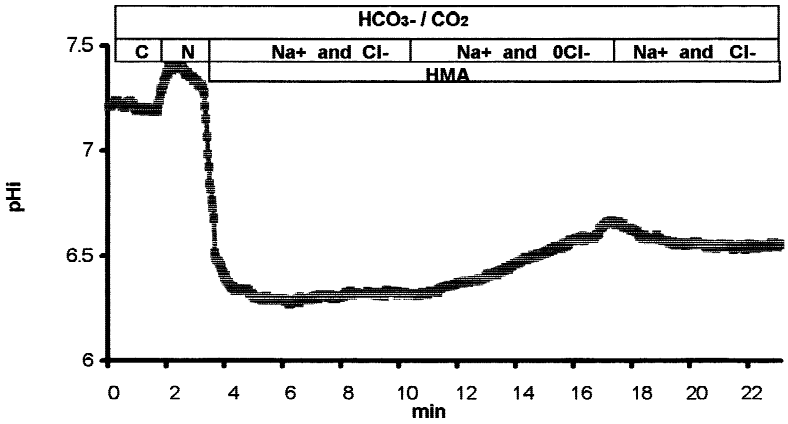

Fig. 4. Superfusion of T84 cells with HCO_3^-/CO_2 Ringer (C) and application of an ammonium pulse (N), followed by 0.1 mM HMA. When Cl^- is removed from the solution (sol. 6), the cells alkalinize, probably by HCO_3^- influx via the Cl^-/HCO_3^- exchanger. Returning to Cl^- Ringer (sol. 4) the system returns toward its original state, extruding bicarbonate via the exchanger and thereby reducing pH.

pH is observed after the acidification caused by the ammonium pulse (*see* Fig. 1). A similar behavior had been observed in IEC-6 and HT29 cells.

The absence of cell pH recovery after an acid pulse in Na^+-free medium (*see* Fig. 1) suggests that other mechanisms for H^+ extrusion such as the vacuolar H^+-ATPase and H^+-K^+ ATPase, are apparently not present in the cell membranes of T84 cells. The vacuolar H^+-ATPase has not been found in a number of other cells derived from intestinal epithelia (Sabolic et al., 1997; Feldman & Ickes, Jr., 1997). On the other hand, H^+-K^+ ATPase has been found in colon cells (Sangan et al., 1997). These transporters, however, have been described in cultured cells of renal origin (Oberleithner et al., 1990; Fernández & Malnic, 1998; Sangan et al., 1999).

The colon is known to absorb or secrete chloride and bicarbonate ions, and the presence of Cl^-/HCO_3^- exchange and Na^+-HCO_3^- cotransport has been described in this intestinal segment (Rajendran & Binder, 1999). As shown in Fig. 3, the role of HCO_3^-/CO_2 in T84 cells was studied by preincubating the cells in medium containing these components, and then substituting this medium by a HCO_3/CO_2 free solution buffered by Hepes. This leads to a marked initial alkalinization due to loss of CO_2, followed by acidification, which might be due to reaction of cell HCO_3^- with H^+ generated by metabolism, or due to outflux of the HCO_3^- remaining within the cells. We demonstrated that this outflux depended on Cl^- in the extracellular environment, and that it was impaired by DIDS, an anion-exchange blocker (Fig. 3 and Table 2). Under such conditions, the cells remained alkaline for a markedly prolonged period. These findings are compatible with the presence of a Cl^-/HCO_3^- exchanger in the plasma membrane of T84 cells. This ion-exchanger had been detected in colon crypt cells, being responsible for approximately $\frac{1}{4}$ of Cl^- reabsorption by the colon (Davis et al., 1983). The presence of Cl^-/HCO_3^- exchange has also been detected in the intestinal cell lines HT_{29} and IEC-6, with the function of extrusion of bicarbonate after an alkaline load (Wenzl et al., 1989; Koettgen et al., 1994). It has been shown recently that the isoforms AE-2 and AE-3 of the anion exchanger are expressed only at the basolateral membrane of colon cells (Alrefai et al., 1997). Besides this evidence favoring the presence of Cl^-/HCO_3^- exchange in T84 cells, in our study no evidence for the presence of Cl^-/OH^- exchange was obtained, since cell pH did not change significantly in bicarbonate/CO_2-free medium in which Cl^- ions were substituted by gluconate, during continued inhibition of Na^+/H^+ exchange by HMA. It is true that in these experimental conditions cell pH is considerably below normal levels due to inhibition of Na^+/H^+ exchange, thus reducing cellular OH^- levels and possibly

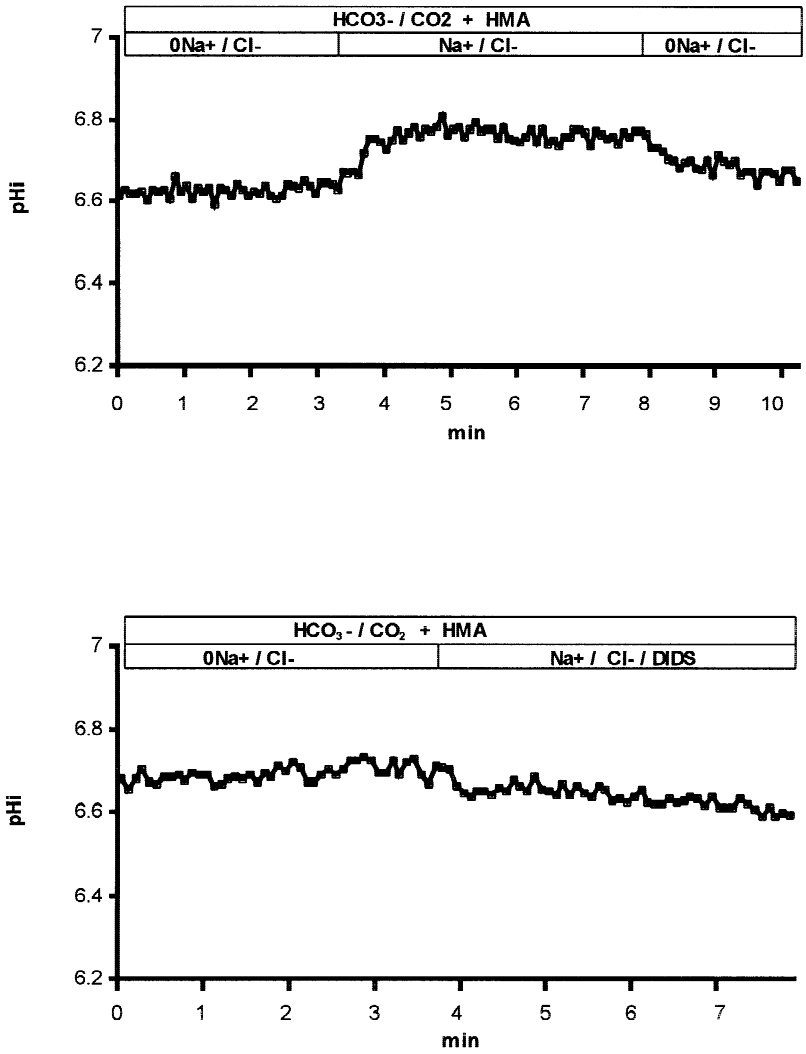

Fig. 5. Evidence for the presence of a Na^+/HCO_3^- cotransporter in T84 cells. *Upper graph:* superfusion with HCO_3^-/CO_2 Ringer with 0 Na^+ (sol. 7) plus 0.1 mM HMA. A significant alkalinization is observed when Na^+ Ringer is superfused (sol. 4), which is reverted when returning to 0 Na^+, (sol. 7). With sol. 4, cells are loaded with bicarbonate by the cotransporter. *Lower graph:* the observed alkalinization is abolished when DIDS is added to solution 4 (Na^+ Ringer).

Table 3. Role of Na^+/HCO_3^- cotransport in T84 cell pH regulation

Experiment	0 Na^+/HMA	Na^+/HMA	Δ pH_i	*n*
Control	6.69 ± 0.018	6.88 ± 0.040*	0.19 ± 0.04	11
With DIDS (0.2 mM)	6.72 ± 0.016	6.72 ± 0.025$^{@}$	0.01 ± 0.02#	4

Cells were superfused with 0 Na^+ CO_2/HCO_3^- Ringer, followed by Na^+ Ringer, both containing 0.1 mM HMA.
Means ± SE, *n*, number of used monolayer preparations. $\Delta pH_i = (pH + Na^+) - (pH\ 0\ Na^+)$
DIDS, 4,4-diisothiocyanatostilbene-2,2-disulfonate; HMA, Hexamethylene-amiloride (0.1 mM)
*$P < 0.05$ *vs.* pH 0 Na^+, $^{@}P < 0.05$ *vs.* pH Na^+ control, #$P < 0.05$ *vs.* ΔpH_i control.

inhibiting anion exchange. As a consequence, we could be underestimating Cl^-/OH^- exchange. However, Fig. 4 shows that in bicarbonate Ringer at similar cell pH the removal of Cl^- leads to a marked increase in cell pH. Nevertheless, in the absence of bicarbonate cell pH did not increase, as would be expected for a Cl^-/OH^- exchange mechanism. It has to be remembered, in addition, that at the pH of 7.4 of the superfusing solution bicarbonate concentration is much higher than that of OH^-.

In several cell lines and tissues, Na^+ dependent Cl^-/HCO_3^- exchange mechanisms have been described (Orsenigo, Tosco & Faelli, 1994; Kim, Brokl & Dantzler, 1997). We have not found a significant Na^+ dependence

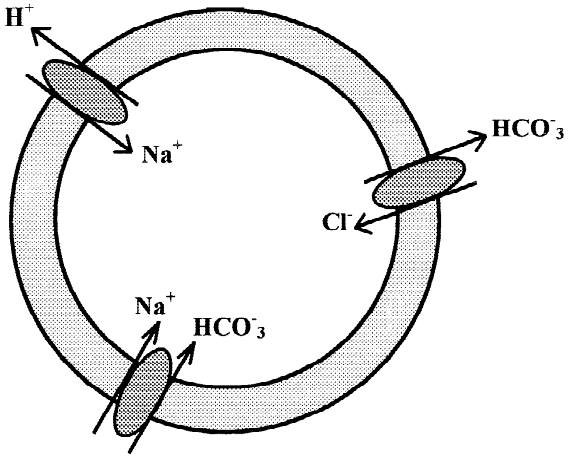

Fig. 6. Schematic drawing of T84 cell presenting in its membrane a Na^+/H^+ and a Cl^-/HCO_3^- exchanger, as well as a Na^+/HCO_3^- cotransporter. Cell pH depends on the balance of these transporters.

of the exchanger in T84 cells in the present study, which is in accordance with previous studies on colonocites (Calonge & Ilundáin, 1998).

In addition, studies of the regulation of cell pH in CO_2/HCO_3^- containing medium in HMA-pretreated T84 cells, that is, after blocking Na^+/H^+ exchange, have shown the presence of a Na^+ and HCO_3^- dependent mechanism of cell pH regulation (*see* Fig. 5), independent of changes in Cl^- concentration. When the cells were perfused under these conditions in Na^+-free solution, their pH was reduced to a mean value of 6.69, and the addition of Na^+ caused an increase of 0.19 ± 0.04 (n = 11) pH units, suggesting flow of $NaHCO_3$ into the cells, by means of a Na^+/HCO_3^- cotransporter, as proposed for other cell systems (Akiba et al., 1986; Lopes et al., 1987; Townsley & Machen, 1989). It has also been shown that this cotransporter may work in the direction of charging cells with HCO_3^-, which is equivalent to acid extrusion, in experimental conditions similar to those described above (Wenzl et al., 1989). On the other hand, this cotransporter has been described in several tissues to work as a HCO_3^- extruder, particularly in acid secreting epithelia such as the renal tubule, with a $3HCO_3^-/1Na^+$ stoichiometry (Boron & Boulpaep, 1983; Yoshitomi, Burckhardt & Froemter, 1985). However, when working in the opposite direction (alkalinizing the cell) this stoichiometry may be 2:1 (Planelles, Thomas & Anagnostopoulos, 1993).

The schematic drawing of Fig. 6 illustrates the function of the acid/base transporters that we have detected in T84 cells. They regulate their pH by means of Na^+/H^+ exchange in nominally bicarbonate/CO_2 free media (buffered by HEPES), no evidence for the activity of other H^+ transporters having been found under these conditions. The present experimental setup does not allow us to determine on which of the cell surfaces the observed transporters are located. However, it must be remembered that in colon cells in general the NHE1 isoform is located at the basolateral membrane (Vilella et al., 1992), while at the apical membrane the presence of NHE2 and NHE3 has been described (Greger et al., 1997). On the other hand, in media buffered by bicarbonate/CO_2, the presence of Na^+ independent Cl^-/HCO_3^- exchange as well as of Na^+/HCO_3^- cotransport was demonstrated. No evidence for Cl^-/OH^- exchange was obtained. Since colon cells have been shown to be able to both secrete and reabsorb bicarbonate, these transport mechanisms may be located on both cell surfaces. However, T84 cells have been shown to be predominantly secretory (Barrett, 1993). Therefore, the Na^+/HCO_3^- cotransporter is expected to be located mainly on the basolateral membrane, and the Cl^-/HCO_3^- exchanger predominantly on the luminal membrane.

In Fig. 6, only acid/base transport mechanisms are shown. It is known that Cl^- channels, including CFTR, are present in T84 cells (Barrett, 1993). They may participate in acid-base balance by interacting with the Cl^-/HCO_3^- exchanger. This possibility will be approached in a future paper.

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