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HCO_3^--dependent ion transport systems and intracellular pH regulation in colonocytes from the chick

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Abstract

The current study examines the presence of the Na^+/HCO_3^- cotransporter and of the Cl^-/HCO_3^- exchanger in chicken colonocytes and their role in cytosolic pH (pH_i) homeostasis. pH_i was measured with 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF) at 25°C. Basal pH_i was 7.16 in HEPES-buffered solutions and 7.06 in those buffered with HCO_3^-. Removal of external Cl^- increased pH_i and Cl^- reinstatement brought the pH_i towards resting values. These Cl^--induced pH_i changes were Na^+-independent, inhibited by H_2-DIDS and faster in the presence than in the absence of HCO_3^-. Cells recovered from alkaline loads by a mechanism that was Cl^--dependent, Na^+-independent and inhibited by H_2-DIDS. This rate of Cl^--dependent cell acidification decreased as the pH_i decreased, with a Hill coefficient value close to 4. Removal of external Na^+ decreased pH_i and readdition of Na^+ brought pH_i towards the control values. The rate of the Na^+-induced changes was not modified by the presence of HCO_3^- and was prevented by EIPA and unaffected by H_2-DIDS. In the presence of EIPA cells partially recovered from a moderate acid load only when both Na^+ and HCO_3^- were present. The EIPA resistant Na^+- and bicarbonate-dependent pH_i recovery was inhibited by H_2-DIDS and occurred at equal rates in both Cl^--containing and Cl^--free solutions. It is concluded that in chicken colonocytes bathed in HCO_3^--buffered solutions, both the Na^+/H^+ exchanger and the Cl^-/HCO_3^- exchanger participate in setting the resting pH_i value. The latter transporter helps the cells to recover from alkaline loads and the first transporter, together with the Na^+/HCO_3^- cotransporter, is involved in pH_i recovery from an acid load.

Keywords: Colonocyte; Intracellular pH; pH_i; Cl^-/HCO_3^- exchanger; Na^+/HCO_3^- cotransporter

1. Introduction

The concentration of cytosolic H^+ is finely regulated. In the short term, intracellular pH (pH_i) regulation depends on different intracellular buffers. In the long term, pH_i is maintained by proton transport across the cell membrane [1–5]. Several studies have investigated the mechanisms involved in pH_i regulation of epithelial cells (see Ref. [3] for review). However, studies on colonocytes pH_i regulation have been scarce and limited to either culture cell lines derived from mammalian colon [6–8] or to mammalian colonic crypt cells [9,10].

Previous studies [11] designed to investigate the role of the Na^+/H^+ exchanger in pH_i regulation of

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colonocytes isolated from the chick were carried out in the absence of CO_2/HCO_3^- to minimize the contribution of HCO_3^--dependent mechanisms. Studies done under more physiological conditions, that is in the presence of bicarbonate, have revealed the presence in chicken colonocytes of Cl^-/HCO_3^- exchange and Na^+/HCO_3^--cotransport, both involved in pH_i homeostasis.

2. Materials and methods

2.1. Solutions

Solutions composition is shown in Table 1. HCO_3^--free solutions were titrated to pH = 7.4 with HEPES–Tris and equilibrated at room temperature with air. HCO_3^--buffered solutions were equilibrated with 95% O_2/5% CO_2 to yield pH 7.4. In experiments with gluconate, the calcium concentration was increased to 6 mmol l^{-1}.

2.2. Intracellular pH measurements

Hubbard chickens, four- to six-weeks old, were killed by decapitation. Colonocytes were isolated by hyaluronidase incubation as described in Ref. [12]. Cell viability was assessed by determining the fraction of the cell population able to exclude 0.2% trypan blue and usually ranged from 60% to 75%.

Table 1
Composition of solutions (mM)

	A	B	C	D	E	F	G	H
Mannitol	100	100	100	260	100	100	100	240
NaCl	80	0	0	0	55	0	0	0
$CaCl_2$	1	0	1	0	1	0	1	0
$MgCl_2$	1	0	1	0	1	0	1	0
K_2HPO_4	3	3	3	3	3	3	3	3
$NaHCO_3$	0	0	0	0	25	25	0	0
HEPES–Tris	20	20	20	20	5	5	5	5
CholineCl	0	0	80	0	0	0	55	0
$CholineHCO_3$	0	0	0	0	0	0	25	25
NaGluconate	0	80	0	0	0	55	0	0
MgGluconate	0	1	0	1	0	1	0	1
CaGluconate	0	6	0	1	0	6	0	1

All solutions contained (in mM) 0.5 β-hydroxybutyrate, 10 fructose and 1 L-glutamine.

pH_i was measured fluorimetrically at 25°C with 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF), as described in Ref. [13]. Fluorescence ratios (500/450) were correlated with pH_i at the end of each recording session by permeabilizing the cell membrane with 70 μM digitonin and constructed a calibration curve. We have previously reported for chicken colonocytes that the digitonin-based calibration, as compared to the nigericin null point procedure, underestimates the actual pH_i values by 0.15 pH units [11]. Therefore, a correction of 0.15 pH units was applied to the results.

The initial pH_i change after an experimental maneuver is defined as the change in pH_i that occurred during the first minute. Initial ion flux rates (J_{OH}^- or J_H^+) in nanomol per minute per milligram protein were calculated according to the formula:

$$J_{ion} = (dpH_i/dt)V\beta_t$$

where V is cell volume, dpH_i/dt is the rate of change of pH_i and β_t is the total intracellular buffering capacity. β_t is the sum of the intrinsic buffer capacity (β_i) and the buffering capacity of the intracellular HCO_3^-/CO_2 system (β_{CO_2}), and is given by the following equation:

$$\beta_t = \beta_i + 2.3[HCO_3^-]_i$$

In HCO_3^--free solutions, $\beta_t = \beta_i$. β_t and β_i were calculated from the increase in pH_i after the addition of 20 mmol l^{-1} NH_4Cl.

2.3. Acid-loading

For acid loading, the ammonium chloride technique was used [14]. The cells were incubated for 15 min in the Na^+-free solutions (C or G) containing 30 mmol l^{-1} NH_4Cl. The cells were then centrifuged and resuspended in the respective NH_4Cl- and Na^+-free solution and immediately transferred into the cuvette for the fluorescence measurement. After the fluorescence ratio had stabilized, 40 mmol l^{-1} NaCl was added to the cuvette and the rate of alkalinization was determined as described above. When the effect of Cl^--free conditions was tested the cells were preincubated for 20 min in Cl^--free solutions (D or H). The inhibitors, if any, were already present during incubation in the Na^+-free solutions.

2.4. Chemicals

BCECF-AM and H_2-DIDS were purchased from Molecular Probes (Eugene, OR); digitonin, hyaluronidase and all the salts used in the current study were obtained from Sigma Chemical, Madrid, Spain. 5-(*N*-ethyl-*N*-isopropyl)-amiloride (EIPA) was purchased from Merck, Sharp and Dohme. None of the chemicals used in the current work interfered with the BCECF fluorescence.

The BCECF-AM (1.45 mM), digitonin (14 mM), EIPA (4 mM) and H_2-DIDS (100 mM) were prepared in DMSO. They were stored for up to 30 days at −20°C without loss in potency. Solvent concentration did not exceed 0.5% (v:v) and did not affect the fluorescence of BCECF.

2.5. Calculations and statistics

Results are expressed as mean ± S.E.M. Statistical significance was evaluated by the two-tailed Student's *t*-test for unpaired observations.

3. Results

3.1. Intracellular resting steady-state pH and intracellular buffering capacity of colonocytes

We have previously reported [11] that resting steady-state pH_i of chicken colonocytes, measured at 25°C, in the nominal absence of CO_2/HCO_3^- (solution A) was 7.16 ± 0.02 ($n = 15$). The current work shows that in the presence of 25 mM HCO_3^-/5% CO_2 (solution E) resting steady-state pH_i was 7.06 ± 0.02 ($n = 10$).

Total intracellular buffering power, β_t, calculated as described in Section 2, was 83 ± 7 mM per pH unit ($n = 10$) in the nominal absence of HCO_3^- and 124 ± 8 mM per pH unit ($n = 10$) in the presence of 25 mM HCO_3^-/5% CO_2. These values are higher than those reported for other cells [3,5] and close to that reported for chicken breast muscle [15].

3.2. Cl^-/HCO_3^- exchanger and pH_i regulation

To test whether the chicken colonocytes possess a functional Cl^-/HCO_3^- exchanger at resting pH_i, the transmembrane Cl^- gradient was inverted by removal of extracellular Cl^-. The experiments were carried out in the presence of 25 mM HCO_3^-/5% CO_2. Cells were incubated for 15 min in Cl^--containing solution (solution E) and transferred to a Cl^--free medium (solution F) at the beginning of the fluorescence recording. This maneuver would reverse the ion gradient for Cl^- and an operational Cl^-/HCO_3^- exchanger in the membrane would produce a net influx of HCO_3^-. Fig. 1A and Table 2 show that the cells alkalinized following removal of extracellular Cl^-. This suggest that a gradient-driven efflux of chloride induced influx of base equivalents.

In another set of experiments cells were incubated for 15 min in Cl^--free medium (solution F) and transferred to a Cl^--containing medium (solution E)

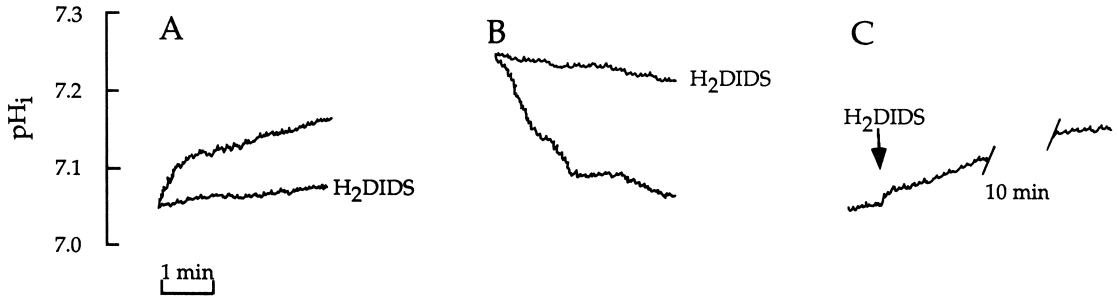

Fig. 1. Effect of H_2-DIDS and external Cl^- on resting pH_i in isolated chicken colonocytes. (A) At the beginning of each trace, dye-loaded cells incubated for 15 min in standard solution (solution E) were suspended in Cl^--free solution (solution F) with or without 0.5 mM H_2-DIDS. (B) At the beginning of each trace, dye-loaded cells incubated for 15 min in Cl^--free solution (solution F) were suspended in Cl^--containing solution (solution E) with or without 0.5 mM H_2-DIDS. (C) Dye-loaded cells incubated in standard solution (solution A) were suspended in the same solution and at the time indicated by the arrow 0.5 mM H_2-DIDS was added. Each trace is representative of six independent experiments.

Table 2
Effect of external Cl^- on pH_i

Saline solution	Alkalization on Cl^- removal		Acidification on Cl^- addition	
	dpH_i/dt (U min^{-1}) $\times 10^{-2}$	J^-_{OH}	dpH_i/dt (U min^{-1}) $\times 10^{-2}$	J^-_{OH}
HCO_3^-/CO_2 buffer				
Control	7 ± 0.6	32 ± 3	8 ± 1	32
With H_2-DIDS	$0.5 \pm 0.1*$	$2.3 \pm 0.4*$	$0.5 \pm 0.5*$	$2.3 \pm 2*$
Na^+-free	6 ± 0.5	27.6 ± 2	9 ± 2	28 ± 6
HEPES-buffer				
Control	5 ± 1	$15.3 \pm 3**$	6 ± 1	$18.4 \pm 4**$

Alkalization and acidification are measured as initial rates of change in pH_i observed upon removal and reinstatement of Cl^-, respectively.
The concentration of H_2-DIDS was 0.5 mM.
The net base flux, J^-_{OH}, is given in nmol min^{-1} mg^{-1}.
Means $\pm$ S.E.M of six independent determinations.
$* p < 0.001$, $** p < 0.01$, significant differences with the control in the presence of HCO_3^- (first row).

at the beginning of the fluorescence recording. Lack of chloride induced cell alkalinization and reinstatement of the anion reverted the pH_i to normal values (Fig. 1B and Table 2).

The chloride-dependent pH_i changes just described were inhibited by H_2-DIDS, an inhibitor of anion exchanger in other cells [16] (Fig. 1 and Table 2).

Repetition of the experiments above described in the nominal absence of sodium (solutions G and H) led to changes in pH_i identical to those found in the presence of Na^+ (Table 2).

In the nominal absence of HCO_3^- (solutions A and B) the chloride-dependent pH_i changes were in the same direction as those above described but they occur at a lower rate (Table 2).

All these results together suggest that chicken colonocytes present a Na^+-independent Cl^-/HCO_3^- exchanger operative under resting conditions, that can also work in HEPES-buffered solutions.

Since the other H_2-DIDS-sensitive and bicarbonate-dependent mechanisms described in the current work does not operate under resting conditions (see below), the significant cell alkalinization caused by H_2-DIDS favors the view that the Cl^-/HCO_3^- exchanger is functional under resting conditions (Fig. 1C).

3.3. Cl^-/HCO_3^- exchanger and pH_i recovery from an alkaline load

An alkaline load was imposed by incubation of the cells in 25 mM HCO_3^-/5% CO_2 (solution E) for 15

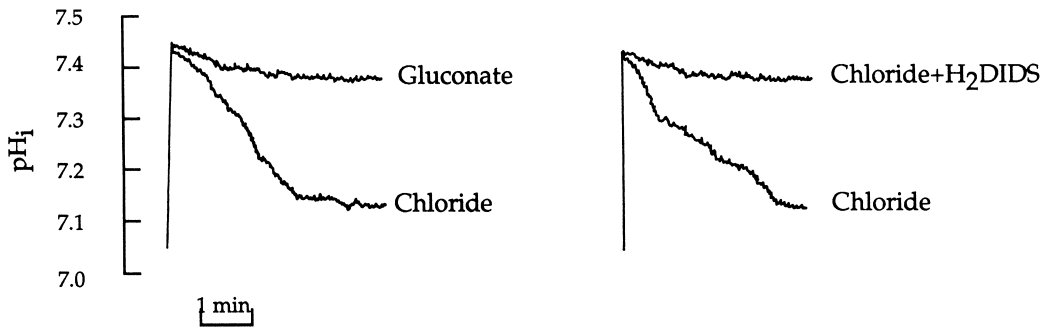

Fig. 2. External Cl^- and pH_i recovery from alkaline load. Dye-loaded cells were incubated for 15 min in the presence of 25 mM HCO_3^-/5% CO_2 (solution E), with or without 0.5 mM H_2-DIDS and transferred at the beginning of the trace to HEPES-buffered solution (solution A or B) containing the indicated modifiers. The experiments were carried out in the presence of Na^+. Each trace is representative of the number (n) of independent experiments indicated in Table 3.

Table 3
Effect of Cl^-, Na^+ and H_2-DIDS (0.5 mM) on pH_i recovery from an alkaline load

Recovery solution (HEPES)	*n*	pH_i		
		At peak	1 min after peak	3 min after peak
With Na^+				
Chloride	10	7.45 ± 0.02	7.31 ± 0.02	7.28 ± 0.01
Gluconate	9	7.45 ± 0.04	7.42 ± 0.01 *	7.40 ± 0.02 *
Cl^- + H_2-DIDS	4	7.45 ± 0.06	7.41 ± 0.02 * *	7.38 ± 0.02 *
Without Na^+				
Chloride	4	7.36 ± 0.02	7.23 ± 0.02	7.11 ± 0.02
Gluconate	4	7.34 ± 0.01	7.32 ± 0.01 * *	7.28 ± 0.02 *
Cl^- + H_2-DIDS	4	7.35 ± 0.01	7.31 ± 0.01 * *	7.22 ± 0.02 *

Experimental conditions as for Fig. 2.
Means ± S.E.M. in (*n*) independent experiments.
* $p < 0.001$, * * $p < 0.01$, significant differences with its own control (first row in each case).

min and transfer to HEPES-buffered solution nominally free of CO_2. This transfer led to an immediate alkalinization (Fig. 2 and Table 3), attributed to the rapid efflux of CO_2, equivalent to a net proton extrusion. This alkalinization was transient in the presence of chloride (transfer to solution A) and pH_i

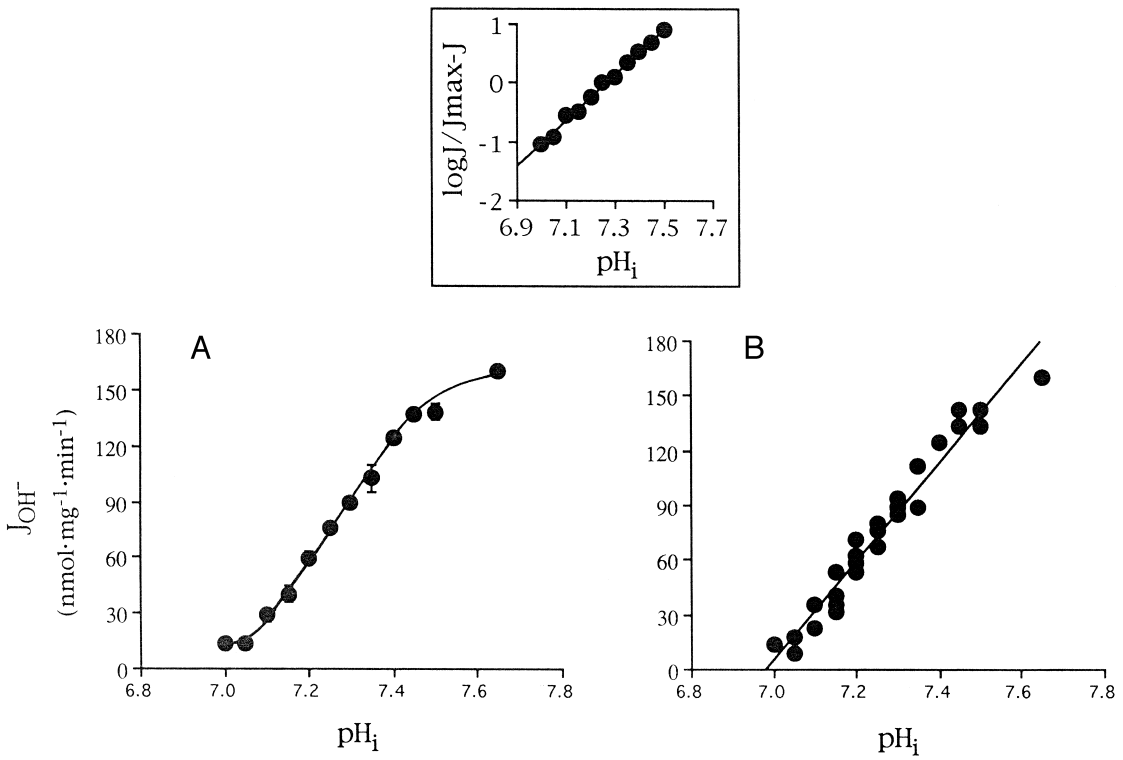

Fig. 3. Relationship between initial rate of Cl^--dependent pH_i recovery from an alkaline load and initial pH_i. Dye-loaded cells were alkalinized as described in Fig. 2. From the trace obtained in the presence of Cl^- in the recovery solution, the rate of pH_i recovery was calculated at the different pH_i values. (A) Means ± S.E.M. ($n = 5$) of rates of OH^- efflux (nmol mg^{-1} min^{-1}) against the means of pH_i at 0.05 pH unity intervals. (B) All the individual data are plotted and adjusted to a line by computer. Insert: Hill plot of data in (A). J_{max} was calculated from the Lineweaver–Burk plot of the data. The line was calculated by linear regression analysis, $Y = -28.27 + 3.89X$, $r = 0.99$.

decreased towards base line. This pH_i recovery was nearly abolished by H_2-DIDS or in Cl^--free solutions (solution B instead of A). Repetition of the experiments in the absence of Na^+ (transfer to solution C or D) did not modify the responses (Table 3). These results suggest that a Na^+-independent, Cl^-/HCO_3^- exchanger is responsible for most of the pH_i recovery from an alkaline load. We have no explanation for the residual acidification observed in the absence of Cl^- or in the presence of H_2-DIDS. Similar acidification was observed in chicken small intestinal epithelial cells [13].

3.4. Relationship between pH_i and Cl^-/HCO_3^- exchanger activity

The dependence of Cl^-/HCO_3^- exchange activity on pH_i was studied in colonocytes alkalinized as described above. The results show (Fig. 3) that the activity of the exchanger increases with increasing pH_i. The relationship between net OH^- efflux (nmol mg^{-1} min^{-1}) and pH_i did not follow simple Michaelis–Menten kinetics (Fig. 3). The Hill plot (Fig. 3, insert) gives an interaction coefficient of 3.89 and a $[H^+]_{0.5}$ of 54 nM. In Fig. 3B all the individual values are plotted and fitted to a line by computer. The extrapolated pH_i value at which no measurable cell acidification occurred, set-point, is approx. 6.97.

3.5. Na^+/HCO_3^- cotransporter and pH_i regulation

The experiments were started with two batches of dye-loaded cells kept in HEPES- or HCO_3^--buffered solutions on ice. After incubation of a cell sample for 15 min at 25°C in a Na^+-free solution (solution C or G respectively of Table 1), with or without inhibitors, the cells were washed in the same solution and the sample was transferred into a cuvette of 2 ml volume for recording the fluorescence ratio. One to two min later 20 μl of 4 mol l^{-1} NaCl were added and the recording was continued for at least 3 min. Exposure of the cells to Na^+-free solutions for 15 min, a maneuver that would reverse the ion gradient for Na^+, acidified the cells (Table 4). Readdition of Na^+ resulted in a rapid increase in pH_i. The Na^+-induced pH_i changes occurred nearly at equal rates in both, HCO_3^--containing and HCO_3^--free solutions and were prevented by EIPA and unaffected by H_2-DIDS.

These observations indicate that in chicken colonocytes only one Na^+-dependent alkalinizing mechanism is operative under resting conditions, the EIPA-sensitive Na^+/H^+ exchanger.

3.6. pH_i recovery from an acid load

We have previously reported [11] that in HEPES-buffered solutions (nominally CO_2/HCO_3^--free) pH_i

Table 4
Effect of Na^+ removal and readdition on pH_i

Conditions	Acidification on Na^+ removal		Alkalinization on Na^+ readdition	
	pH_i	Total acidification	dpH_i/dt (U min^{-1}) $\times 10^{-2}$	J_H^+
HEPES buffer (reference pH_i in sol. A = 7.16 ± 0.02)				
Control	6.92 ± 0.02	43 ± 5	11.0 ± 1	34 ± 3
HCO_3^-/CO_2 buffer (reference pH_i in sol. E = 7.06 ± 0.02)				
Control	6.98 ± 0.03	36.7 ± 4	10 ± 1	46 ± 6
EIPA	7.08 ± 0.05	9.2 ± 9 *	1 ± 0.5 *	4.6 ± 1 *
H_2-DIDS	6.96 ± 0.05 #	45.8 ± 9	10 ± 2	46 ± 6

Cells adapted to HEPES buffer or HCO_3^-/CO_2 buffer, that in presence of Na^+ would have $pH_i = 7.16$ or 7.06, respectively, were incubated for 15 min in Na^+-free solutions, then transferred into the fluorimeter to measure pH_i (column 1) and to determine the change in pH_i (column 3) after adding Na^+ to a final concentration of 40 mmol l^{-1}.

Total acidification, in nmol mg $protein^{-1}$, represents the total apparent increase in cytosolic proton content following 15 min incubation in Na^+-free conditions and it was calculated as indicated in Section 2.

Net proton efflux, J_H^+, in nmol mg $protein^{-1}$ min^{-1} was calculated as indicated in Section 2. The concentration of H_2-DIDS was 0.5 mmol l^{-1} and that of EIPA 100 μmol l^{-1}.

Values are means ± S.E.M. of four individual samples measured.

* $p < 0.001$ as compared with the control in the presence of bicarbonate.

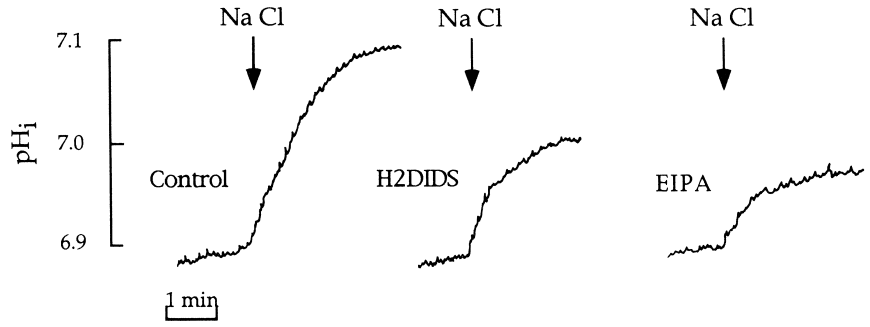

Fig. 4. pH_i recovery of acid loaded cells following addition of Na^+ to Na^+-free solutions. All cells were acidified with the NH_4Cl technique in conjunction with the preincubation in Na^+-free solutions in the presence (solution G) and absence (solution C) of bicarbonate. As a result pH_i was close to 6.88 in all series of experiments immediately prior to addition of 40 mmol l^{-1} Na^+ (average pH_i value varied only between 6.87 ± 0.02 and 6.90 ± 0.02 in the individual series). When tested, the inhibitors were present in the ammonium prepulse. Following withdrawal of NH_4^+ cells were immediately suspended in Na^+-free solutions containing the indicated inhibitors. The concentration of H_2-DIDS was 0.5 mmol l^{-1} and that of EIPA 100 μmol l^{-1}. At the time indicated by the arrow 40 mmol l^{-1} NaCl was added. Each trace is representative of five independent experiments.

recovery from a moderate acid load was totally dependent on the activity of the Na^+/H^+ exchanger, since the recovery was Na^+-dependent, inhibited by EIPA and unaffected by H_2-DIDS. We have now repeated and extended these experiments.

The rate of Na^+-dependent pH_i recovery from an acid load was monitored in either HEPES- or HCO_3^--buffered solutions. As shown in Fig. 4 and Table 5, in the absence of bicarbonate the rate of pH_i recovery was suppressed by EIPA. Bicarbonate significantly increased the rate of Na^+-dependent regulatory cell alkalinization and both, EIPA and H_2-DIDS inhibited it and the effects of the inhibitors were additive.

In another set of experiments, cells were acidified in a nominally Na^+- and Cl^--free, bicarbonate-buffered solutions (solution H) following 20 min incubation in Cl^--free, bicarbonate-buffered solutions (solution F). This procedure served to nominally deplete the cells of intracellular chloride. Cells were then placed in Na^+ and Cl^--free solutions (solution H). The rates of Na^+-dependent pH_i recovery were not different from those obtained in Cl^--containing solutions (Table 5).

Table 5
Initial rate of pH_i recovery of acid-loaded cells following addition of Na^+ to Na^+-free solutions

Conditions	Alkalinization on Na^+ readdition		
	dpH_i/dt (U min^{-1}) $\times 10^{-2}$	J_H^+ (nmol min^{-1} mg $protein^{-1}$)	Inhibition %
HEPES buffer			
Control	12 ± 1	36.8 ± 3	
EIPA	1 ± 0.5 *	3 ± 1.5 *	92
HCO_3^-/CO_2 buffer			
Control	14 ± 1	62 ± 4 #	
EIPA	4 ± 0.5 *	18.3 ± 2 *	71
H_2-DIDS	6.5 ± 0.5 *	29.8 ± 2 *	50
EIPA + H_2-DIDS	0.5 ± 0.5 *	2.3 ± 2.3 *	100
Cl^--free, HCO_3^-/CO_2 buffer			
Control	13 ± 0.3	59.6 ± 1.4 #	
EIPA	6 ± 0.5 *	27.5 ± 2 *	46
H_2-DIDS	6 ± 0.5 *	27.5 ± 2 *	46

Net proton flux, J_H^+, in nmol mg $protein^{-1}$ min^{-1}, was calculated as indicated in Section 2.
Inhibition refers to the observed J_H^+ value in the presence of inhibitor compared to respective control value of each series in absence of inhibitors.
Other details as in Fig. 4.
Means $\pm$ S.E.M. of five independent determinations.
* $p < 0.001$, as compared with its own control (first row in each case).
# $p < 0.001$, as compared with values obtained in HEPES-buffered solution in the absence of modifiers (first row of the table).

4. Discussion

The resting pH_i values of chicken colonocytes reported herein are lower than those reported for rabbit [9] and human [10] colonic crypt cells and for rabbit colonocytes [17], similar to those measured in the colon carcinoma cell line, HT_{29}, [7] and slightly more acidic in HCO_3^- than in HEPES-buffered solutions ($p < 0.01$).

Chicken colonocytes possess a Na^+/H^+ exchanger operative under resting conditions and responsible for pH_i recovery from an acid load in the nominal absence of HCO_3^- [11]. The current work presents evidence for the existence in the same cells of a Na^+-independent Cl^-/HCO_3^- exchanger and a Na^+/HCO_3^- cotransporter.

The observations listed below are consistent with the presence of a Na^+-independent Cl^-/HCO_3^- exchanger which is involved in setting the resting pH_i and in pH_i recovery from an alkaline load: (i) intracellular alkalinization following external Cl^- removal, (ii) return to resting pH_i values upon reinstatement of Cl^-, (iii) recovery from alkaline loads required external Cl^-, and (iv) all these Cl^--dependent processes were Na^+-independent and inhibited by H_2-DIDS. The activity of the exchanger observed under resting conditions may be responsible for the slight decrease in pH_i due to external HCO_3^-. Although the involvement of Cl^-/HCO_3^- exchanger in pH_i regulation is well established in several epithelial cell types (see Ref. [3] for review), there are only two reports for the large intestine. The exchanger regulates pH_i in HT29 cells [7], but not in the human colonic crypt cells [10].

The Cl^-/HCO_3^- exchanger may have an absolute requirement for bicarbonate or only a preference for bicarbonate over OH^- (see Ref. [3] for review). The present results show that the Cl^--dependent changes in pH_i occurred under HCO_3^--free conditions, although at a lower rate than those observed in HCO_3^--containing solutions. This indicates that, as demonstrated in apical membrane vesicles of rat distal colon [18] and in HT_{29} cells [7], the Cl^-/HCO_3^- exchanger of chicken colonocytes has not an absolute requirement for exogenous bicarbonate since it also permits transport of OH^-. Another reason for the observed Cl^--dependent alkalinization under HCO_3^--free conditions might be that metabolism produces sufficient endogenous CO_2/HCO_3^- to support the slow activity of a exchanger without recourse to OH^- ions.

The set point of the Cl^-/HCO_3^- exchanger (no net flux) of chicken colonocytes is a pH_i approx. 6.96, a value below the observed resting pH_i value. This observation agrees with those described above which suggest that the Cl^-/HCO_3^- exchanger is functional under resting conditions. The relationship between net base efflux and pH_i shows that at resting pH_i the Cl^-/HCO_3^- exchanger functions at a low rate and its activity increases with increasing pH_i. This relationship has a Hill coefficient value greater than 1 ($n = 3.89$), indicating a positive cooperative mechanism for the influence of cytosolic base on Cl^-/HCO_3^- exchanger. Similar observations have been reported for other cell types (see Ref. [3] for a review).

The current results also indicate that chicken colonocytes possess at least two Na^+-dependent acid/base transporters operating during pH_i recovery from acid loads. One is the EIPA-sensitive Na^+/H^+ exchanger, which also functions under resting conditions, and the other is an EIPA-resistant mechanism. This latter pH_i regulatory mechanism has all the properties of the Na^+/HCO_3^- cotransporter in that recovery was absolutely dependent on the presence of bicarbonate and Na^+, it was inhibited by H_2-DIDS and its rate of operation was unaffected by Cl^--removal. This last criterion distinguishes between Na^+/HCO_3^- cotransporter and Na^+-dependent Cl^-/HCO_3^- exchanger. Under resting conditions the Na^+/HCO_3^- cotransporter does not appear to be functional, since the rate of Na^+-induced pH_i changes was prevented by EIPA and unaffected neither by H_2-DIDS nor by the presence of bicarbonate.

Na^+/HCO_3^- cotransporter, originally described in the basolateral membrane of kidney proximal tubules in the tiger salamander [19], has later been described in a variety of other cells, including rat distal colon [20] and human colonic crypt cells [10]. In some cells the cotransporter is quiescent when assayed in nominally CO_2/HCO_3^--free solutions [21,22], but operates in other cells, apparently supported by metabolically generated HCO_3^- [23,24]. The Na^+/HCO_3^- cotransporter present in chicken colonocytes has absolute requirement for exogenous bicarbonate, since it did not work at an appreciable rate in HEPES-buffered solutions.

Our study shows for the first time the presence of a Cl^-/HCO_3^- exchanger and a Na^+/HCO_3^- cotransporter in avian colonocytes. Among the two, only the Cl^-/HCO_3^- appears to be functional under resting conditions and therefore, together with the Na^+/H^+ exchanger, participates in setting the resting pH_i. In addition the Cl^-/HCO_3^- exchanger is involved in pH_i recovery from an alkaline load, whereas the Na^+/HCO_3^- cotransporter plays a role in pH_i recovery from an acid load. The results offer no clues on

the cellular localization of the transporters. The Cl^-/HCO_3^- exchanger is present in the brush border membrane, but not in the basolateral membrane, of rat colonocytes [18], whereas in HT_{29} cells [25] the Cl^-/HCO_3^- exchanger is localized in the basolateral membrane. The Na^+/HCO_3^- cotransporter has been found in the basolateral membrane of rat distal colon [20]. In some cells the Na^+/HCO_3^- cotransporter functions as a base loader and in others as an acid loader (see Ref. [3] for a review). Since we have not addressed the issue of electrogenicity and stoichiometry of the cotransporter we cannot say whether in vivo the cotransporter operates as a base loader or as a base extruder.

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