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Cl^- permeability of the basolateral membrane of the *Rana esculenta* epithelium: activation of Cl^-/HCO_3^- exchange by alkaline intracellular pH

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We have investigated Cl^- transport mechanism(s) located in the basolateral membranes of the frog skin epithelium and in particular activation of Cl^-/HCO_3^- exchange following an alkaline load. We found that 87% of the total ^{36}Cl uptake by the epithelial cells occurs across the basolateral membranes (J_{Cl}^b-) and submitting the epithelium to an alkaline load (HCO_3^--Ringer solution, pH 8.1) increased J_{Cl}^b-. Intracellular Cl^- activity (a_{Cl}^i-), measured with ion-sensitive microelectrodes, increased when the Ringer solution bathing the basolateral membranes was changed from a Ringer solution equilibrated in air (pH 7.4) to one containing CO_2/HCO_3^- (pH 7.4). pH_i recovery following an alkaline load was dependent on Cl^- since it did not occur in serosal Cl^--free media, indicating the presence of a Cl^--dependent regulatory mechanism. Acid loading of the epithelial cells (5% CO_2, HCO_3^--free Ringer) produced no change in J_{Cl}^b- but stimulated an amiloride-sensitive ^{22}Na uptake across the basolateral membranes of the epithelium, compatible with an activation of a Na^+/H^+ exchanger, previously described in this tissue. J_{Cl}^b- was partially blocked by SITS ($5 \cdot 10^{-4}$ mmol/l), niflumic acid ($5 \cdot 10^{-5}$ mmol/l), furosemide or bumetanide. Simultaneous addition of furosemide and niflumic acid produced an inhibition of J_{Cl}^b- which was not different with furosemide alone. Substitution of Na^+ by choline had no effect on J_{Cl}^b- and furosemide did not block the $^{22}Na^+$ uptake, suggesting that J_{Cl}^b- is not a Na^+-dependent process (cotransport). We conclude that a significant Cl^- permeability at the basolateral membranes of the epithelial cells is due to the presence of a Cl^-/HCO_3^- exchanger which is essential for the recovery of pH_i following an alkaline load.

Introduction

The frog skin epithelium is involved in hydromineral regulation and acid-base equilibrium because of its capacity to absorb Na^+ and to secrete H^+. The control of epithelial intracellular pH (pH_i) is of prime importance since the skin is (i) a major site of transepithelial proton movement and (ii) undergoes acid-base disturbances which are common to many types of cells and (iii) Na^+ transport is pH_i-sensitive. The transepithelial proton secretion is carried out by the mitochondria-rich cells, by means of an apical proton pump [1,2] in series with an ethoxzolamide sensitive Cl^-/HCO_3^- exchanger at the basolateral membranes [3]. The other cell type of the outermost living cell layer (the granular cells) and the deeper cell layers constitute a syncytium and are involved in transepithelial Na^+ transport. These cells must regulate intracellular pH within narrow limits since the transepithelial sodium transport rate, the apical Na^+ membrane conductance and the basolateral K^+ membrane conductance were all found to be highly sensitive to changes in intracellular pH [4]. A basolateral Na^+/H^+ exchanger regulates pH_i and ion transport when stimulated by intracellular acidification [5,6]. We have also presented some evidence that a Cl^--dependent process is involved in cell pH recovery after an alkaline load of the epithelium [7].

In this study, using kinetic and electrophysiological approaches, we further investigated the nature of Cl^- transport mechanism located in the basolateral membranes, paying special attention to the possible role of Cl^-/HCO_3^- exchange in cell pH regulation.

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Materials and Methods

Rana esculenta frogs (supplied by Couetard, St Hilaire de Riez, France and originating from Yugoslavia) were kept at 15°C without food in running tap water and used within 10 days of arrival in the laboratory. Large sheets of epithelium were isolated from the ventral skin by the technique of Aceves and Erlij [8].

Four different Ringer solutions were used in these experiments: the '$HCO_3^-CO_2$ Ringer' solution contained (in mmol/l): NaCl 85; $NaHCO_3$ 24; KCl 2.5; $CaCl_2$ 2; $MgSO_4$ 2; Na_2HPO_4 2.5; KH_2PO_4 1.2 and glucose 11, gassed with a mixture of 5% CO_2 and 95% O_2; pH 7.34. In the 'Air Ringer' solution, Na_2SO_4 replaced $NaHCO_3$ and was gassed with air, pH 7.34 (final $[HCO_3^-]$ 0.2 mmol/l). 'Alkaline Ringer' solution had a similar composition to normal Ringer containing bicarbonate (24 mmol/l), but was equilibrated in air to give a pH of 8.1. We also used an 'Air Ringer' solution containing 25 mmol/l acetate to load the cells with the permeant non ionized form. 'Acid Ringer' solution was identical to the 'Air Ringer' solution but was gassed with a mixture of 5% CO_2/95% O_2, giving a solution pH of 6.20.

In some experiments Cl^- was replaced by gluconate or SO_4^{2-} in 'Air Ringer' as indicated in the text. The hypotonic shock solution was half strength 'Air Ringer' solution.

Kinetic studies: determination of the Cl and Na uptakes

Details of the techniques, derived from those described originally by Stoddard and Helman [9] and Stoddard, Jakobson and Helman [10] have been given in a previous paper [5]. Briefly, disks of epithelium (16 pieces of 1.13 cm^2 obtained from one skin in 'beaker' experiments, or 6 pieces of 1.54 cm^2 in 'Using chamber' experiments) were sandwiched between two nylon meshes (type AP32, Millipore U.S.A.) to avoid curling and to maximise the exposed surface area.

Na^+ uptake through the basolateral cell membranes (J^b) of the epithelium was measured in 'beaker' experiments in the presence of phenamil (10^{-6} M). This amiloride analogue was used to block the apical conductive Na^+ entry, and in this condition the ^{22}Na loading of the epithelium (during 1 min) occurs essentially through the basolateral membranes [5]. The $^{22}Na^+$ efflux was then measured in a solution containing furosemide (10^{-3} M), ouabain ($5 \cdot 10^{-4}$ M) and amiloride (10^{-3} M). Plotting the ^{22}Na cell content as a function of time made it possible to distinguish between the extracellular and the cellular components. The evaluation of the initial ^{22}Na quantity (Q_o) before washing the epithelium was determined from linear regression analysis of the cellular ^{22}Na washout component and J^b, expressed in nequiv. $h^{-1}cm^{-2}$, was calculated from the value of Q_o.

The determination of the Cl^- uptake from the apical side (J^a) or the basolateral side (J^b) of the epithelium was similar in principle to that described by Stoddard et al. [10] and similar to that used for measuring the Na^+ uptake except that furosemide (10^{-3} M) was the sole agent added to the washing solution to reduce the Cl^- efflux from the epithelium. Cl^- uptake was measured by loading for 1 min with ^{36}Cl (10 μCi/ml) from the apical or the basolateral Ringer solution in Ussing chambers. The epithelium was then washed rapidly and incubated in 'Air Ringer' solution containing 10^{-3} M furosemide. The washout of ^{36}Cl from the tissue was followed over a 32 min period by sampling this solution every 4 min. Furosemide was used to reduce the rate of $^{36}Cl^-$ efflux from the epithelium [10]. The double-exponential from the washout kinetics corresponds to a two-compartment system consisting of an extracellular space and a single-cellular compartment (see Refs. 9 and 10). Extrapolation of the second exponential washout to time zero gives the initial-time ^{36}Cl quantity (Q_o) which was used to calculate J^a or J^b expressed in nequiv. $h^{-1}cm^{-2}$. In other experiments, the epithelia were loaded with isotope in beakers, since it was found that most of the ^{36}Cl uptake occurred through the basolateral membranes of the epithelium.

Measurement of cell membrane potential, cell Cl^- activity ($a^i_{Cl^-}$) and cell pH (pH_i)

Microelectrode experiments were carried out under short-circuit conditions (SC) in which the spontaneous transepithelial electrical potential difference was clamped at zero volt using an automatic voltage clamp (Model VC600, Physiologic instrument, Houston, TX, USA), or in some cases under open-circuit conditions (OC). Impalements were performed from the basolateral side of the isolated epithelium. Cell membrane potential and the intracellular pH (pH_i) or intracellular Cl^- activity ($a^i_{Cl^-}$) were measured with double-barrelled H^+ ion-sensitive microelectrodes using the proton ionophore tridodecylamine as H^+ sensor (Proton cocktail 82500 Fluka Switzerland) or the Cl^--selective liquid ion exchanger (Corning 477913), respectively. Manufacture and calibration of pH and Cl^- microelectrodes were similar to those previously described in detail [11,3,4]. The ion-sensitive and reference barrels were coupled via Ag/AgCl wires to a high-input impedance dual microprobe amplifier (FD 223, World Precision Instruments, New Haven, USA) connected to a chart recorder (Linseis L 2065, Selb, F.R.G.).

Drugs

Amiloride (Merck, Sharp and Dohme, Research Laboratories. West Point, PA, U.S.A.). Ouabain and furosemide and SITS (4-acetomido-4′-isothiocyanostilbene-2,2′-disulfonic acid) were purchased from Sigma

Chemical Co. (St. Louis, MO, U.S.A.); niflumic acid was a gift from UPSA laboratories.

Results

Localization of Cl^- uptake

In preliminary experiments we determined the Cl^- permeability of the apical and the basolateral cell membranes of the isolated epithelium by measuring Cl^- influxes across both sides of the epithelium. Paired pieces of isolated epithelium of the same frog skin were mounted in Ussing chambers (1.54 cm^2 exposed surface area), bathed with an 'Air Ringer' solution under open-circuit conditions (OC) and the isotope ^{36}Cl was added for 1 min either on the apical or on the basolateral side of the epithelium. The isotope washout was then followed as a function of time (for 32 min) and the Cl^- uptake across the apical membranes ($J^a_{Cl^-}$) or through the basolateral membranes ($J^b_{Cl^-}$) of the epithelium was calculated as described in Methods. Large differences in the chloride transport rates were found depending on which side the $^{36}Cl^-$ was added. The uptakes from the apical and basolateral sides were 124 ± 32 nequiv.h^{-1} cm^{-2} and 795 ± 158 nequiv.$h^{-1}cm^{-2}$, respectively ($n = 4$). This finding of a $J^b_{Cl^-}$ 6.5-times higher than $J^a_{Cl^-}$ is in agreement with the data of Stoddard et al. [10] who concluded that the Cl^- permeability of the apical membranes of the frog *R. pipiens* skin epithelium is much lower than that of the basolateral membranes. It must be remarked that $J^a_{Cl^-}$ and $J^b_{Cl^-}$ are calculated per cm^2 of flat epithelium. Considering the multilayer nature of the epithelium and the location of the tight junctions at the outermost living cell layer, the total cell membrane surface area accessible to the isotope is much larger on the basolateral side than on the apical side. Moreover, basolateral membrane electrical capacitance is 50-times larger than that of the apical membrane [30]. Therefore, the ratio of chloride uptake from apical and basolateral sides does not reflect a permeability ratio, but depends on the surface area accessible to the isotope during the loading period.

Considering that total Cl^- uptake by the epithelium is mainly that of Cl^- transport through the basolateral membranes, we performed 'beaker' experiments in preference to Ussing chambers since the former has the advantage that several pieces of the same epithelium (12 to 16 pieces each with a surface area of 1.13 cm^{-2}) can be tested under different experimental conditions.

Basolateral Cl^- uptake activated by cell alkalization

A basolateral Cl^-/HCO_3^- exchanger has already been implicated in maintaining intracellular Cl^- activity at a higher level than that predicted by the membrane potential [3]. We investigated further the participation of such an exchanger in basolateral Cl^- uptake by determining the effect on $J^b_{Cl^-}$ of procedures designed to change intracellular pH. An alkaline Ringer solution (pH 8.2, HCO_3^- 24 mmol/l and CO_2-free) was used to raise pH_i to a pH_i of 7.37 and an acid Ringer solution (pH 6.2, HCO_3^--free and gassed with 5% CO_2), to acidify the epithelium to a pH_i of 6.96 [5]. In the the 'HCO_3^-/CO_2 Ringer' solution (pH 7.4, HCO_3^- 24 mmol/l, CO_2 5%) and the 'Air Ringer' solution (pH 7.4, HCO_3^-- and CO_2-free), the epithelial cells bathed on both sides in either of these solutions, have about the same pH_i of 7.20–7.28 [12,4]. An additional procedure of alkalinisation was also used which consisted of preincubating the epithelium in an 'Air Ringer' solution containing 25 mmol/l of acetate for 90 min followed by return to 'Air Ringer' solution. During the latter period, the rapid loss of the neutral form of acetate from loaded cells leads to an intracellular alkalinization as anionic acetate combines with protons [31]. After incubation of the epithelium for a 90 min period in one of the above solutions, ^{36}Cl uptake was measured for one minute immediately on return to control conditions, i.e., in epithelia replaced in an 'Air Ringer' solution. Under the two conditions expected to alkalanize the cells, $J^b_{Cl^-}$ was significantly increased (Figs. 1A and 1B). Taking the 'Air Ringer' as control, the alkaline solution stimulated $J^b_{Cl^-}$ by $36 \pm 12\%$ ($P < 0.01$, $n = 8$ for the difference with control), whereas a smaller increase ($29 \pm 8\%$; $P < 0.05$, $n = 8$ for the difference with control) was observed during the recovery phase after preincubation of the epithelium with an 'Acetate Ringer'. No significant changes were observed in $J^b_{Cl^-}$ after incubating the epithelia in conditions known to acidify the cells nor was $J^b_{Cl^-}$ significantly different in epithelia incubated in a Ringer solution buffered with CO_2 and HCO_3^-. It must be remarked that the amplitude of the variations in $J^b_{Cl^-}$ were relatively small compared to the steady-state values of the unidirectional fluxes.

Effect of HCO_3^- on intracellular Cl^- activity

Ion-sensitive microelectrodes were used to measure intracellular Cl^- activity ($a^i_{Cl^-}$) and basolateral membrane potential (V_b) under OC or SC in order to identify the Cl^- uptake mechanism at the basolateral membranes. In a previous paper [3] we found that substituting Cl^- by gluconate in the serosal 'CO_2/HCO_3^- Ringer' solution, produced a reversible and rapid decrease in $a^i_{Cl^-}$ in parallel with an increase in pH_i and small changes in the basolateral membrane potential (V_b). Here we investigated the effects of CO_2/HCO_3^- in the Ringer solution bathing the serosal side of the short-circuited epithelium, on intracellular Cl^- activity. In SC epithelia, changing from a Cl^--free 'Air Ringer' solution to a normal 'Air Ringer' solution increased $a^i_{Cl^-}$ from 7.7 ± 2.7 nequiv./l ($n = 8$ cells) to 13.5 ± 2.7 nequiv./l ($n = 7$ cells). Subsequent perfusion with CO_2/HCO_3^- Ringer produced an additional increase in $a^i_{Cl^-}$ (17.3 ± 2.4 nequiv./l, $n = 4$ cells) which

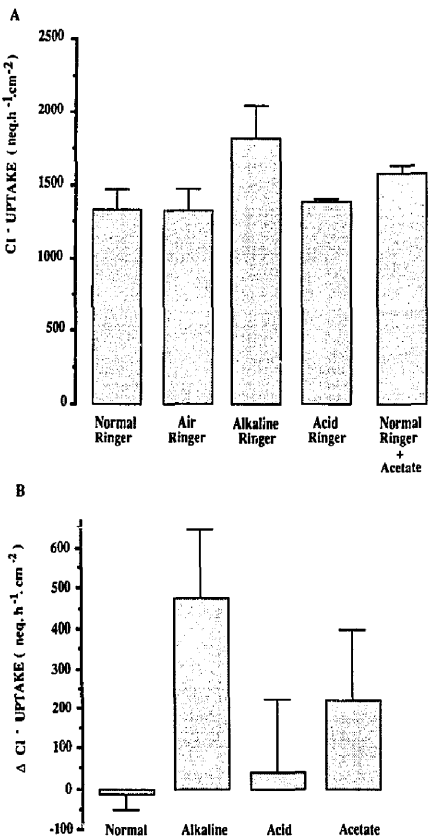

Fig. 1. (A) Effects of pH_i disturbances on the basolateral Cl^- uptake. Epithelia were incubated for 90 min in the Ringer solutions shown and $^{36}Cl^-$ uptake was measured on return to 'Air Ringer' solution. Epithelial incubated with a Ringer solution containing 25 mmol/l acetate, were washed for 1 min with an 'Air Ringer' solution before the determination of Cl^- uptake ($n = 8$). (B) Effects of pH_i disturbances on basolateral Cl^- uptake expressed as the difference between $J^b_{Cl^-}$ in ('Air Ringer') solution and the experimental Ringer solutions as described in text.

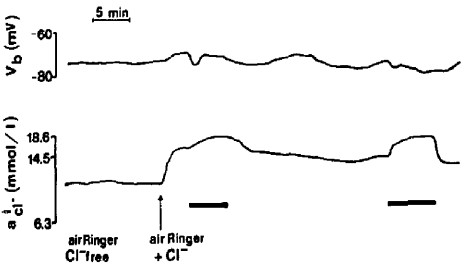

Fig. 2. Effect of perfusing the serosal side with a Cl^--free solution, 'Air Ringer' and of CO_2/HCO_3^--buffered Ringer on cell Cl^- activity in frog skin epithelium. After a preincubation period in a serosal Cl^--free Ringer, $a^i_{Cl^-}$ increased rapidly upon changing the perfusion to a normal Cl^--containing Ringer (arrow). Interruption of the 'Air Ringer' perfusion and switching to a CO_2/HCO_3^--buffered Ringer (bars) produced an additional increase in $a^i_{Cl^-}$. Little change in the intracellular potential (V_b) was observed during these substitutions.

was reversible upon return to an 'Air Ringer' solution, as seen in a typical experiment given in Fig. 2. The CO_2/HCO_3^- dependent increase in $a^i_{Cl^-}$ was also observed when the frog skin epithelium was maintained in open-circuit conditions (Fig. 3), which was the electrical potential condition used for $^{36}Cl^-$ uptake experiments. As in SC, the changes in membrane potential produced by Cl^- substitution were small. The increase in $a^i_{Cl^-}$ following exposure to a 'HCO_3^-/CO_2 Ringer' can be interpreted as occurring via an electroneutral Cl^-/HCO_3^- exchanger activated by loading the cells with CO_2/HCO_3^- and functioning in the direction Cl^- in and HCO_3^- out.

Cl^- dependence of pH_i regulation

A second electrophysiological approach was undertaken to test the Cl^- dependence of pH_i regulation following a transient alkaline load of the epithelium produced by pre-incubating the isolated epithelium for 10 min in CO_2/HCO_3^--buffered Ringer solution (pH 7.4) on the basolateral side and then rapidly replacing this solution with an 'Air Ringer' solution (Fig. 4). The rapid loss of CO_2 relative to HCO_3^- produced a transient alkaline shift in pH_i of 0.3 unit, a hyperpolarisation of the basolateral membranes (V_b) and an increase in short-circuit current (I_{sc}). The changes in V_b and I_{sc} have been explained by a combined stimulation of apical Na^+ and basolateral K^+ conductances at raised pH_i [4]. Recovery of pH_i from the alkaline load (Fig. 4)

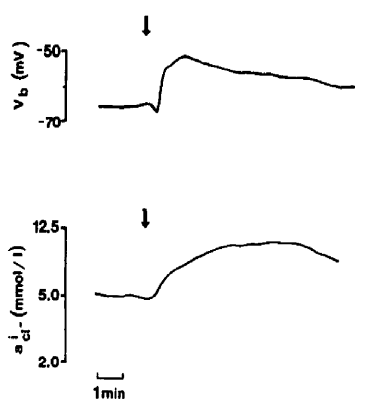

Fig. 3. Effect of CO_2/HCO_3^- on cell Cl^- activity under open-circuit conditions. The epithelium was first incubated in an 'Air Ringer' solution. Changing the serosal perfusion to a CO_2/HCO_3^--buffered Ringer (arrows) produced an increase in $a^i_{Cl^-}$. The changes in membrane potential are a consequence of cell pH effects on basolateral membrane K^+ conductance (Ref. 4).

was absent when the basolateral Ringer solution was Cl^--free. The subsequent return to standard Cl^--containing Ringer solution (Fig. 4) produced a rapid recovery in pH_i, showing that the loss of alkali from the cell is Cl^- dependent. These findings are consistent with the operation of a Cl^-/HCO_3^- exchange in pH_i regulation. It should be noted that passing from an 'Air Ringer' to a 'CO_2/HCO_3^- Ringer' solution slightly increased pH_i, and did not result in a cell pH_i decrease as expected from an eventual stimulation of the anion exchanger to drive Cl^- in and HCO_3^- out. This result may be explained by the rapid loading of the cell with CO_2/HCO_3^- which produced a doubling in the intracellular buffering power.

Independence of Cl^-/HCO_3^- and Na^+/H^- exchangers

Intracellular acidification activates a basolateral Na^+/H^+ exchanger [5,6] and stimulates a transient amiloride-sensitive ^{22}Na uptake across the basolateral membranes (Fig. 5) without producing significant effects on Cl^- uptake (Fig. 1).

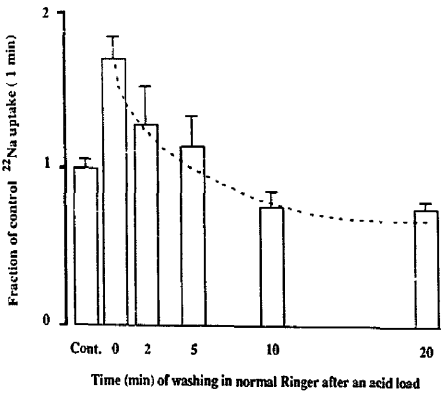

Fig. 5. Recovery from an acidification of the epithelium induced a transient stimulation of an amiloride-sensitive $^{22}Na^+$ uptake. Epithelia were incubated for 90 min in an 'acid Ringer' in presence of phenamil (10^{-5} mol/L). The uptake of $^{22}Na^+$ was followed after different times of washing with an 'Air Ringer' solution ($n = 6$). An half time of $^{22}Na^+$ uptake of 2 min can be estimated from the best fit exponential to the data (dashed line).

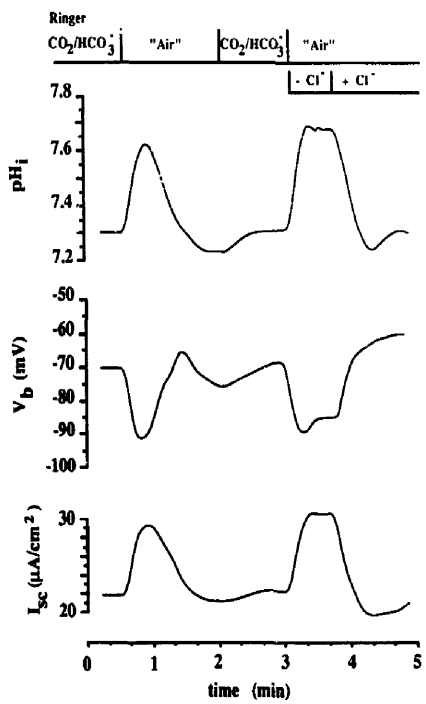

Fig. 4. pH_i recovery from an alkaline load is dependent on the presence of Cl^- in the serosal Ringer solution. The isolated epithelium was first pre-incubated for 10 min in CO_2/HCO_3^--buffered solution (pH 7.4) on the serosal side. Replacement with an 'Air Ringer' solution produced a transient alkalinization, a hyperpolarization of V_b and an increase in I_{sc}. The pH_i did not recover from the alkaline load when the basolateral Ringer solution was Cl^- free.

This result indicates that Cl^-/HCO_3^- and Na^+/H^+ exchangers are not linked like the SITS-sensitive $Na:HCO_3^-/Cl^-:H^+$ exchange mechanism described in excitable tissues [25]. Therefore, Cl^-/HCO_3^- and Na^+/H^+ exchangers are independent and complementary mechanisms of pH_i regulation in frog skin epithelium.

Sensitivity of Cl^- uptake to blockers of Cl^- transport mechanisms

We tested the effects on $J^b_{Cl^-}$ of known inhibitors of anion exchange, cotransporters and Cl^- channels. All experiments were performed on epithelia which were first incubated in 'alkaline Ringer' solution. The relatively specific anion exchanger inhibitor, SITS ($5 \cdot 10^{-4}$ M) caused a slight block of $J^b_{Cl^-}$ (17% inhibition), whereas niflumic acid ($5 \cdot 10^{-5}$ M) produced a 47% inhibition (Fig. 6). Furosemide (10^{-7} M to 10^{-3} M) and bumetanide (10^{-6} M, $5 \cdot 10^{-6}$ M) which are considered as cotransport blockers both inhibited $J^b_{Cl^-}$ (Fig. 6B), with the latter being most effective. Furosemide had a maximal inhibitory effect of $53\% \pm 4\%$ and its dose-response curve is given in Fig. 7. Furosemide also had an inhibitory effect on epithelia bathed in an 'Air Ringer' solution ($41\% \pm 7\%$ of inhibition at 10^{-3} M, $n = 6$). The same concentration of the drug added to the apical side had no effect on the small Cl^- permeability of the apical membranes ($J^a_{Cl^-} = 124 \pm 31$ nequiv.$h^{-1}cm^{-2}$ and $J^a_{Cl^-} = 128 \pm 39$ nequiv.$h^{-1}cm^{-2}$ for control and furosemide, respectively, $n = 4$). The inhibition of $J^b_{Cl^-}$ produced by furosemide (10^{-4} M) and niflumic acid ($5 \cdot 10^{-5}$ M) was not significantly different from that observed with furosemide alone

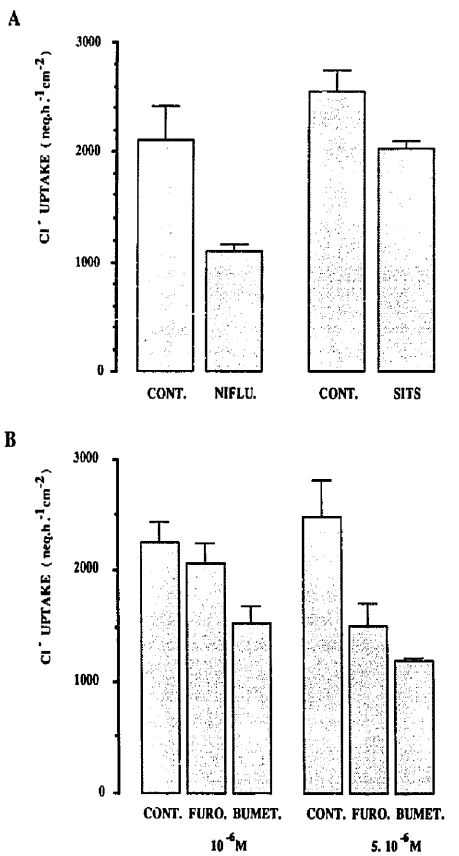

Fig. 6. (A) Comparative effects of SITS ($5 \cdot 10^{-4}$ mmol/l) and niflumic acid ($5 \cdot 10^{-5}$ mmol/l) on basolateral Cl^- uptake; ($n = 4$). (B) Comparative effects of furosemide and bumetamide tested at 10^{-6} mmol/l and $5 \cdot 10^{-6}$ mmol/l ($n = 4$). Epithelia were incubated for 90 min in 'alkaline Ringer' and the drugs were added 15 min before Cl^- uptake measurements.

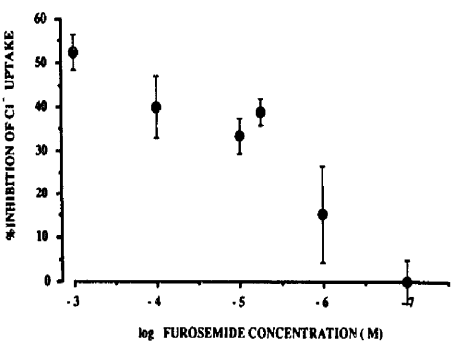

Fig. 7. Log-dose response curve of basolateral chloride uptake (expressed in % inhibition of Cl^- uptake) versus furosemide concentration. Epithelia were incubated for 90 min in 'alkaline Ringer' and the drug was added 15 min before Cl^- uptake measurements ($n = 8$).

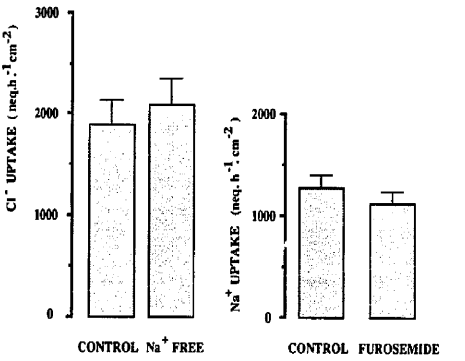

Fig. 8. (A) The effect of Na^+-free solution on basolateral Cl^- uptake. Epithelia were incubated 90 min in 'alkaline Ringer' and rinsed for 5 min in an 'alkaline Ringer' in which Na^+ was replaced by choline. The $^{36}Cl^-$ uptake was performed in a Na^+-free 'Air Ringer' ($n = 9$). (B) The effect of furosemide (10^{-3} mmol/l) on basolateral Na^+ uptake. Epithelia were incubated for 90 minutes with the Na^+ channel blocker, phenamil (10^{-5} mmol/l) and furosemide was added 15 min before the Na^+ uptake measurements ($n = 5$).

(39% ± 6% of inhibition with furosemide alone and 35% ± 5% with furosemide plus niflumic acid, $n = 4$).

Since these drugs can act on various anion transport systems, we tested whether they inhibited $J^b_{Cl^-}$ by acting on Na^+- or K^+-dependent Cl^- cotransporters. The dependence of $J^b_{Cl^-}$ on external Na^+ was determined by substituting sodium by choline in the Ringer solution in alkali-loaded epithelia. As can be observed in Fig. 8A, Na^+ substitution did not affect $J^b_{Cl^-}$. In addition, Na^+ uptake through the basolateral membranes ($J^b_{Na^+}$) was insensitive to furosemide (10^{-3} M) (Fig. 8B) indicating that transport of Na^+ and Cl^- through the basolateral membranes occur by independent mechanisms which do not involve Na/K/Cl or NaCl cotransports.

Discussion

The aim of this study was to determine the nature of Cl^- transport across the basolateral membranes of the frog skin epithelium.

Our results indicate that a fraction of the Cl^- permeability of these membranes depends on Cl^-/HCO_3^- exchange. The presence of this exchanger is supported by the following evidence: (1) $^{36}Cl^-$ uptake across basolateral cell membranes is markedly increased after an alkaline load (2) intracellular Cl^- activity is increased in the presence of HCO_3^- in the serosal Ringer solution, (3) pH_i regulation following an alkaline load is dependent on Cl^- in the serosal solution. Under steady-state conditions, the presence of a basolateral Cl^-/HCO_3^- exchanger, has already been demonstrated in *R. pipiens* skin [10,13,14]. In previous studies [3], we showed that a Cl^-/HCO_3^- exchanger, working in series

with the apical electrogenic proton pump produced an alkalinisation of the serosal solution bathing *R. esculenta* epithelium (an apparent Cl^--dependent transepithelial HCO_3^- flux of 150 to 200 equiv.$h^{-1}cm^{-2}$)). The proton pump in the frog skin and the turtle urinary bladder is restricted to the mitochondria-rich cells (for reviews, see Refs. 15 and 16) and the apical proton pump and its linked basolateral HCO_3^- reabsorption via the anion exchanger are sensitive to carbonic anhydrase inhibitors [17,3]. It is most likely that MR cells which contain this enzyme [18] also possess a Cl^-/HCO_3^- exchanger located on the basolateral membranes. Here, we measured Cl^- uptake across the basolateral membranes of the entire frog skin epithelium and not a transepithelial ion transport. Considering the magnitude of the bicarbonate-dependent Cl^- uptake (500 equiv.$h^{-1}cm^{-2}$), it is reasonable to assume that the Cl^- permeability stimulated by an alkaline load of the epithelium, occurs in a much larger cell population than the mitochondria-rich cells and probably involves all the epithelial cells. Microelectrode impalements from the basolateral side produce recordings from the deeper granular cell layers and confirm the presence of Cl^-/HCO_3^- exchange in these cells.

In previous studies, we found that a Na^+/H^+ exchanger was stimulated by an acid-load [5,6]. The stimulation of a Cl^-/HCO_3^- exchange after an alkaline load would provide a complementary mechanism of pH regulation. In addition to their cell pH regulatory role, these exchangers can have an indirect effect on transepithelial Na^+ transport which is highly sensitive to pH_i [7].

It should be stressed that the pharmacological approach did not provide clear-cut experiments to distinguish between the different transport mechanisms which create the anion permeability in the basolateral membranes. Although $J^b_{Cl^-}$ was partially inhibited by blockers of Cl^-/HCO_3^- exchange such as niflumic acid [19] and SITS [20], the less specific agents furosemide and bumetanide also inhibited $J^b_{Cl^-}$. Since no additional effect was noted after the simultaneous addition of furosemide and niflumic acid, these two drugs are expected to act on the same mechanism of Cl^- transport. Furosemide and bumetanide are considered to be rather specific cotransport blockers [22] when used at relatively low concentrations (10^{-6} to 10^{-5} M). They have been shown to be non-specific when used at higher concentrations, for example, by inhibiting the erythrocyte Cl^-/HCO_3^- exchange [23,24]. We found an effect of these drugs at relatively low doses: The possibility of a Cl^- uptake mediated by an electroneutral Na:Cl or 2Cl/Na/K cotransport system is excluded however, since the basolateral membrane Cl^- transport rate was unchanged in a sodium-free Ringer solution and Na^+ uptake through the basolateral membranes was insensitive to furosemide. Furthermore, since the Cl^-/HCO_3^- exchange was shown to be independent of external Na^+ and Cl^- uptake was unchanged when Na^+/H^+ exchange was stimulated, it must differ from the mechanism of coupled $Na^+/H^+ : Cl^-/HCO_3^-$ exchange observed in other cell types (for review, see Ref. 25).

The stimulation of a normally quiescent 2Cl/Na/K cotransporter has been reported to be involved in cell volume regulation [25,27] following cell shrinkage. Ussing and Kristensen produced this effect by submitting the epithelium to a serosal hypoosmotic shock (a 50% Ringer solution) and observed cell shrinkage followed by regulatory cell volume increase upon return to a 'normal' Ringer solution. Using a similar experimental protocol, we found a small and transient stimulation of $J^b_{Cl^-}$: under isotonic conditions $J^b_{Cl^-}$ was 529 ± 51 equiv.$h^{-1}cm^{-2}$ ($n = 12$) which increased at 5 min after recovery in 'normal' Ringer to 723 ± 99 equiv.$h^{-1}cm^{-2}$ ($n = 5$), returning 40 min later to 528 ± 47 equiv.$h^{-1}cm^{-2}$ ($n = 11$). These results are compatible with the stimulation of a cotransport mechanism which is active during cell volume regulation [27] but normally quiescent under steady-state conditions.

We conclude that a basolateral Cl^-/HCO_3^- exchange accounts for a fraction of Cl^- permeability and is involved in cell pH regulation.

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