Journal of Physiology, (1989), **419**, *pp.* 539–553
With 7 text-figures
Printed in Great Britain

RHEOGENIC SODIUM–BICARBONATE CO-TRANSPORT ACROSS THE RETINAL MEMBRANE OF THE FROG RETINAL PIGMENT EPITHELIUM

BY MORTEN LA COUR

From the Department of General Physiology and Biophysics, University of Copenhagen, Panum Institute, Blegdamsvej 3, 2200 Copenhagen N, Denmark

(*Received 6 February 1989*)

SUMMARY

1. Na^+ and HCO_3^- transport across the retinal membrane of the frog retinal pigment epithelium was studied by means of double-barrelled Na^+- and pH-selective microelectrodes. Transient changes in the intracellular pH and in the intracellular Na^+ activity were monitored in response to abrupt changes in the Na^+ concentration and in the HCO_3^- concentration on the retinal side of the epithelium, and in response to transepithelial currents.

2. Removal of Na^+ from the retinal side of the epithelium caused a depolarization of the membrane potential across the retinal membrane, a decrease in the intracellular Na^+ activity and a decrease in the intracellular pH.

3. An increase in the HCO_3^- concentration on the retinal side of the epithelium from 27·5 to 50 mM caused a hyperpolarization of the membrane potential across the retinal membrane, an increase in the intracellular Na^+ activity and an increase in the intracellular pH.

4. Passage of a transepithelial current of 30 μA in the choroid-to-retina direction caused an increase in the intracellular Na^+ activity and an increase in the intracellular pH.

5. The data are interpreted as evidence for rheogenic co-transport of Na^+, HCO_3^- across the retinal membrane of the frog retinal pigment epithelium.

6. The transient changes described under 2, 3 and 4 above were blocked by 0·5 mM-4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS). The Na^+–HCO_3^- co-transport was not inhibited by 1 mM-amiloride.

INTRODUCTION

The retinal pigment epithelium separates the photoreceptors from the choriocapillaries; it is thus an important part of the blood–retinal barrier. The retinal membrane of the retinal pigment epithelium constitutes the outer border of the subretinal space which is the extracellular space surrounding the photoreceptors. Since the outer neuroretina is avascular, transport across the retinal pigment

epithelium has a major influence on the composition of the fluid in the subretinal space and thus on the extracellular environment of the visual cells.

It has been found that changes in extracellular HCO_3^- concentration have important consequences for the function of the photoreceptors *in vitro* (Besharse & Dunis, 1983). In the present study I address the question of acid–base transport across the retinal membrane of the frog retinal pigment epithelium; in particular I investigate whether this membrane incorporates a rheogenic Na^+–HCO_3^- co-transport system similar that described in the basolateral membrane of the proximal tubules in the kidney (see below). I have applied double-barrelled Na^+- and pH-selective microelectrodes to study transport of Na^+ and HCO_3^-. In a set of experiments, the intracellular parameters were monitored while the electrochemical gradients for Na^+ and for HCO_3^- across the retinal membrane were transiently altered by ion substitutions and by passage of transepithelial currents. The results were fully compatible with the hypothesis that this membrane incorporates a rheogenic Na^+–HCO_3^- co-transport system.

Rheogenic Na^+–HCO_3^- co-transport was originally described in the basolateral membrane of the proximal tubules of the tiger salamander (Boron & Boulpaep, 1983), and later in proximal tubules of the rat (Alpern, 1985; Yoshitomi, Burckhardt & Fromter, 1985), the rabbit (Biagi & Sohtell, 1986; Sasaki, Shiigai, Yoshiyama & Takeuchi, 1987) and *Necturus* (Lopes, Siebens, Giebisch & Boron, 1987). Rheogenic Na^+–HCO_3^- co-transport has also been described in cultured bovine corneal endothelial cells (Jentsch, Keller, Koch & Wiederholt, 1984*a*; Jentsch, Koch, Bleckmann & Wiederholt, 1984*b*) and in a continuous cell line (BSC-1) derived from monkey kidney (Jentsch, Schill, Schwarz, Matthes, Keller & Wiederholt, 1985; Jentsch, Matthes, Keller & Wiederholt, 1986). Recently a rheogenic Na^+–HCO_3^- co-transport system has been found in oxyntic cells of frog gastric mucosa (Curci, Debellis & Froemter, 1987), and in glial cells in the central nervous system of the leech (Schlue & Deitmer, 1988).

METHODS

Double-barrelled ion-selective microelectrodes were made as described by Zeuthen (1980). The ion-selective barrel of the microelectrodes was filled with Na^+ cocktail A (Fluka) or with pH cocktail A (Fluka). The reference barrels were filled with 2 M-KCl. The Na^+ electrodes were calibrated in solutions containing 120 mM-Cl^- and 2, 5, 10, 20 or 40 mM-Na^+, with K^+ as the substituting ion. The sensitivity of the Na^+ electrodes between 10 and 20 mM-Na^+ were 44 ± 3 mV (average $\pm$ S.D., $n = 16$) per 10-fold change in the Na^+ concentration. The intracellular Na^+ activities are reported as apparent concentrations, assuming that the intracellular activity coefficient for Na^+ equals the activity coefficient for Na^+ in the calibrating solution. The pH electrodes were calibrated in solutions which contained 105 mM-KCl, 15 mM-Na^+ and 15 mM-phosphate. The pH of the calibrating solutions for the pH electrodes were 6·84, 7·15 and 7·60. The sensitivity of the pH electrodes were 55 ± 5 mV (average $\pm$ S.D., $n = 15$) per pH unit. In a few control experiments single-barrelled microelectrodes were used. These electrodes were filled with 2 M-KCl and they had resistances of 50–80 MΩ.

Large bull-frogs (*Rana catesbeiana*) were obtained from the Kons Scientific Co. (German Town, WI, USA). Dark-adapted frogs were killed by decapitation and double pithing. The eyes were enucleated after decapitation. The retinal pigment epithelium and choroid were excised and mounted as a unilateral preparation in a mini-Ussing chamber as previously described (LaCour, Lund-Anderson & Zuethen, 1986). A surface area of 0·07 cm^2 of the epithelium was exposed. The

retinal side of the epithelium was perfused by means of a peristaltic pump. The perfusate could be changed within 10 s between different solutions. The choroidal compartment contained *ca* 30 μl standard solution and was not perfused.

Current pulses of 10 μA were passed across the epithelium in the retina-to-choroid direction every 20 s. The deflections in the membrane potential across the retinal membrane (dE_r) and in the transepithelial potential (dTEP) caused by the current pulses were used to calculate the voltage divider ratio (VDR): $VDR = dE_r/(dTEP - dE_r)$. The voltage divider ratio is the ratio of the resistance of the retinal membrane to the resistance of the choroidal membrane.

TABLE 1. Solutions (concentrations in mM)

	Na^+	K^+	Mg^{2+}	Ca^{2+}	$NMDGH^+$	Cl^-	HCO_3^-	HEPES	Glu
Solution 1	110	2	1	1·8	0	90·1	27·5	0	5
Solution 2	110	2	1	1·8	0	67·6	50	0	5
Solution 3	0	2	1	1·8	110	90·1	27·5	0	5
Solution 4	110	2	1	1·8	0	117·6	0	10	5
Solution 5	0	2	1	1·8	110	117·6	0	10	5

Glu = glucose, $NMDGH^+$ = *N*-methyl-D-glucamine, acid.

The composition of the solutions used to perfuse the epithelium is stated in Table 1. The control solution (solution 1), the Na^+-free solution (solution 3) and the solution containing 50 mM-HCO_3^- (solution 2) were gassed with 95% O_2 and 5% CO_2. The HCO_3^--free solution (solution 4) and the HCO_3^-- and Na^+-free solution (solution 5) were gassed with air. Solution 2 had a pH of 7·66 ± 0·02. The other solutions were gassed and titrated to pH 7·40 before each experiment. 4-Acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS) and ouabain were obtained from Sigma, amiloride was a gift from Sharp and Dohme. These were of Reagent grade.

All experiments were carried out at room temperature (23–25 °C).

Results are presented as average ± S.D. (n = number of experiments).

RESULTS

The cells were punctured while the tissue was perfused with the control solution. During the first 2–6 min after penetration with the microelectrode the cells were studied in steady state. Thereafter a transient experiment was performed. Table 2 summarizes the results of the three basic transient experiments: (1) reducing the Na^+ concentration in the perfusate to 0 mM, (2) passing transepithelial currents and (3) increasing the HCO_3^- concentration in the perfusate from 27·5 to 50 mM.

Steady-state measurements

Under control conditions the membrane potential across the retinal membrane (E_r) was −84·9 ± 3·0 mV (n = 56), the intracellular Na^+ activity (a^i_{Na}) was 11 ± 3 mM (n = 29), the intracellular pH (pH_i) was 7·08 ± 0·08 (n = 27), the voltage divider ratio was 0·42 ± 0·11 (n = 56), the transepithelial potential was 9·5 ± 2·1 mV, retinal side positive (n = 31) and the transepithelial resistance was 143 ± 27 Ω cm² (n = 31).

Effects of removing Na^+ from the perfusate

Figure 1 shows the effects on the intracellular Na^+ activity and the membrane potential across the retinal membrane of removing Na^+ from the perfusate. Figure 2

shows the effects on the intracellular pH and the membrane potential across the retinal membrane of reducing the Na^+ concentration in the perfusate from 110 mM to nominally 0, 10, 20 or 40 mM.

When the Na^+ concentration in the perfusate was reduced to 0 mM, the membrane

TABLE 2. Summary of results

	ΔE_r (mV)	Δa^i_{Na} (μM/s)	ΔpH_i (10^{-3} units/s)
		Removal of Na^+	
0 Na^+, control	12·2±1·9 (51)	−197±68 (23)	−6·5±2·2 (28)
0 Na^+, 0 HCO_3^-	0·3±2·5 (16)	−106±41 (8)	−0·0±0·0 (8)
0 Na^+, SITS	3·6±2·0 (8)	−117±57 (4)	−2·1±1·3 (4)
0 Na^+, amiloride	12·5±1·2 (8)	−212±34 (5)	−6·4±2·5 (3)
		Passage of currents	
Current, control	21·6±5·8 (52)	26±12 (21)	3·0±1·0 (31)
Current, 0 HCO_3^-	26·7±4·1 (18)	0±0 (8)	1·1±0·4 (10)
Current, SITS	26·7±10·3 (13)	0±0 (6)	0·0±0·0 (7)
Current, amiloride	19·8±6·4 (12)	28±7 (4)	2·5±0·7 (8)
		Increase in HCO_3^- concentration from 27·5 to 50 mM	
50 HCO_3^-, control	−10·8±1·3 (34)	34±12 (19)	6·0±2·4 (15)
50 HCO_3^-, SITS	−6·8±1·6 (11)	10±7 (7)	1·9±1·2 (4)
50 HCO_3^-, amiloride	−10·9±1·4 (10)	26±11 (6)	4·5±1·9 (4)

Initial change in the membrane potential across the retinal membrane (ΔE_r), initial rate of change in the intracellular Na^+ activity (a^i_{Na}) and in the intracellular pH (ΔpH_i). The three panels shows results of the three basic types of experiments: removal of Na^+, passage of currents and increase in the HCO_3^- concentration, done under the different conditions (control conditions, HCO_3^--free, in presence of SITS, in presence of amiloride). Results are presented as average±S.D. (number of experiments).

potential across the retinal membrane depolarized rapidly (Figs 1 and 2, Table 2). The initial depolarization was followed by a slower phase lasting 1–2 min in which the membrane potential recovered towards its control value. The removal of Na^+ also caused a decrease in the intracellular Na^+ activity, and a decrease in the intracellular pH (Figs 1 and 2, Table 2). As shown in Fig. 2, smaller depolarizations and lower rates of intracellular acidification were observed when the Na^+ concentration was reduced to 10, 20 or 40 mM. Within the first minute after Na^+ was removed (i.e. reduced to 0 mM) from the perfusate the voltage divider ratio increased by 2·2±0·5 ($n = 51$). When the perfusate was changed back to the control solution, the intracellular parameters returned to their control values.

The effect of Na^+ removal on the membrane voltage was also investigated 2–11 min after the tissue was poisoned with ouabain. In the presence of 1 mM-ouabain, Na^+ removal caused a depolarization of the membrane potential across the retinal membrane of 13±3 mV ($n = 6$). This is not significantly different from the depolarization caused by Na^+ removal in the absence of ouabain (t test, $P > 0·1$).

Effects of transepithelial currents

Figure 3 shows the effects of a transepithelial current of 30 μA, passed in the choroid-to-retina direction. This caused a depolarization of the membrane potential

across the retinal membrane, an increase in the intracellular Na^+ activity, and an increase in the intracellular pH (Fig. 3, Table 2).

Effects of changing the HCO_3^- concentration in the perfusate

Increase in HCO_3^-

Figure 4 shows two experiments in which the HCO_3^- concentration in the perfusate was increased from 27·5 to 50 mM at constant P_{CO_2}. This caused a rapid

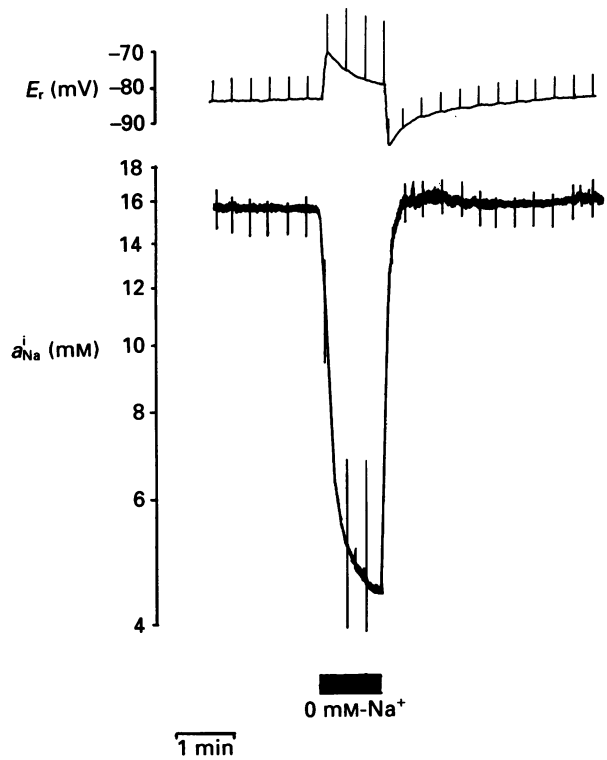

Fig. 1. The changes in the intracellular Na^+ activity (a^i_{Na}) and in the membrane potential across the retinal membrane (E_r) in a frog retinal pigment epithelial cell in response to a reduction in the Na^+ concentration on the retinal side of the tissue from 110 mM to nominally 0 mM.

hyperpolarization of the membrane potential across the retinal membrane. The initial hyperpolarization was followed by a slower phase lasting 1–2 min during which the membrane potential depolarized by 2–4 mV. The increase in the HCO_3^- concentration also caused an increase in the intracellular Na^+ activity, and an increase in the intracellular pH (Fig. 4, Table 2). Within the first minute after the HCO_3^- concentration in the perfusate had been increased from 27·5 to 50 mM the voltage divider ratio decreased by $0{\cdot}13 \pm 0{\cdot}09$ ($n = 34$). When the perfusate was returned to the control solution the intracellular parameters returned to their control values.

The effect on the membrane potential of increasing the HCO_3^- concentration was

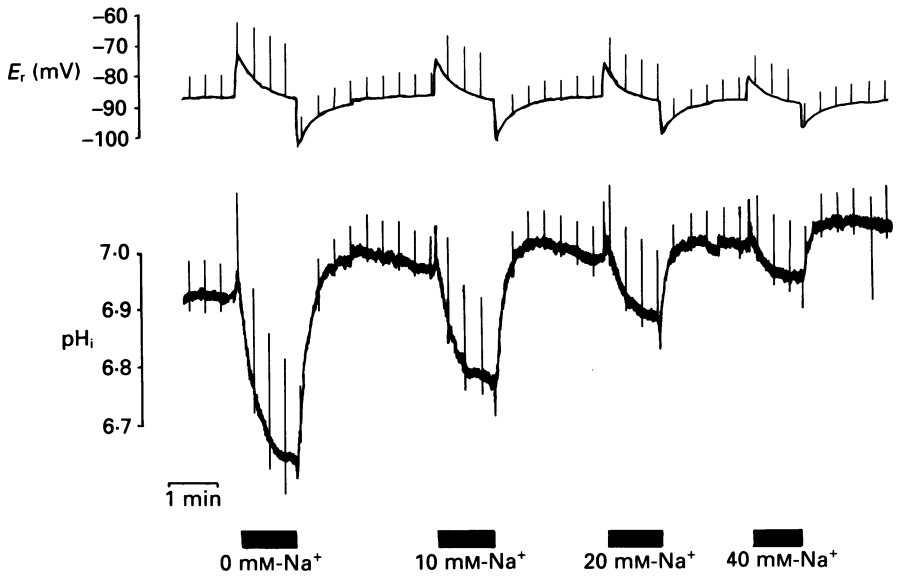

Fig. 2. The changes in the intracellular pH (pH_i) and in the membrane potential across the retinal membrane (E_r) in a frog retinal pigment epithelial cell in response to reductions in the Na^+ concentration on the retinal side of the tissue from 110 mM to subsequently nominally 0, 10, 20 and 40 mM.

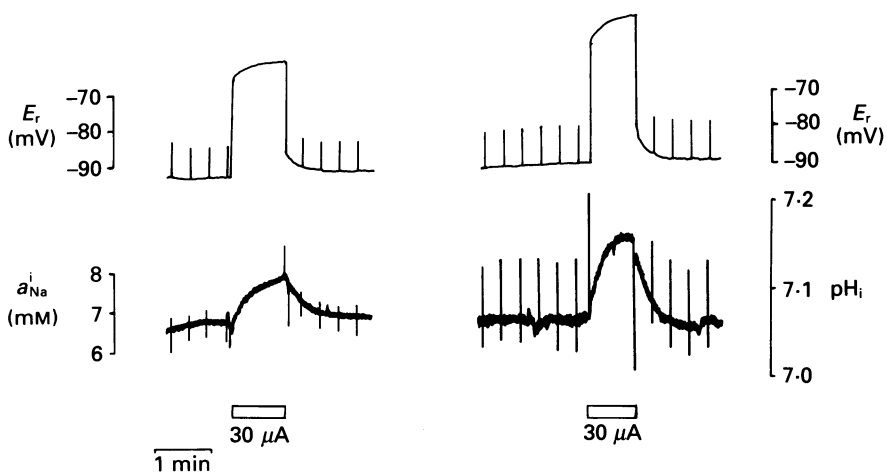

Fig. 3. The changes in the intracellular Na^+ activity (a^i_{Na}), in the intracellular pH (pH_i) and in the membrane potential across the retinal membrane (E_r) in a frog retinal pigment epithelial cell in response to a transepithelial current of 30 μA passed in the choroid-to-retina direction. Left panel shows a recording obtained with a Na^+ selective microelectrode; right panel shows a recording obtained with a H^+-selective microelectrode.

also investigated 4–12 min after the 1 mM-ouabain was added to the perfusate. In the presence of ouabain, an increase in the HCO_3^- concentration from 27·5 to 50 mM caused a hyperpolarization of the membrane potential across the retinal membrane of 9·6 ± 1·7 mV ($n = 7$). This is 1·2 mV less than the hyperpolarization caused by a similar increase in the HCO_3^- concentration in the absence of ouabain, the difference being significant (t test, $P < 0{\cdot}01$).

Removal of HCO_3^-

Figures 5 and 6 show two experiments in which the HCO_3^- concentration in the perfusate was reduced from 27·5 mM to nominally 0 mM at a constant pH of 7·4. This caused a depolarization of the membrane potential across the retinal membrane.

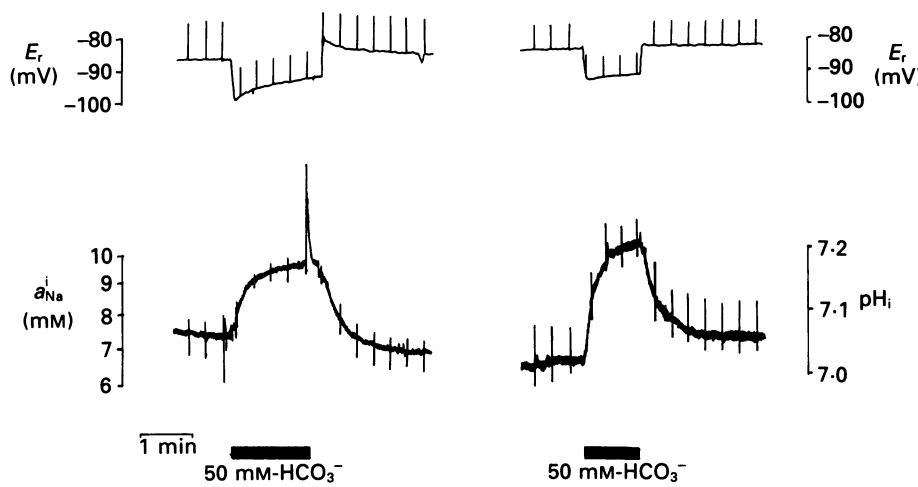

Fig. 4. The changes in the intracellular Na^+ activity (a^i_{Na}), in the intracellular pH (pH_i) and in the membrane potential across the retinal membrane (E_r) in a frog retinal pigment epithelial cell in response to an increase in the HCO_3^- concentration on the retinal side of the tissue from 27·5 to 50 mM at constant P_{CO_2}. Left panel shows a recording obtained with an Na^+-selective microelectrode, right panel shows a recording obtained with an H^+-selective microelectrode.

Initially this depolarization reached 18 ± 4 mV ($n = 23$) but it was followed by a slower phase lasting 2–3 min, during which the membrane potential repolarized to within 2–4 mV of its initial value. The intracellular Na^+ activity decreased with an initial rate of change of 0·06 ± 0·02 mM/s ($n = 11$). This was followed by a phase lasting several minutes during which the intracellular Na^+ activity slowly rose again towards the value found under control conditions. The intracellular pH increased with an initial rate of change of 0·012 ± 0·007 pH unit/s ($n = 12$); there was no sign of recovery towards control values even after prolonged exposure (9–10 min). Within the first minute after the HCO_3^- concentration in the perfusate was reduced from 27·5 mM to nominally 0 mM the voltage divider ratio increased by 0·6 ± 0·3 ($n = 23$).

The membrane voltage transient caused by HCO_3^- removal was also investigated

in the presence of ouabain. Three to fourteen min after 1 mM-ouabain was added to the perfusate, HCO_3^- removal caused a depolarization of the membrane potential across the retinal membrane of 18 ± 5 mV ($n = 7$). This is not significantly different from the depolarization caused by HCO_3^- removal in unpoisoned tissues (t test, $P > 0{\cdot}1$).

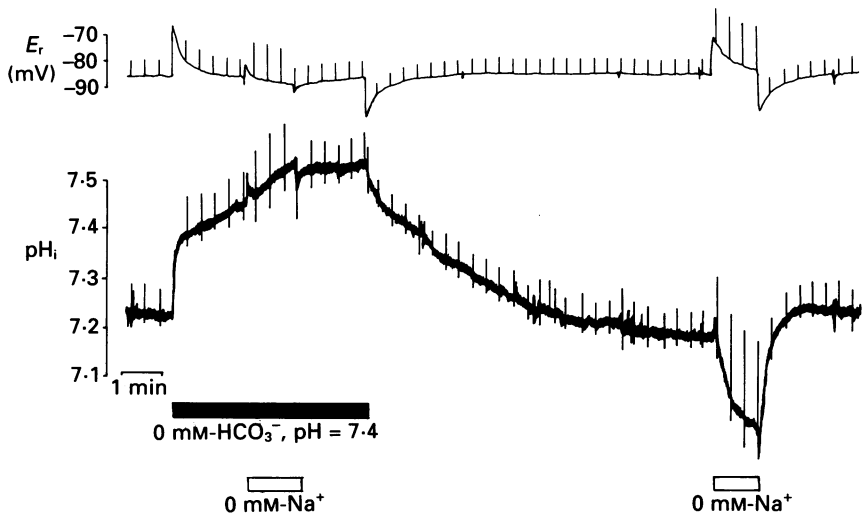

Fig. 5. The changes in the intracellular pH (pH_i) and in the membrane potential across the retinal membrane (E_r) in a frog retinal pigment epithelial cell in response to a reduction in the HCO_3^- concentration on the retinal side of the tissue from 27·5 mM to nominally 0 mM at constant pH = 7·4 (filled bar), and to the subsequent reduction of the Na^+ concentration on the retinal side of the tissue from 110 mM to nominally 0 mM (open bar). Also shown (right) are the effects on pH_i and on E_r in the same cell of a reduction in the Na^+ concentration on the retinal side of the tissue from 110 mM to nominally 0 mM in the presence of 27·5 mM-HCO_3^-.

While the tissue was perfused with the nominally HCO_3^--free solution, the effects of Na^+ removal were investigated. Figure 5 shows an experiment in which the perfusate was first changed from the control solution (solution 1, Table 1) to the HCO_3^--free solution (solution 4, Table 1); 2 min later the perfusate was changed from the HCO_3^--free solution to the HCO_3^-- and Na^+-free solution (solution 5, Table 1). Also shown on Fig. 5 (right) are the effects of Na^+ removal in the presence of 27·5 mM-HCO_3^- in the same cell. When Na^+ was removed under HCO_3^--free conditions (Table 2) no significant change in the membrane potential across the retinal membrane was observed (t test, $P > 0{\cdot}1$). The intracellular Na^+ activity decreased with an initial rate of change which was about half the rate observed when Na^+ was removed in the presence of 27·5 mM-HCO_3^-, the difference being significant (unpaired t test, $P < 0{\cdot}005$). There was no change in the intracellular pH when Na^+ was removed under HCO_3^--free conditions (Fig. 5).

Further the effects of transepithelial currents were studied while the epithelium was perfused with the HCO_3^--free solution. Figure 6 shows an experiment in which the perfusate was changed from the control solution to the HCO_3^--free solution; 2 and 4 min later transepithelial currents of 30 μA were passed. Also shown in Fig. 6

are the effects of a similar current in the presence of 27·5 mM-HCO_3^- (left). While the current always caused an increase in the intracellular pH and an increase in the intracellular Na^+ activity when the epithelium was perfused with the control solution (27·5 mM HCO_3^-), no change was observed in the intracellular Na^+ activity, and only

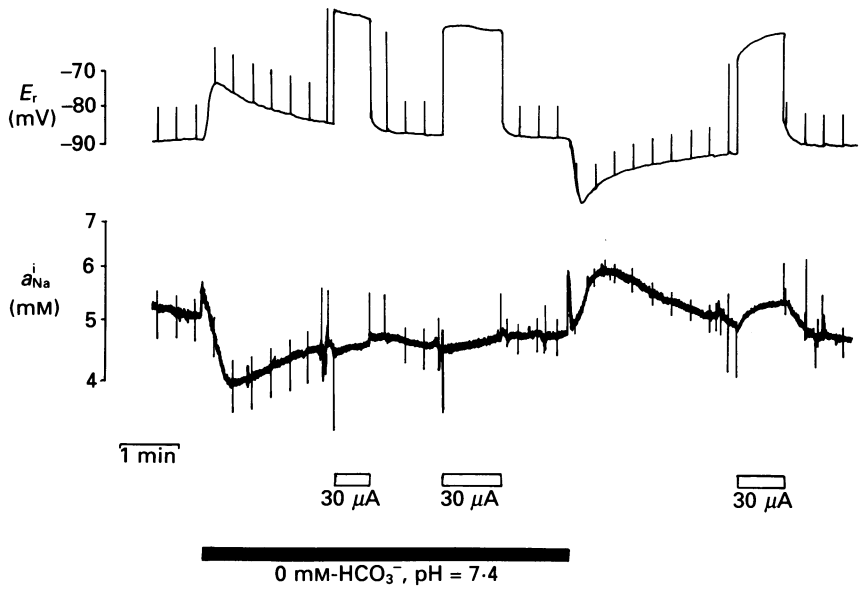

Fig. 6. The changes in the intracellular Na^+ activity (a^i_{Na}) and in the membrane potential across the retinal membrane (E_r) in a frog retinal pigment epithelial cell in response to a reduction in the HCO_3^- concentration on the retinal side of the tissue from 27·5 mM to nominally 0 mM at constant pH = 7·4 (filled bar), and to the passage of transepithelial currents of 30 μA in the choroid-to-retina direction (open bars).

a small increase in the intracellular pH, when the current was passed during perfusion with the HCO_3^--free solution (Table 2). The rate of increase in the intracellular pH observed when the current was applied under HCO_3^--free conditions was significantly less than the rate observed in the presence of 27·5 mM-HCO_3^- (unpaired t test, $P < 0{\cdot}005$).

When the perfusate was returned from the HCO_3^--free solution to the control solution, the membrane potential hyperpolarized within 10 s and then depolarized towards its control value (Figs 5 and 6). The intracellular Na^+ activity increased initially and then decreased towards its control value (Fig. 6). The intracellular pH decreased over several minutes towards its control value (Fig 5). An acid undershoot in the intracellular pH was never observed upon returning to the control solution.

Effects of SITS

When 0·5 mM-SITS was administered to the perfusate (Fig. 7) the membrane potential across the retinal membrane depolarized by 1 ± 1 mV ($n = 21$), the intracellular pH increased with an initial rate of $0{\cdot}002 \pm 0{\cdot}001$ pH units/s ($n = 7$) and the voltage divider ratio increased by $0{\cdot}5 \pm 0{\cdot}4$ ($n = 21$). There was no change in the intracellular Na^+ activity ($n = 14$).

When Na^+ was removed from the perfusate in the presence of 0·5 mM-SITS (Table 2) the depolarization of the membrane potential across the retinal membrane, the initial rate of decrease in the intracellular Na^+ activity, and the initial rate of decrease in the intracellular pH were all significantly smaller when compared to the effects of Na^+ removal in the absence of SITS (*t* tests, $P < 0{\cdot}025$).

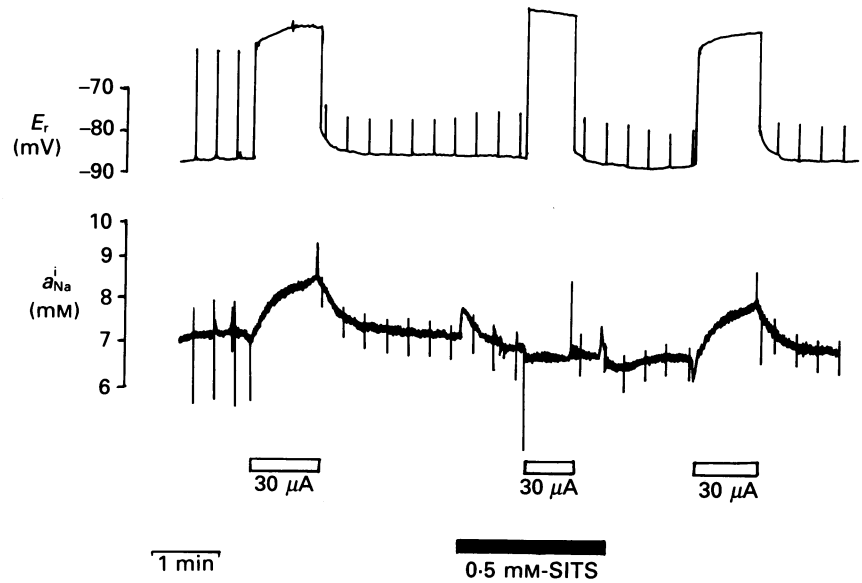

Fig. 7. The changes in the intracellular Na^+ activity (a^i_{Na}) and in the membrane potential across the retinal membrane (E_r) in a frog retinal pigment epithelial cell in response to the administration of 0·5 mM-SITS (filled bar) and to the subsequent passage of a transepithelial current of 30 μA in the retina-to-choroid direction. Also shown are the effects of the passage of transepithelial currents of 30 μA in the absence of SITS (left and right).

When the HCO_3^- concentration in the perfusate was increased from 27·5 to 50 mM in the presence of 0·5 mM-SITS (Table 2) the depolarization of the membrane potential across the retinal membrane, the initial rate of increase in the intracellular Na^+ activity, and the initial rate of increase in the intracellular pH were all significantly smaller than in the absence of SITS (*t* test, $P < 0{\cdot}005$).

In the absence of SITS transepithelial currents (30 μA) caused an increase in the intracellular Na^+ activity and an increase in the intracellular pH. In the presence of 0·5 mM-SITS, similar currents (Fig. 7) had neither effects on the intracellular Na^+ activity nor on the intracellular pH (Table 2).

SITS (0·5 mM) was present for periods of 2–14 min. The effects on the membrane potential, the intracellular Na^+ activity and the intracellular pH were reversible upon removal of the drug (Fig. 7).

Effects of amiloride

Administration of 1 mM-amiloride to the perfusate caused no change in the membrane potential across the retinal membrane, no change in the intracellular Na^+

activity and a slight increase in the intracellular pH (less than 0·06 pH units). The effects of removing Na^+ from the perfusate or of increasing the HCO_3^- concentration in the perfusate from 27·5 to 50 mM or of passing a transepithelial current of 30 μA in the presence of 1 mM-amiloride were not significantly different from the corresponding effects observed in the absence of amiloride (unpaired t test, $P > 0{\cdot}1$).

DISCUSSION

Distribution of Na^+ and HCO_3^- in the steady state

The average intracellular Na^+ activity was 11 mM; this agrees well with what has been found in other epithelial cells (e.g. Zeuthen, 1981). The low intracellular Na^+ activity implies that a large inwardly directed electrochemical gradient exists for Na^+ across the retinal membrane.

The average intracellular pH of 7·08 is identical to the intracellular pH in cultured bovine retinal pigment epithelial cells, recently measured by fluorescence technique (Keller, Jentsch, Janicke & Wiederholt, 1988). This value is also in good agreement with what has been found in other epithelial cells (e.g. Ross & Boron, 1981).

From the Henderson–Hasselbalch equation and the assumption that P_{CO_2} is the same in the intracellular fluid and in the extracellular fluid, the average intracellular HCO_3^- concentration calculated as 13 mM. Intracellular HCO_3^- was thus above electrochemical equilibrium.

Transient experiments

Effects of Na^+ removal

The intracellular acidification, which was caused by Na^+ removal (Fig. 2, Table 2), shows that Na^+-coupled transport of base equivalents takes place across the retinal membrane. The concomitant depolarization of the membrane potential across the retinal membrane (Figs 1 and 2, Table 2) cannot be explained as a result of reduced Na^+–K^+ pumping, since the membrane potential also depolarized when Na^+ was removed in the presence of ouabain (1 mM ouabain in the perfusate completely blocks the Na^+–K^+ pump, since in frog retinal pigment epithelial cells this pump is exclusively located in the retinal membrane: Bok, 1982). The depolarization is exactly the opposite of what would be expected if the retinal membrane was Na^+ permeable. It can however be explained as the result of increased outward rheogenic co-transport of Na^+, HCO_3^- and net negative charge.

Under nominally HCO_3^--free conditions Na^+ removal causes no significant changes in the membrane potential across the retinal membrane and no change in the intracellular pH (Fig. 3). The Na^+-coupled transport of base equivalents and of electrical charge thus requires the presence of HCO_3^-. This substantiates that a rheogenic Na^+–HCO_3^- co-transporter is present in the retinal membrane. When Na^+ removal was done under nominally HCO_3^--free conditions, the initial rate of decrease in the intracellular Na^+ activity was only reduced by 44%. This suggests the presence of an electroneutral Na^+ transport system which is not involved in acid–base transport and which exists in the retinal membrane alongside the rheogenic Na^+–HCO_3^- co-transport system.

Effects of currents

Rheogenic Na^+–HCO_3^- co-transport is assumed to incorporate transport of more than one HCO_3^- ion for each Na^+ ion transported. As opposed to electroneutral Na^+–H^+ exchange, rheogenic Na^+–HCO_3^- co-transport should therefore be affected by changes in the membrane potential. Accordingly, I observed a simultaneous increase in the intracellular Na^+ activity and in the intracellular pH when the membrane potential across the retinal membrane was depolarized by a transepithelial current (Fig. 4, Table 2). No detectable increase in the intracellular Na^+ activity was found when currents were passed under nominally HCO_3^--free conditions (Fig. 6). The rate of intracellular alkalinization was reduced by about two-thirds when currents were passed under nominally HCO_3^--free conditions. The influx of base equivalents was reduced even more since the intracellular buffer power was lower under these conditions than in the presence of 5% CO_2. Complete removal of HCO_3^- from the surface of the epithelium is virtually impossible. Thus the small influx of base equivalents caused by currents under nominally HCO_3^--free conditions were probably caused by HCO_3^- influx through the retinal membrane via rheogenic Na^+–HCO_3^- co-transport; the concomitant influx of Na^+ would be too small to be detected.

The hypothesis of rheogenic Na^+–HCO_3^- co-transport across the retinal membrane requires that the combined presence of Na^+ and HCO_3^- contributes to the conductance of this membrane. Accordingly, the voltage divider ratio increased when either HCO_3^- or Na^+ was removed from the perfusate, and it decreased when the HCO_3^- concentration in the perfusate is increased from 27·5 to 50 mM. It should be noted that the changes in the voltage divider ratio cannot be explained by the presence of a pH-dependent K^+ conductance in either the retinal or choroidal membrane. In that case the voltage divider ratio should not change in the same direction in the experiments in which Na^+ is removed as in the experiments in which HCO_3^- is removed, since the intracellular pH changes in opposite directions in these two types of experiments.

Effects of changes in the HCO_3^- concentration

When the HCO_3^- concentration in the perfusate was increased (Fig. 3 and Table 2), there was a simultaneous increase in the intracellular Na^+ activity, and the intracellular pH as well as a hyperpolarization of the membrane potential across the retinal membrane. The hyperpolarization of the membrane potential cannot be attributed to increased Na^+–K^+-pumping, since it was also observed (albeit reduced by 11%) after the pump was poisoned with ouabain. These observations corroborate that a coupling exists between transport of Na^+, transport of base equivalents and transport of net negative charge across the retinal membrane.

When HCO_3^- was removed from the perfusate, efflux of Na^+ and net negative charge via the rheogenic Na^+–HCO_3^- co-transport can explain both the observed decrease in the intracellular Na^+ activity and the observed depolarization of the membrane potential across the retinal membrane (Figs 3 and 6, Table 2). The simultaneous increase in the intracellular pH was caused by CO_2 exit. That the intracellular pH remained alkaline and did not show sign of recovery while the tissue

was perfused with the HCO_3^--free solution, could indicate that the Na^+–HCO_3^- co-transporter mediated an acid influx into the cells in the steady state under control conditions.

The slow time course of the decline in the intracellular pH and the lack of an acid undershoot in the intracellular pH upon returning from the HCO_3^--free solution to the control solution is surprising. It was not caused by slow perfusion since the hyperpolarization of the membrane potential were always completed within 10 s. The slow time course of the intracellular pH decline may be caused by very rapid intracellular pH regulation in the CO_2-containing medium.

Modes of Na^+ and HCO_3^- transport

Rheogenic Na^+–HCO_3^- co-transport is suggested as a mode of transport of Na^+ and HCO_3^- across the retinal membrane of the frog retinal pigment epithelium.

An electroneutral, amiloride-sensitive Na^+–H^+ exchanger has recently been described in cultured bovine retinal pigment epithelial cells (Keller *et al.* 1988). In the retinal membrane of the frog retinal pigment epithelium, such a transport system can only be of little significance since no intracellular acidification was observed when Na^+ was removed in the absence of HCO_3^- and since 1 mM-amiloride had no effects on the rates of change in the intracellular parameters in the transient experiments. However, it cannot be excluded that a Na^+–H^+ exchanger is present in the choroidal membrane of the frog retinal pigment epithelium.

Other electroneutral transport systems such as Na^+-dependent Cl^-–HCO_3^- exchange (Thomas, 1984), cannot explain the experimentally induced changes in the membrane potential and in the voltage divider ratio. Neither do the electroneutral transport systems explain the influx of Na^+ and the influx of HCO_3^- which were observed when a transepithelial current was passed. Electroneutral, Na^+-carrying transport systems, which are not involved in acid–base transport, may however co-exist with the rheogenic Na^+–HCO_3^- co-transporter in the retinal membrane.

Effects of SITS

In the transient experiments, the presence of 0·5 mM-SITS inhibited the rheogenic Na^+–HCO_3^- co-transport: the rate of change in both the intracellular Na^+ activity and the intracellular pH were reduced when 0·5 mM-SITS was present. Smaller doses of SITS were not effective since in two control experiments, the presence of 0·1 mM-SITS did not reduce the rate of intracellular acidification observed when Na^+ was removed from the perfusate (data not shown). Also in other tissues high concentration (0·1–1 mM) of the stilbene derivatives SITS and 4,4′-diisothiocyanostilbene-2,2′-disulphonic acid (DIDS) have been reported to inhibit rheogenic Na^+–HCO_3^- co-transport (kidney: Baron & Boulpaep, 1983; Yoshitomi *et al.* 1985; Biagi & Sohtell, 1986; Lopes *et al.* 1987; cultured corneal endothelium: Jentsch *et al.* 1984*b*; BSC1 cells: Jentsch *et al.* 1986; oxyntic cells: Curci *et al.* 1987; glial cells: Schlue & Deitmer, 1988).

It is surprising that the effects of SITS were reversible upon removal of the drug. In rabbit proximal tubules, 0·1 mM-SITS caused a slow and irreversible hyperpolarization of the membrane potential. This was not observed in the present preparation.

It can be concluded that the retinal membrane of the frog retinal pigment epithelium incorporates a transport system, which mediates coupled transport of Na^+, HCO_3^- and net negative charge. The transport system is inhibited by large concentrations (0·5 mM) of SITS, but is not affected by 1 mM-amiloride. The physiological significance of the transport system remains to be investigated.

Note added in proof. As this paper went to press a report (Hughes, Adorante, Miller & Lin, 1989) from another laboratory appeared, in which Na^+–HCO_3^- co-transport in the retinal membrane of the bull-frog retinal pigment epithelium was studied by means of single-barrelled microelectrodes and fluorescence measurements of intracellular pH.

Drs S. Dissing, H. Lund-Andersen, J. U. Prause and T. Zeuthen are thanked for useful comments on the manuscript. T. Soland is thanked for technical assistance. This research was supported by the Danish Medical Research Council and Landsforeningen til bekæmpelse af Øjensygdomme og Blindhed.

REFERENCES

ALPERN, R. J. (1985). Mechanism of basolateral $H^+/OH^-/HCO_3^-$ transport in the rat proximal convoluted tubule. A sodium-coupled electrogenic process. *Journal of General Physiology* **86**, 613–636.

BESHARSE, J. C. & DUNIS, D. A. (1983). Rod photoreceptor disc shedding in eye cups: relationship to bicarbonate and amino acids. *Experimental Eye Research* **36**, 567–580.

BIAGI, B. A. (1985). Effects of the anion transport inhibitor, SITS, on the proximal straight tubule of the rabbit perfused *in vitro*. *Journal of Membrane Biology* **88**, 25–31.

BIAGI, B. A. & SOHTELL M. (1986). Electrophysiology of basolateral bicarbonate transport in the rabbit proximal tubule. *American Journal of Physiology* **250**, F267–272.

BOK, D. (1982). Autoradiographic studies on the polarity of plasma membrane receptors in retinal pigment epithelial cells. In *The Structure of the Eye*, ed. HOLLYFIELD, J. G., pp. 247–256. New York: Elsevier North-Holland.

BORON, W. F. & BOULPAEP, E. L. (1983). Intracellular pH regulation in the renal tubule of the salamander. Basolateral HCO_3^- transport. *Journal of General Physiology* **81**, 53–94.

CURCI S., DEBELLIS, L. & FROEMTER, E. (1987). Evidence for rheogenic sodium bicarbonate transport in the basolateral membrane of oxyntic cells of frog gastric fundus. *Pflügers Archiv* **408**, 497–504.

HUGHES, B. A., ADORANTE, J. S., MILLER, S. S. & LIN, H. (1989). Apical electrogenic Na^+:HCO_3^--cotransport. A mechanism for HCO_3^- absorption across the retinal pigment epithelium. *Journal of General Physiology* **94**, 125–150.

JENTSCH, T. J., KELLER, S. K., KOCH, M. & WIEDERHOLT, M. (1984*a*). Evidence for coupled transport of bicarbonate and sodium in cultured bovine corneal endothelial cells. *Journal of Membrane Biology* **81**, 189–204.

JENTSCH, T J., KOCH, M., BLECKMANN, H. & WIEDERHOLT, M. (1984*b*). Effect of bicarbonate, pH, methazolamide and stilbenes on the intracellular potentials of cultured bovine corneal endothelial cells. *Journal of Membrane Biology* **78**, 103–117.

JENTSCH, T. J., SCHILL, B. S., SCHWARTZ, P., MATTHES H., KELLER, S. K., WIEDERHOLT, M. (1985). Kidney epithelial cells of monkey origin (BSC-1) express a sodium bicarbonate co-transport. Characterization by $^{22}Na^+$ flux measurements. *Journal of Biological Chemistry* **260**, 15554–15560.

JENTSCH, T. J., MATTHES, H., KELLER, S. K. & WIEDERHOLT, M. (1986). Electrical properties of sodium bicarbonate symport in kidney epithelial cells (BSC-1). *American Journal of Physiology* **251**, F954–968.

KELLER, S. K., JENTSCH, T. J., JANICKE, I. & WIEDERHOLT, M. (1988). Regulation of intracellular pH in cultured bovine retinal pigment epithelia cells. *Pflügers Archiv* **411**, 47–52.

LA COUR, M., LUND-ANDERSEN, H. & ZEUTHEN, T. (1986). Potassium transport of the frog retinal pigment epithelium: autoregulation of potassium activity in the subretinal space. *Journal of Physiology* **375**, 461–479.

LOPES, A. G., SIEBENS, A. W., GIEBISCH, G. & BORON, W. F. (1987). Electrogenic Na^+/HCO_3^- co-transport across basolateral membrane of isolated perfused *Necturus* proximal tubule. *American Journal of Physiology* **253**, F340–350.

ROSS, A. & BORON, W. F. (1981). Intracellular pH. *Physiological Reviews* **61**, 297–421.

SASAKI, S., SHIIGAI, T., YOSHIYAMA, N. & TAKEUCHI, J. (1987). Mechanism of bicarbonate exit across basolateral membrane of rabbit proximal straight tubule. *American Journal of Physiology* **252**, F11–18.

SCHLUE, W. R. & DEITMER, J. W. (1988). Ionic mechanisms of intracellular pH regulation in the nervous system. In *Proton Passage Across Cell Membranes*, ed. BOCK, G. & MARSH J., pp. 47–69. Ciba Foundation Symposium **139**. Chichester: Wiley.

THOMAS, R. C. (1984). Experimental displacement of intracellular pH and the mechanism of its subsequent recovery. *Journal of Physiology* **354**, 3–22P.

YOSHITOMI, K., BURCKHARDT, B.-CH. & FROMTER, E. (1985). Rheogenic sodium bicarbonate co-transport in the peritubular cell membrane of rat renal proximal tubules. *Pflügers Archiv* **405**, 360–366.

ZEUTHEN, T. (1980). How to make and use double barreled microelectrodes. In *Current Topics in Membranes and Transport*, vol. 13, eds: BRONNER, F., KLEINZELLER, A. & BOULPAEP, E. L., pp. 31–47, London: Academic Press.

ZEUTHEN, T. (1981). Ion transport in leaky epithelia studied with ion-selective microelectrodes. In *The Application of Ion-selective Microelectrodes*, ed. ZEUTHEN, T., pp. 27–46. New York: Elsevier North-Holland.