J. exp. Biol. 150, 381–394 (1990)
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BICARBONATE TRANSPORT SYSTEMS IN THE INTESTINE OF THE SEAWATER EEL

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Accepted 1 February 1990

Summary

Utilizing a pH-stat method, the rates of mucosal and serosal alkalinization were measured separately in the seawater eel intestine. These two rates were dependent on contralateral HCO_3^- concentration and were inhibited by contralateral application of DIDS, an inhibitor of HCO_3^- transport, indicating that the mucosal and serosal alkalinization are due to HCO_3^- secretion and absorption, respectively. The mucosal alkalinization was enhanced after inhibiting $Na^+/K^+/Cl^-$ cotransport by treatment with bumetanide, furosemide or Ba^{2+}, with a latent period of more than 10 min, suggesting that HCO_3^- absorption from mucosa to serosa depends on $Na^+/K^+/Cl^-$ cotransport. The serosal alkalinization caused by HCO_3^- absorption was completely abolished after mucosal application of bumetanide. After pretreatment with bumetanide, mucosal omission of Cl^- halved the enhanced rate of mucosal alkalinization, and Na^+ omission had no effect on it; this indicates that the exit of HCO_3^- into the lumen depends on luminal Cl^-, i.e. on the existence of the usual Cl^-/HCO_3^- exchange on the brush-border membrane. When serosal Na^+ was removed under the same conditions, mucosal alkalinization was reduced, indicating that HCO_3^- entry from the serosal fluid depends on Na^+. Serosal omission of Cl^- did not reduce mucosal alkalinization. In addition, serosal alkalinization was enhanced by serosal removal of Na^+ but not of Cl^-. These results suggest that there is a Na^+/HCO_3^- cotransport on the basolateral membrane. A possible model for HCO_3^- transport systems in the seawater eel intestine is proposed, and a possible role for these transport systems is discussed in relation to Na^+, Cl^- and water transport.

Introduction

In the preceding paper (Ando, 1990), it was proposed that HCO_3^- transport systems may contribute to a homeostasis in the intracellular H^+ concentration (pHi), which will control $Na^+/K^+/Cl^-$ cotransport *via* pHi-sensitive K^+ channels on the brush-border membrane of the epithelium in the intestine of the seawater eel. The present study aimed to elucidate how HCO_3^- is transported across

Key words: HCO_3^-, Cl^-/HCO_3^- exchange, Na^+/HCO_3^- cotransport, $Na^+/K^+/Cl^-$ cotransport, pH-stat, eel intestine.

the intestinal epithelium. However, the HCO_3^- flux cannot be detected directly by using radioisotopes, because labels on HCO_3^- are promptly dispersed into CO_2 and H_2O. Therefore, in the present study, the HCO_3^- transport rate was estimated from the rate of alkalinization of the bathing fluid.

HCO_3^- transport in the fish intestine has been little studied. So far as we know, the only study is that of Dixon and Loretz (1986), who observed HCO_3^- secretion in the goby intestine using a pH-stat method. However, they clamped the pH manually, and therefore they were not able to analyse precisely the time course of HCO_3^- secretion. Using an automatic pH-stat, we analysed more precisely the time course of HCO_3^- secretion as well as HCO_3^- absorption, and examined the effects of Na^+, Cl^-, 4,4′-diisothiocyanostilbene-2,2′-disulphonic acid (DIDS) and bumetanide on HCO_3^- transport. The results obtained indicate that some HCO_3^- absorption is linked with the $Na^+/K^+/Cl^-$ cotransport system, and that at least two kinds of HCO_3^- transport system exist in the seawater eel intestine.

Materials and methods

Japanese cultured eels *Anguilla japonica*, weighing 200–240 g, were kept in seawater aquaria (20 °C) for more than 1 week before use. After decapitation, the intestine was removed and stripped of its serosal muscle layers. The stripped intestine was opened by cutting longitudinally and mounted as a flat sheet in an Ussing–Rehm chamber with an exposed area of 0.785 cm^2. One side of the intestine was bathed with normal HCO_3^- Ringer's solution (6.5 ml), and the other side was bathed with an unbuffered Ringer's solution (5.0 ml). Both solutions were kept at 20 °C and circulated continuously; they were gassed with a 95 % O_2/5 % CO_2 gas mixture or 100 % O_2.

Table 1 shows the composition of the Ringer's solutions used in this experiment. Solution A is the normal HCO_3^- Ringer's solution. In Na^+-free Ringer's solution (solution B), all Na^+ was replaced with choline$^+$. Cl^--free Ringer's solution (solution C) was made by replacing NaCl, KCl and $CaCl_2$ with sodium gluconate, KNO_3 and $Ca(NO_3)_2$, respectively. These HCO_3^--buffered Ringer's solutions were bubbled with a 95 % O_2/5 % CO_2 gas mixture (pH 7.4). Solution D is phosphate-buffered Ringer's solution, gassed with 100 % O_2 (pH 7.4). Solution E is the standard unbuffered Ringer's solution, in which HCO_3^- is replaced with gluconate and acetate, and $MgCl_2$ is substituted for $MgSO_4$. In low-Na^+ unbuffered Ringer's solution (solution F), Na^+ was replaced with choline$^+$, and this solution was used within 1 week. Cl^--free unbuffered Ringer's solution (solution G) was made by replacing NaCl, KCl, $CaCl_2$ and $MgCl_2$ with sodium gluconate, KNO_3, $Ca(NO_3)_2$ and magnesium acetate, respectively. These unbuffered solutions were gassed with 100 % O_2 and the pH was clamped at 7.4 using a pH-stat (TOA, HSM-10A).

The rate of alkalinization (J^{OH}) was calculated from the amount of 20 mmol l^{-1} HCl titrated automatically to clamp the unbuffered fluid pH at 7.4 using the pH-stat. The amount of HCl titrated was recorded automatically (TOA, EPR-

Table 1. *Composition of experimental solutions (mmol l^{-1})*

	A HCO_3^-	B Na^+-free	C Cl^--free	D Phosphate	E Unbuffered	F Low-Na^+	G Cl^--free
NaCl	118.5			137.4	118.5		
Choline chloride		118.5				118.5	
Sodium gluconate			118.5		24.3	24.3	142.8
KCl	4.7	4.7		4.7	2.3	2.3	
KNO_3			4.7				4.7
Potassium acetate					3.6	3.6	3.6
$CaCl_2$	3.0	3.0		3.0	3.0	3.6	
$Ca(NO_3)_2$			3.0				3.0
KH_2PO_4	1.2	1.2	1.2	0.6			
$MgSO_4$	1.2	1.2	1.2	1.2			
$MgCl_2$					1.2	1.2	
Magnesium acetate							1.2
$NaHCO_3$	24.9		24.9				
Choline bicarbonate		24.9					
Na_2HPO_4				2.5			
D-Glucose	5.0	5.0	5.0	5.0	5.0	5.0	5.0
L-Alanine	5.0	5.0	5.0	5.0	5.0	5.0	5.0

121A) and the pH in the unbuffered medium was monitored throughout the experiment with a polyrecorder (TOA, EPR-10A). A similar technique has been used for measuring H^+ secretion rate in the eel stomach (Ando *et al.* 1986). The transepithelial potential difference (PD) was recorded with the polyrecorder (TOA, EPR-121A) as the serosal potential with respect to the mucosa through a pair of calomel electrodes (A. H. Thomas Co.). The PD was short-circuited every 10 min for less than 10 s and the tissue resistance (R_t) was calculated from the ratio of the PD to the short-circuit current (I_{sc}). Under short-circuit conditions, current flow from mucosa to serosa is reported as a positive I_{sc}. The fluid resistance was 18.8 Ωcm^2 and this factor was also used to correct each I_{sc} and R_t value as usual.

After these four variables had reached steady levels under the standard condition, 4-4′-diisothiocyanostilbene-2-2′-disulphonic acid (DIDS, Sigma), acetazolamide (Sigma) or bumetanide (a gift from Sankyo Co., Tokyo) was added to either the serosal or the mucosal fluid.

Results

Mucosal and serosal alkalinization are due to HCO_3^- transport

When the mucosa was bathed with standard unbuffered Ringer's solution (solution E), while the serosa was bathed with normal HCO_3^- Ringer's solution (solution A), the mucosal fluid was alkalinized at a constant rate (Fig. 1). The serosa-negative PD and I_{sc} were maintained under these conditions. After replacement of the HCO_3^--buffered solution with phosphate-buffered solution,

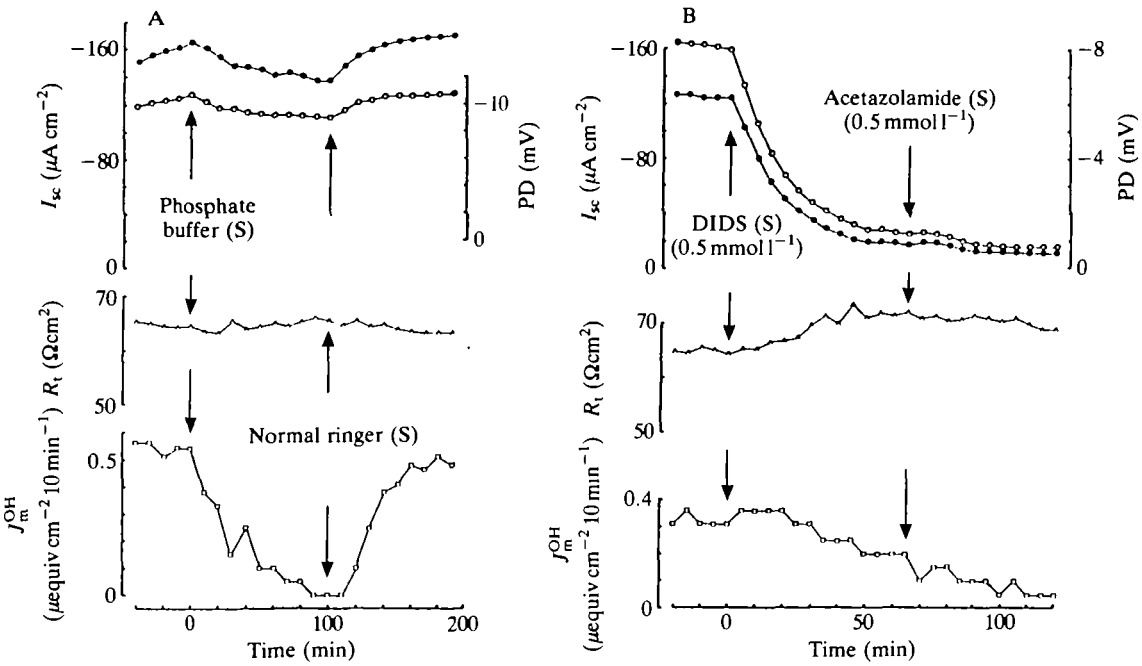

Fig. 1. Effects of serosal HCO_3^- and DIDS on the rate of mucosal alkalinization (J_m^{OH}, □), the transepithelial potential (PD, ○), the short-circuit current (I_{sc}, ●) and the tissue resistance (R_t, △). (A) After bathing the mucosa and the serosa of the intestine with the standard unbuffered solution (solution E) and normal HCO_3^- Ringer's solution (solution A), respectively, the serosal fluid was replaced with phosphate-buffered Ringer's solution (solution D) at time zero. At the second arrows, HCO_3^- was reintroduced to the serosal fluid. S in parentheses denotes that the serosal fluid is replaced. (B) After a steady state had been reached, 0.5 mmol l^{-1} DIDS was added to the serosal fluid (first arrows). At the second arrows, 0.5 mmol l^{-1} acetazolamide was further added to the serosal medium. S in parentheses denotes that each drug is applied to the serosal side of the intestine.

the rate of mucosal alkalinization (J_m^{OH}) was reduced to zero, accompanied by a decrease in PD and I_{sc}. The tissue resistance (R_t) tended to increase.

When DIDS, an inhibitor of HCO_3^- transport, was added to the serosal fluid under the same conditions, J_m^{OH} decreased gradually, accompanied by a decrease in PD and I_{sc} and by an increase in R_t (Fig. 1B). Addition of acetazolamide, an inhibitor of carbonic anhydrase, enhanced the inhibitory effects of DIDS. When DIDS was applied to the mucosal fluid under the same conditions, J_m^{OH} decreased slightly, accompanied by a slight decrease in PD and I_{sc}, whereas R_t did not change significantly (data not shown).

Similar experiments were performed after bathing the mucosa and the serosa with normal HCO_3^- Ringer and standard unbuffered Ringer, respectively (Fig. 2). After removal of HCO_3^- from the mucosal fluid, the rate of serosal alkalinization (J_s^{OH}) was reduced to zero, accompanied by a decrease in PD and I_{sc}

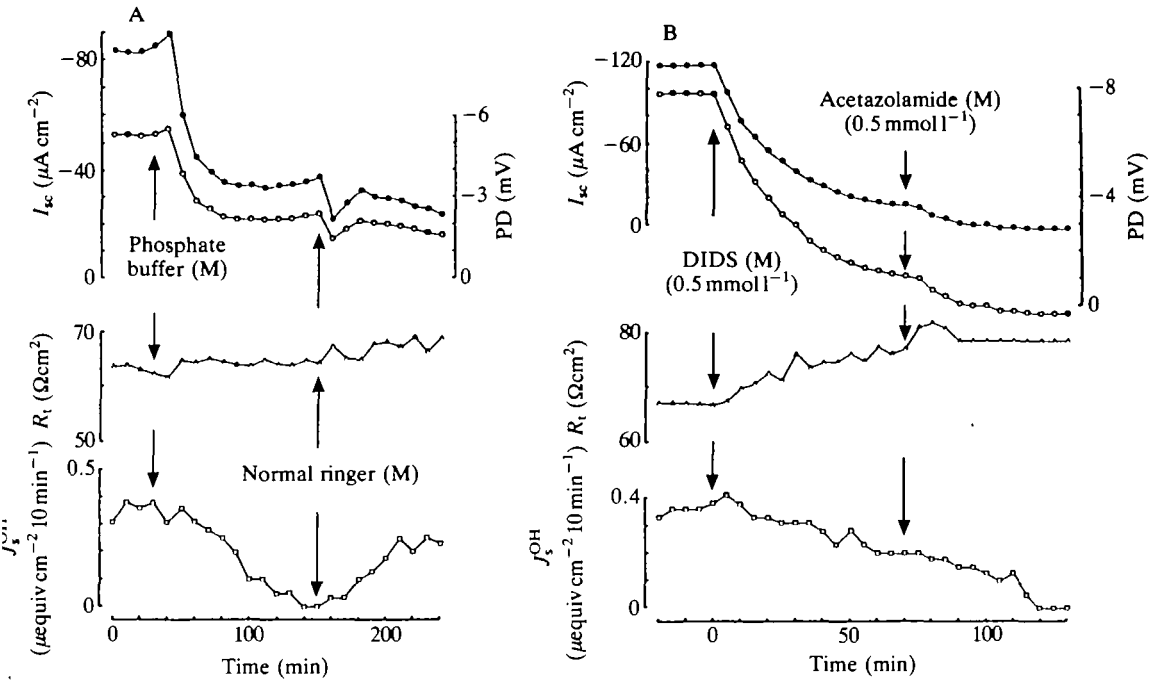

Fig. 2. Effects of mucosal HCO_3^- and DIDS on the rate of serosal alkalinization (J_s^{OH}, □), PD (○), I_{sc} (●) and R_t (△). (A) After bathing the mucosa and the serosa with solution A and solution E, respectively, the mucosal fluid was replaced with phosphate-buffered Ringer's solution (solution D) at the first arrows. After 150 min (second arrows), HCO_3^- was reintroduced to the mucosal fluid. M in parentheses denotes that the mucosal fluid is replaced. (B) At time zero, 0.5 mmol l^{-1} DIDS was added to the mucosal fluid (first arrows). At the second arrows, 0.5 mmol l^{-1} acetazolamide was added to the mucosal fluid.

and by an increase in R_t (Fig. 2A). When DIDS was added to the mucosal fluid, J_s^{OH} decreased gradually (Fig. 2B). PD and I_{sc} also decreased after treatment with DIDS, accompanied by an increase in R_t. Acetazolamide also enhanced the inhibitory effects of mucosal DIDS on these four parameters. Serosal addition of DIDS inhibited J_s^{OH} slightly, accompanied by a slight decrease in PD and I_{sc} (data not shown).

Effects of inhibition of $Na^+/K^+/Cl^-$ cotransport

To clarify the relationship between HCO_3^- transport and $Na^+/K^+/Cl^-$ cotransport, the following experiments were performed. Whilst bathing the mucosa and the serosa with standard unbuffered Ringer's solution and normal HCO_3^- Ringer's solution, respectively, 1 μmol l^{-1} bumetanide, an inhibitor of $Na^+/K^+/Cl^-$ cotransport, was added to the mucosal fluid (Fig. 3A). After addition of bumetanide, PD and I_{sc} decreased immediately and R_t increased more slowly, indicating that $Na^+/K^+/Cl^-$ cotransport is blocked by this drug and that the luminal K^+ channels are blocked secondarily. The mucosal alkalinization

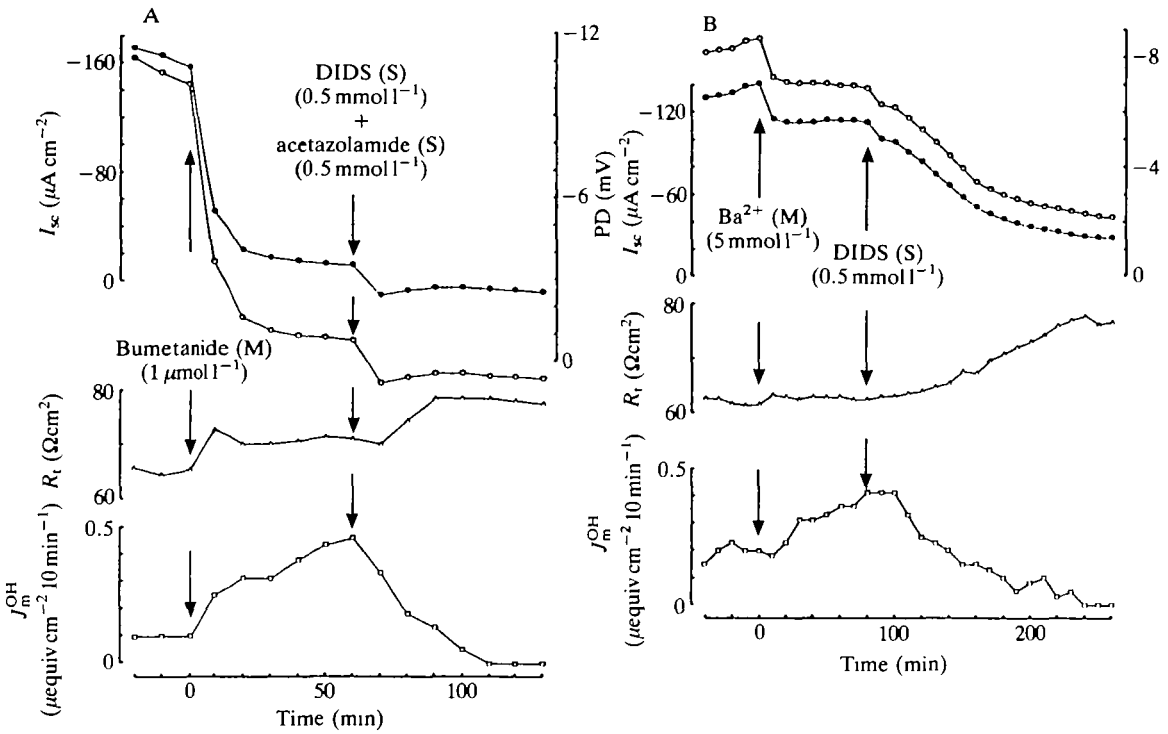

Fig. 3. Effects of bumetanide and Ba^{2+} on mucosal alkalinization (J_m^{OH}, □), PD (○), I_{sc} (●) and R_t (△). (A) After bathing the mucosa and the serosa with solution E and solution A, respectively, 1 μmol l^{-1} bumetanide was added to the mucosal fluid (first arrows). At the second arrows, 0.5 mmol l^{-1} DIDS and 0.5 mmol l^{-1} acetazolamide were added to the serosal fluid. (B) At time zero, 5 mmol l^{-1} $BaCl_2$ was added to the mucosal fluid (first arrows). At the second arrows, 0.5 mmol l^{-1} DIDS was added to the serosal fluid.

(J_m^{OH}) increased gradually after a latent period of 10.0±1.0 min (N=14). This enhancement in J_m^{OH} was completely blocked by DIDS and acetazolamide added to the serosal fluid. A similar increase in DIDS-sensitive J_m^{OH} was also observed after application of furosemide (10 μmol l^{-1}) to the mucosal fluid. When Ba^{2+}, a well-known blocker of K^+ channels, was added to the mucosal fluid, the DIDS-sensitive J_m^{OH} was also enhanced with a latent period of 18.8±1.9 min (N=5). However, PD and I_{sc} decreased immediately, accompanied by an immediate increase in R_t (Fig. 3B). Since bumetanide, furosemide and Ba^{2+} are known to inhibit $Na^+/K^+/Cl^-$ cotransport, these results suggest that inhibition of the cotransport either stimulates HCO_3^- secretion or inhibits HCO_3^- absorption. The following result supports the latter explanation.

Fig. 4 shows the 'sidedness' of the effects of bumetanide. In this experiment, the serosal HCO_3^- was omitted and bumetanide was added either to the serosal side or to the mucosal side. Although serosal addition of bumetanide had no effects on any of the four parameters (PD, I_{sc}, R_t and J_s^{OH}), mucosal application abolished

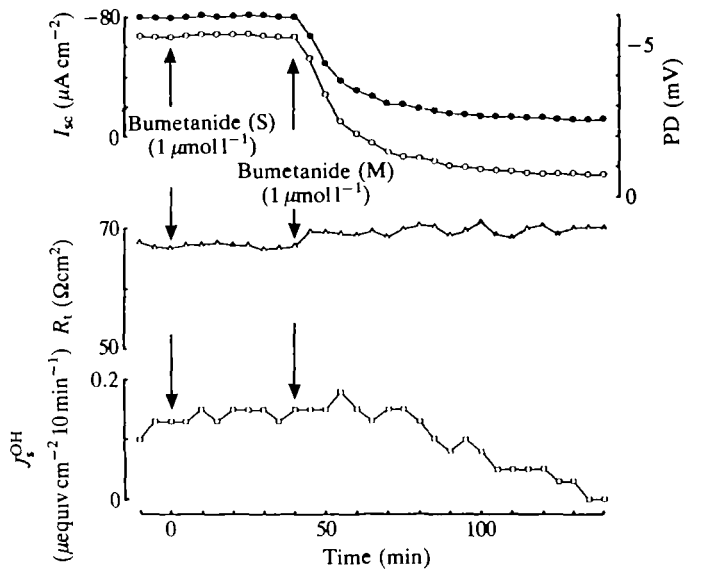

Fig. 4. The 'sidedness' of the effect of bumetanide on serosal alkalinization (J_s^{OH}, □), PD (○), I_{sc} (●) and R_t (△). After bathing the mucosa and the serosa with solution A and solution E, respectively, 1 μmol l^{-1} bumetanide was added to the serosal fluid (first arrows). At the second arrows, 1 μmol l^{-1} bumetanide was added to the mucosal fluid.

J_s^{OH}, reduced PD and I_{sc}, and caused an increase in R_t. These changes in the electrical parameters were similar to those shown in Fig. 3A.

Effects of Na^+ and Cl^- on HCO_3^- transport systems

Since HCO_3^- reabsorption was blocked by mucosal bumetanide, as shown in Figs 3 and 4, the following experiments were designed to clarify the mechanisms of HCO_3^- secretion in the presence of bumetanide. Fig. 5A shows the effects of removal of mucosal Cl^- on mucosal alkalinization (J_m^{OH}) after pretreatment with bumetanide. When Cl^- was omitted from the mucosal solution, J_m^{OH} was reduced by 50 %; it recovered after the reintroduction of Cl^- into the mucosal fluid. In the absence of Cl^- in the mucosal fluid, PD and I_{sc} shifted their polarity to become serosa-positive and R_t increased significantly. These three electrical parameters recovered to their original levels after reintroduction of Cl^- into the mucosal fluid.

The effects of mucosal Na^+ on mucosal alkalinization were also examined (Fig. 5B). J_m^{OH} was not affected by lowering the mucosal Na^+ concentration. When the mucosal Na^+ concentration was lowered, the serosa-negative PD and I_{sc} increased dramatically and R_t also increased significantly. These three electrical parameters returned to their original levels after reintroduction of the standard solution into the mucosal fluid.

Under the same conditions, when serosal Na^+ was removed, however, J_m^{OH} was gradually reduced by 40 % (Fig. 6A). PD and I_{sc} became more serosa-positive and

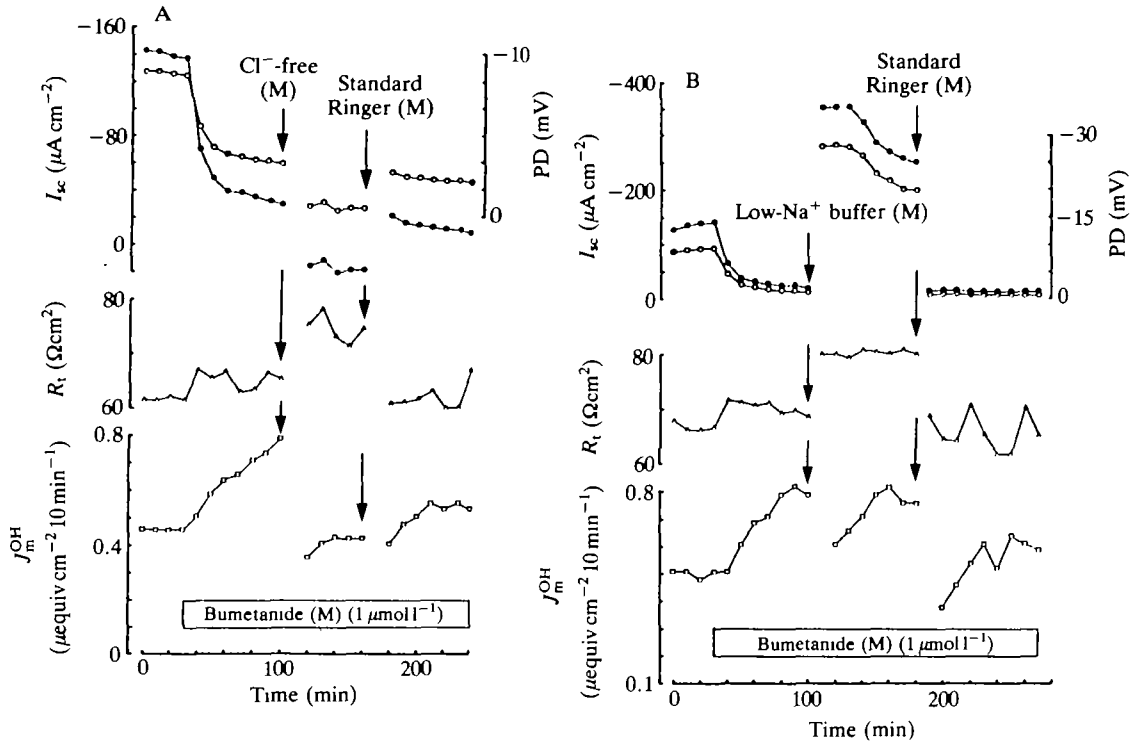

Fig. 5. Effects of mucosal Cl^- and Na^+ on the mucosal alkalinization (J_m^{OH}, □), PD (○), I_{sc} (●) and R_t (△). (A) After bathing the mucosa and the serosa with solution E and solution A, respectively, 1 μmol l^{-1} bumetanide was applied to the mucosal fluid at 30 min. In the presence of bumetanide, mucosal Cl^- was removed by replacement with solution G (first arrows). At the second arrows, the standard unbuffered Ringer's solution (solution E) was reintroduced to the mucosal side. Discontinuous lines denote that measurements were interrupted for more than 20 min, which is the time required until titration starts, since the pH in the unbuffered fluid is lower than 7.0. (B) After pretreatment with bumetanide (1 μmol l^{-1}), the mucosal fluid (solution E) was replaced with low-Na^+ Ringer's solution (solution F) at 100 min. At the second arrows, the standard unbuffered Ringer's solution (solution E) was reintroduced to the mucosal side.

R_t increased significantly. After reintroduction of Na^+ into the serosal fluid, all these four parameters returned to their original levels.

In contrast, serosal omission of Cl^- did not affect mucosal alkalinization (Fig. 6B). PD and I_{sc} increased gradually and R_t increased dramatically after removal of Cl^- from the serosal fluid. When normal Ringer's solution was reintroduced, these electrical parameters recovered to their original levels.

After bathing the mucosa and the serosa with normal HCO_3^- Ringer's solution and with standard unbuffered Ringer's solution, respectively, the effects of serosal Na^+ or Cl^- on serosal alkalinization (J_m^{OH}) were examined (Fig. 7). When the serosal Na^+ concentration was lowered from 142.8 to 24.3 mmol l^{-1}, J_s^{OH}

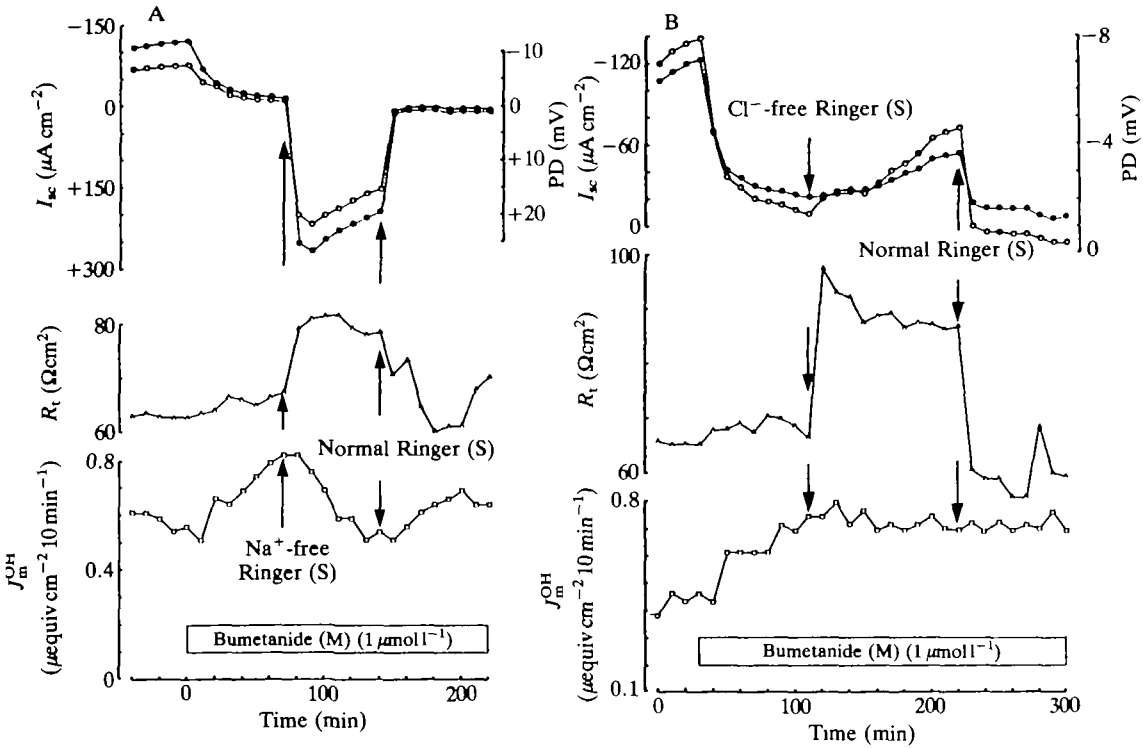

Fig. 6. Effects of serosal Na^+ and Cl^- on mucosal alkalinization (J_m^{OH}, □), PD (○), I_{sc} (●) and R_t (△). (A) After pretreatment with bumetanide (1 μmol l^{-1}), the serosal fluid (solution A) was replaced with Na^+-free Ringer's solution (solution B) at the first arrows. After 70 min normal HCO_3^- Ringer's solution (solution A) was reintroduced to the serosal side. (B) After pretreatment with bumetanide (1 μmol l^{-1}), the serosal fluid (solution A) was replaced with Cl^--free Ringer's solution (solution C) at the first arrows. After 110 min, solution A was reintroduced to the serosal side.

increased significantly (Fig. 7A). PD and I_{sc} become more serosa-positive and R_t also increased significantly. When the standard solution was reintroduced into the serosal fluid, all these four parameters returned to their initial levels.

In contrast, when serosal Cl^- was omitted, J_s^{OH} was not affected (Fig. 7B). PD and R_t increased significantly but I_{sc} increased only slightly. After reintroduction of the standard solution into the serosal fluid, R_t returned to its original level, but PD and I_{sc} were slightly lower than their original values.

Discussion

The present study demonstrates that mucosal and serosal alkalinization in the seawater eel intestine are due to HCO_3^- secretion and absorption, respectively, since these two rates of alkalinization depend on contralateral HCO_3^- concentration and are inhibited by contralateral DIDS, an inhibitor of HCO_3^- transport

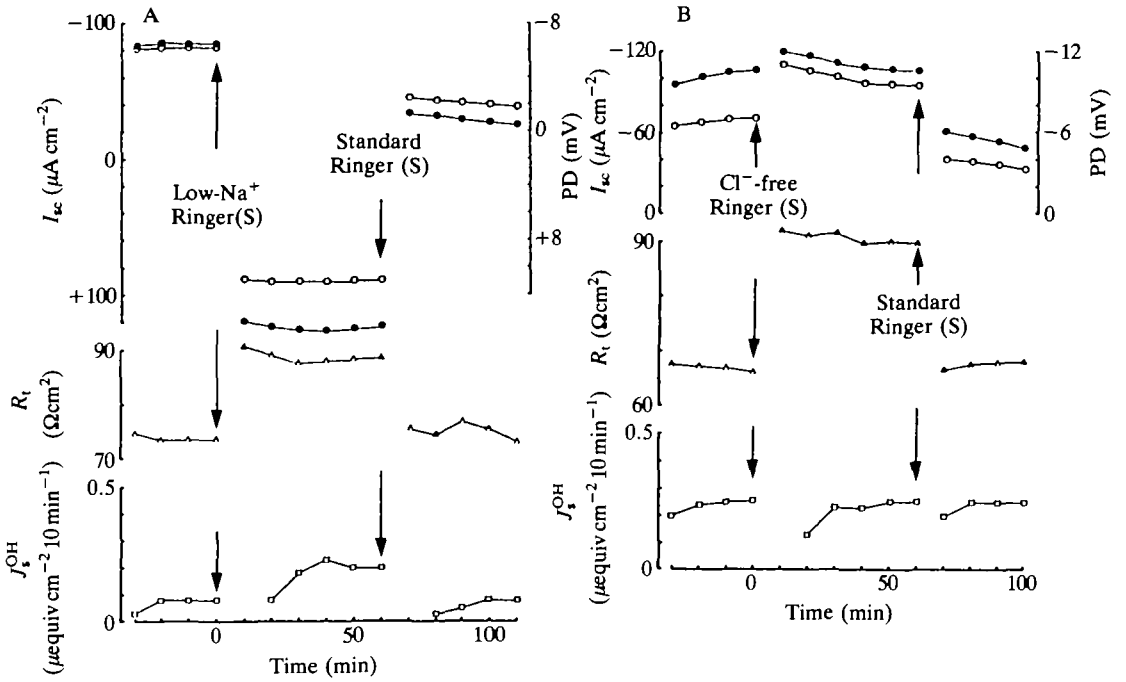

Fig. 7. Effects of serosal Na^+ and Cl^- on serosal alkalinization (J_s^{OH}, □), PD (○), I_{sc} (●) and R_t (△). (A) After bathing the mucosa and the serosa with solution A and solution E, respectively, the serosal fluid was replaced with low-Na^+ Ringer's solution (solution F) at time zero. At the second arrows, standard unbuffered solution (solution E) was reintroduced to the serosal side. (B) After bathing the mucosa and the serosa with solution A and solution E, respectively, the serosal fluid was replaced with Cl^--free Ringer's solution (solution G) at time zero. At the second arrows, solution E was reintroduced to the serosal side.

(Cabantchik and Rothstein, 1972; Marsh and Spring, 1985; Jentsch *et al.* 1988). Acetazolamide, an inhibitor of carbonic anhydrase, enhanced these inhibitory effects of DIDS. When HCO_3^- transport was inhibited in both directions, the serosa-negative PD and I_{sc} decreased and R_t increased simultaneously. These phenomena may be explained by an inhibition of luminal K^+ channels, since the serosa-negative PD is mostly due to K^+ leakage from the cell to the lumen in the seawater eel intestine (Ando and Utida, 1986).

Mucosal alkalinization was enhanced by the addition of bumetanide to the mucosal fluid. Since mucosal bumetanide blocks HCO_3^- absorption from mucosa to serosa (Fig. 4), this enhanced J_m^{OH} seems to be due to the inhibition of HCO_3^- reuptake from the luminal fluid. Similar enhancement in J_m^{OH} was also observed after the addition of furosemide or Ba^{2+} to the mucosal fluid. Since these three drugs are known inhibitors of the $Na^+/K^+/Cl^-$ cotransport system, these results suggest that the HCO_3^- reuptake processes are closely linked with $Na^+/K^+/Cl^-$ cotransport. However, it is unlikely that the cotransport itself carries HCO_3^-,

because the inhibition of HCO_3^- reuptake (enhancement of J_m^{OH}) is delayed by more than 10 min after the initiation of changes in PD, I_{sc} and R_t.

After blocking the HCO_3^- reuptake processes with bumetanide, omission of Cl^- from the mucosal side halved the enhanced J_m^{OH} but Na^+ omission had no effect on it, indicating that the movement of HCO_3^- into the lumen depends on luminal Cl^-. In other words, this suggests that there is Cl^-/HCO_3^- exchange on the brush-border membrane: this idea is also supported by the inhibitory effect of mucosal DIDS on J_m^{OH}, since DIDS is known to inhibit Cl^-/HCO_3^- exchange.

Mucosal alkalinization was reduced by removing Na^+ from the serosal fluid but not by removing Cl^- (Fig. 6), and blocked by serosal DIDS (Fig. 1). In addition, serosal alkalinization (J_s^{OH}) was enhanced by lowering serosal Na^+ concentration, but not by removing serosal Cl^- (Fig. 7). These results indicate that HCO_3^- entry from the serosal fluid depends on Na^+ but not on Cl^-, and suggest that there is a DIDS-sensitive Na^+/HCO_3^- cotransporter which may be driven by the Na^+ gradient across the basolateral membrane. Similar DIDS-sensitive $Na^+/(HCO_3^-)_n$ cotransport has been reported in the renal tubules of amphibians (Boron and Boulpaep, 1983; Guggino *et al.* 1983; Wang *et al.* 1987) and mammals (Good *et al.* 1984; Alpern, 1985; Good, 1985; Yoshitomi *et al.* 1985; Akiba *et al.* 1986; Biagi and Sohtell, 1986; Grassl and Aronson, 1986; Jentsch *et al.* 1986*a*,*b*; Grassl *et al.* 1987; Kondo and Frömter, 1987; Sasaki *et al.* 1987; Ullrich and Papavassiliou, 1987), in the frog gastric fundus (Curci *et al.* 1987) and in bovine corneal endothelial cells (Jentsch *et al.* 1984, 1985; Wiederholt *et al.* 1985).

Although the relationship between $Na^+/K^+/Cl^-$ cotransport and HCO_3^- reuptake across the brush-border membrane is not clear yet, a plausible explanation is a coupling between HCO_3^- reuptake and Cl^- movement out of the cell, such as Cl^-/HCO_3^- exchange, since HCO_3^- absorption (J_s^{OH}) is blocked by mucosal DIDS (Fig. 2B). Considering driving forces for such Cl^-/HCO_3^- exchange, however, the exchanger must be driven by other force(s), such as the Na^+ gradient. Such a DIDS-sensitive $Na^+/(HCO_3^-)_n/Cl^-$ transport has been reported in *Necturus* proximal tubule (Guggino *et al.* 1983; Matsumura *et al.* 1984) and in invertebrate cells (Thomas, 1977; Boron *et al.* 1981). We have no direct information about how HCO_3^- moves from the cell into the serosal fluid, except that this process is independent of serosal Cl^- and inhibited by serosal DIDS.

All the responses of the electrical parameters (PD, I_{sc} and R_t) observed after replacement of Na^+ or Cl^- indicate that this tissue is substantially permeable not only to Na^+ but also to Cl^-, although the permeation pathways are not clear from this study.

Fig. 8 shows a possible model for HCO_3^- transport systems in the seawater eel intestine: the HCO_3^- absorption process ($Na^+/HCO_3^-/Cl^-$ exchange and HCO_3^- conductance) is based on speculation from circumstantial evidence. Since NaCl and water absorption depend on HCO_3^- transport (Ando, 1990) and HCO_3^- transport also depends on $Na^+/K^+/Cl^-$ cotransport (present study), all these transport systems appear to be mutually interrelated. The HCO_3^- transport systems discussed in this paper will control the pHi homeostasis in the intestinal

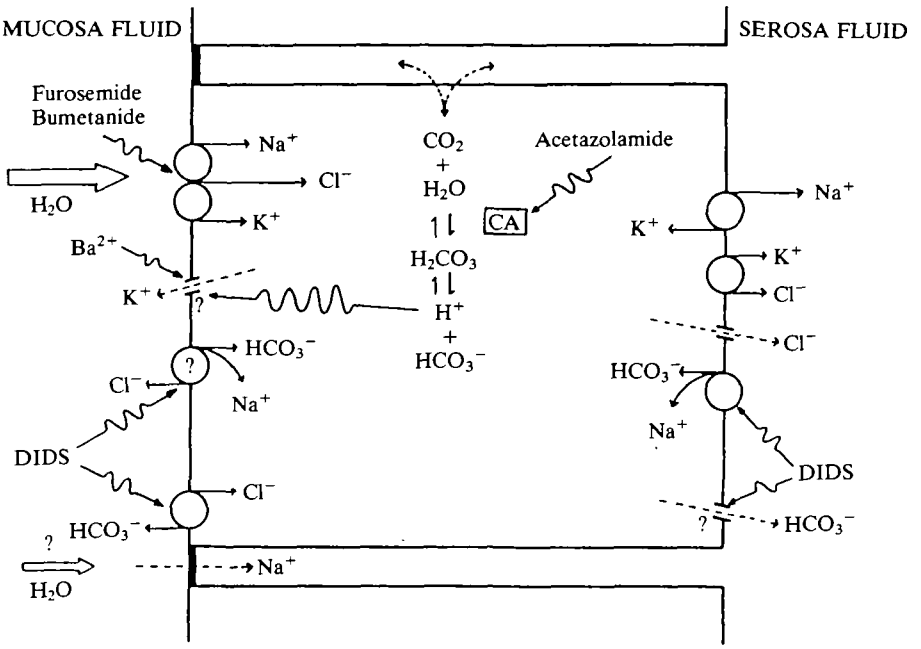

Fig. 8. A possible model for HCO_3^- transport systems in the seawater eel intestine in relation to Na^+, Cl^- and water transport. The direction of each ion flux is indicated by solid arrows and the actions of inhibitors are shown as wavy lines. Dotted arrows indicate diffusional ion fluxes. Water flux is represented by open arrows. Question marks mean that these processes were not directly demonstrated, but are based on speculation from circumstantial evidence. Na^+, K^+, Cl^- and water fluxes are all taken from Ando and Utida (1986). CA, carbonic anhydrase.

epithelium. Although other intracellular organic osmolytes may also control the pHi homeostasis, their contribution may be smaller than that of the HCO_3^-/CO_2 buffer system, since amino acid metabolism is very active in this tissue (Ando, 1988). The amino acid metabolism may continuously acidify the cytoplasm. Intracellular pH may control K^+ channels on the brush-border membrane, and secondarily regulate $Na^+/K^+/Cl^-$ cotransport, as discussed in the preceding paper (Ando, 1990). Among these HCO_3^- transport systems, the Na^+/HCO_3^- cotransport system on the basolateral membrane might be the most important in controlling pHi, since serosal deficiency of HCO_3^- and serosal addition of DIDS effectively inhibit the serosa-negative PD and water absorption (Ando, 1990).

We wish to thank Professors Makoto Kobayashi and Yojiro Muneoka, Faculty of Integrated Arts and Sciences, Hiroshima University, for their helpful advice. This research was supported in part by a Grant-in-Aid no. 01304027 from the Ministry of Education, Science and Culture, Japan.

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