BICARBONATE TRANSPORT SYSTEMS IN THE INTESTINE OF THE SEAWATER EEL

BY MASAAKI ANDO AND M. V. V. SUBRAMANYAM

Laboratory of Physiology, Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima 730, Japan, and Department of Sericulture, Bangalore University, Bangalore 560 001, India

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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
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$_2$O. 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-
HCO₃⁻ transport across eel intestine

Table 1. Composition of experimental solutions (mmol l⁻¹)

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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 (Rt) was calculated from the ratio of the PD to the short-circuit current (Isc). Under short-circuit conditions, current flow from mucosa to serosa is reported as a positive Isc. The fluid resistance was 18.8 Ωcm² and this factor was also used to correct each Isc and Rt 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 HCO₃⁻ transport

When the mucosa was bathed with standard unbuffered Ringer's solution (solution E), while the serosa was bathed with normal HCO₃⁻ Ringer's solution (solution A), the mucosal fluid was alkalinized at a constant rate (Fig. 1). The serosa-negative PD and Isc were maintained under these conditions. After replacement of the HCO₃⁻-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 $I_{sc}$.
HCO₃⁻ transport across eel intestine

Fig. 2. Effects of mucosal HCO₃⁻ and DIDS on the rate of serosal alkalinization ($J_{\text{OH}}^S$, □), 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), HCO₃⁻ 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, $J_{\text{OH}}^S$ 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 $J_{s\text{OH}}^S$ 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 HCO₃⁻ 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 HCO₃⁻ 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 ($J_m^{\text{OH}}$, □), 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.

($J_m^{\text{OH}}$) increased gradually after a latent period of 10.0±1.0 min ($N$=14). This enhancement in $J_m^{\text{OH}}$ was completely blocked by DIDS and acetazolamide added to the serosal fluid. A similar increase in DIDS-sensitive $J_m^{\text{OH}}$ 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 $J_m^{\text{OH}}$ 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 $J_s^{\text{OH}}$), mucosal application abolished
HCO₃⁻ transport across eel intestine

Fig. 4. The 'sidedness' of the effect of bumetanide on serosal alkalinization ($J_m^{OH}$, □), 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.

$J_m^{OH}$, 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 HCO₃⁻ transport systems

Since HCO₃⁻ reabsorption was blocked by mucosal bumetanide, as shown in Figs 3 and 4, the following experiments were designed to clarify the mechanisms of HCO₃⁻ 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\textsuperscript{−} and Na\textsuperscript{+} 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\textsuperscript{−1} bumetanide was applied to the mucosal fluid at 30 min. In the presence of bumetanide, mucosal Cl\textsuperscript{−} 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\textsuperscript{−1}), the mucosal fluid (solution E) was replaced with low-Na\textsuperscript{+} 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\textsuperscript{+} into the serosal fluid, all these four parameters returned to their original levels.

In contrast, serosal omission of Cl\textsuperscript{−} did not affect mucosal alkalinization (Fig. 6B). PD and $I_{sc}$ increased gradually and $R_{t}$ increased dramatically after removal of Cl\textsuperscript{−} 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\textsubscript{3}\textsuperscript{−} Ringer’s solution and with standard unbuffered Ringer’s solution, respectively, the effects of serosal Na\textsuperscript{+} or Cl\textsuperscript{−} on serosal alkalinization ($J_{s}^{OH}$) were examined (Fig. 7). When the serosal Na\textsuperscript{+} concentration was lowered from 142.8 to 24.3 mmol L\textsuperscript{−1}, $J_{s}^{OH}$
HCO$_3^-$ transport across eel intestine

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 $I_{sc}$ became 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, $J_{m}^{OH}$ 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 (\(J^{\text{OH}}_m\), □), PD (○), \(I_{\text{sc}}\) (●) 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.

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 \(I_{\text{sc}}\) 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 \(J^{\text{OH}}_m\) seems to be due to the inhibition of HCO\(_3^-\) reuptake from the luminal fluid. Similar enhancement in \(J^{\text{OH}}_m\) 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\(_3^-\),

(Cabantchik and Rothstein, 1972; Marsh and Spring, 1985; Jentsch et al. 1988).
because the inhibition of HCO₃⁻ reuptake (enhancement of $J_{m}^{OH}$) is delayed by more than 10 min after the initiation of changes in PD, $I_{sc}$ and $R_l$.

After blocking the HCO₃⁻ 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 HCO₃⁻ into the lumen depends on luminal Cl⁻. In other words, this suggests that there is Cl⁻/HCO₃⁻ 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⁻/HCO₃⁻ 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 HCO₃⁻ entry from the serosal fluid depends on Na⁺ but not on Cl⁻, and suggest that there is a DIDS-sensitive Na⁺/HCO₃⁻ cotransporter which may be driven by the Na⁺ gradient across the basolateral membrane. Similar DIDS-sensitive Na⁺/ (HCO₃⁻)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. 1986a,b; Grassl et al. 1987; Kondo and Frömter, 1987; Sasaki et al. 1987; Ulrich 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 HCO₃⁻ reuptake across the brush-border membrane is not clear yet, a plausible explanation is a coupling between HCO₃⁻ reuptake and Cl⁻ movement out of the cell, such as Cl⁻/HCO₃⁻ exchange, since HCO₃⁻ absorption ($J_{s}^{OH}$) is blocked by mucosal DIDS (Fig. 2B). Considering driving forces for such Cl⁻/HCO₃⁻ exchange, however, the exchanger must be driven by other force(s), such as the Na⁺ gradient. Such a DIDS-sensitive Na⁺/(HCO₃⁻)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 HCO₃⁻ 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 $R_l$) 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 HCO₃⁻ transport systems in the seawater eel intestine: the HCO₃⁻ absorption process (Na⁺/HCO₃⁻/Cl⁻ exchange and HCO₃⁻ conductance) is based on speculation from circumstantial evidence. Since NaCl and water absorption depend on HCO₃⁻ transport (Ando, 1990) and HCO₃⁻ transport also depends on Na⁺/K⁺/Cl⁻ cotransport (present study), all these transport systems appear to be mutually interrelated. The HCO₃⁻ transport systems discussed in this paper will control the pH homeostasis in the intestinal
Mucosa fluid

Furosemide

Bumetanide

H₂O

Ba²⁺

K⁺

Cl⁻

HCO₃⁻

Na⁺

CO₂

+ H₂O

↓

H₂CO₃

↓

H⁺

+ HCO₃⁻

Acetazolamide

K⁺

Cl⁻

HCO₃⁻

Na⁺

DIDS

H₂O

Fig. 8. A possible model for HCO₃⁻ 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 HCO₃⁻/CO₂ 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 HCO₃⁻ transport systems, the Na⁺/HCO₃⁻ cotransport system on the basolateral membrane might be the most important in controlling pHᵢ, since serosal deficiency of HCO₃⁻ and serosal addition of DIDS effectively inhibit the serosa-negative PD and water absorption (Ando, 1990).

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