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Is There a Cl^--OH^- Exchange (Cl^--H^+ Cotransport) Mechanism in the Brush-Border Membrane of the Intestine of the Fresh Water Trout (*Salmo gairdneri*, R.)?

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Summary. Experiments were performed to determine the presence of a Cl^--OH^- exchange (Cl^--H^+ cotransport) in the brush-border membranes isolated from the intestinal epithelium of freshwater trout. Determinations of alkaline phosphatase activities have shown that vesicle suspensions had an enrichment factor of about 17 in this enzyme indicating a high degree of purification of the brush-border membrane preparation. Cl^- uptake by vesicles in the presence of a proton gradient occurs against a concentration gradient with an overshoot ratio of about 2 and is inhibited by SITS. Several lines of evidence suggest that the mechanism involved is electrical in nature: (*i*) Cl^- uptake is increased when the proton gradient is increased, but there is a linear relationship between the Cl^- uptake and the Nernst potential of protons. (*ii*) Cl^- uptake is increased when a proton ionophore is added at low concentration and inhibited at high concentration, suggesting that a proton conductance is involved in the Cl^- uptake. (*iii*) there is a linear relationship between the initial speed of the uptake of increasing Cl^- concentrations and the Cl^- concentration. (*iv*) Cl^- uptake can be modulated by different potassium gradients with or without valinomycin. It is concluded that the enterocyte of the freshwater trout is not equipped with a Cl^--OH^- exchange and the Cl^- uptake by vesicles is realized by a Cl^- conductance.

Key Words trout · intestine · brush-border membrane · vesicle · Cl^- conductance

Introduction

Chloride transport by intestine is now well documented in seawater (SW) fish but little is known about Cl^- absorption in freshwater (FW) fish. An active Cl^- transport system has been suggested to be present in SW trout intestine (Lahlou, Smith & Ellory, 1974). This hypothesis was confirmed in the winter flounder intestine by Field et al. (1978) and Frizzell et al. (1979) who demonstrated that this system was constituted in part by an apical Na^+-Cl^- cotransport process, which maintained Cl^- activity in enterocytes three to four times above its equilibrium distribution across the mucosal membrane (Duffey et al., 1979). Furthermore, Musch et al. (1982) and Frizzell et al. (1984) have shown that this process was enhanced by K^+. In European flounder enterocytes, Lau (1985) has demonstrated that intracellular Cl^- activity was well above the equilibrium distribution in SW and in FW, as well. Furthermore, he has shown that the Cl^- activity was maintained in FW even in presence of piretanide, a Na^+-K^+-Cl^- cotransport process inhibitor, which suggested the absence of Na^+-K^+-Cl^- cotransport. Lau (1985) hypothesized the presence of a dual exchanger Na^+-H^+ and Cl^--HCO_3^-, and Zuidema et al. (1985) proposed the same hypothesis for the goldfish. Nonnotte, Aubrée-Pelletier and Colin (1987) have observed that, in the middle intestine of the FW trout, Cl^- fluxes were equivalent to Na^+ fluxes as determined with a perfusion preparation in absence of HCO_3^- in the perfusion media. This finding suggested that the mechanism involved was rather a Cl^--OH^- than a Cl^--HCO_3^- exchange. We have performed a series of experiment on a purified apical membrane preparation to determine the presence of a Cl^--OH^- exchange (Cl^--H^+ cotransport) system in the brush border of trout enterocytes. A preliminary report of some results of this study has already appeared (Aguenaou et al., 1987).

Materials and Methods

Preparation of Brush-Border Membrane Vesicles

The brush-border membranes (BBM) were sampled in middle intestine (Bergot, Solari & Luquet, 1975) of trout (200, 300 g), obtained from a local hatchery (Strasbourg, France) maintained in a well aerated aquarium (12 ± 1°C). Fish were fed daily a synthetic diet (Trouvit, Verdun, France) at a rate of 1 g% of fresh weight.

The BBM vesicles were prepared by the modified method of Di Costanzo et al. (1983). Briefly, fed trout were killed by

Table. Specific activities and enrichment factors of marker enzymes

Enzyme	Homogenate	Vesicle suspension	Enrichment factor	*n*
Alkaline phosphatase	140 ± 10	2464 ± 103	17.6 ± 1.0	148
Leucine aminopeptidase	40 ± 6	624 ± 125	15.9 ± 2.9	5
Na^+-K^+-ATPase	273 ± 94	56 ± 14	0.21 ± 0.15	18
Cytochrome-*c*-oxydase	8.7 ± 2.8	5.4 ± 0.3	0.62 ± 0.10	44

Means ± SD. of specific activities are expressed in μmol substrate/min/g protein or as log (absorbance variation)/min/mg protein for cytochrome-*c*-oxydase. *n* = number of samples.

decapitation and the intestine rinsed with 0.9% NaCl at 4°C. The intestinal mucosa was scraped and homogenized with a Waring blender for 20 sec at low speed in 40 ml of a solution containing: 200 mM sorbitol; 2 mM EGTA; 5 mM TEA; 0.5 mM $MgSO_4$, pH 7.4. After centrifugation at 12,000 × *g* for 10 min, the pellet was homogenized with an Ultra-Turax (2 strokes, 10 sec) in the same solution. After addition of 10 mM $MgSO_4$, the suspension was maintained at 4°C for 15 min and then centrifuged at 5,000 × *g* for 15 min. The supernatant was then centrifuged at 43,000 × *g* for 20 min. The pellet was then suspended with a Teflon Potter (8 strokes) in 10 ml of a buffered solution the composition of which is identical to the intravesicular milieu, given in the figure legends. The last purification step was repeated once, and after the last centrifugation the vesicles were suspended in intravesicular buffer at 10 to 15 mg protein/ml. Protein was determined by the Bradford (1974) method with serum albumin as standard. The vesicle suspension was stored in liquid nitrogen until utilization.

Enzyme Assays

The purity of the BBM was evaluated by determining the enrichment factors of membrane enzyme markers. Alkaline phosphatase activity was determined after Di Costanzo et al. (1983), cytochrome *c* oxydase activity after Cooperstein and Lazarow (1951), Na, K-ATPase activity after Arnold et al. (1976) and leucine aminopeptidase after Appel (1974).

Cl^- Uptake Measurement

Uptake by BBM vesicles of ^{36}ClH neutralized with Tris base was measured at 20°C by a rapid filtration technique with a laboratory-made apparatus. Incubation was initiated by mixing a 10-μl vesicle suspension with 40 or 90 μl of a buffered solution to obtain the extravesicular milieu the composition of which is given in the figure legends. Osmotic pressures were determined with a Roebling osmometer. At specified times, uptake was terminated by rapidly adding 4 ml of a "stop" solution at 4°C containing: 350 mM KCl, 25 mM $MgSo_4$, 10 mM HEPES, 7 mM *n*-butylamine (pH 7.5). The vesicles were immediately collected on a Sartorius filter (0.65 μm, 25 mm) and rinsed with 8 ml of the "stop" solution. The radioactivity of the filters was determined by liquid scintillation counting with a SL 30 Intertechnique. Correction for $^{36}Cl^-$ binding to the filters was made by subtracting the radioactivity measured at zero time in absence of vesicles from the experimental time point. Unless otherwise specified each point was performed in triplicate on vesicles obtained from eight different trout. Results are expressed in nmol/mg protein. All pH and K^+ gradients are given as extravesicular/intravesicular.

Materials

^{36}ClH was purchased from Amersham (UK), carbonyl cyanide *m*-chloro-phenyl-hydrazone (CCCP) was obtained from Aldrich (Strasbourg, France), and valinomycin, ethylenebis (oxyethylenenitrilo)tetraacetic acid (EGTA), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 2-(N-morpholino)-ethanesulfonic acid (MES), 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS), triethanolamine (TEA), tetramethylammonium (TMA) hydroxyde pentahydrate and other chemicals were from Sigma Chemical (St. Louis, MO). TMA gluconate was prepared by neutralization of TMA hydroxide with gluconic acid.

Results

Characterization of Membrane Microvillar Vesicles

The activities of alkaline phosphatase and leucine aminopeptidase, BMM markers, of Na, K-ATPase, a basolateral membrane marker and of cytochrome *c* oxydase, a mitochondrial membrane marker obtained in the crude homogenate of scrapings and in the final suspension of vesicles, are summarized in the Table. The enrichment factors obtained: around 17 for alkaline phosphatase and 16 to the leuucine aminopeptidase and less than 1 for the two other membrane markers demonstrate that the preparation of vesicles is well purified in BBM and that contamination by other membranes can be considered to be negligible.

Chloride Uptake

Effect of pH Gradients

The existence of Cl^--OH^- exchange (equivalent to a Cl^--H^+ cotransport) in the BBM of the trout enterocytes will be assessed by a concentrative uptake generated by a proton gradient (out greater than in) as driving force. Figure 1 illustrates the Cl^- uptake obtained with BBM vesicles when intravesicular and extravesicular pH are equilibrated to 7.5. It can be seen that intravesicular Cl^- concentration increases slowly with time until its value reaches the equilibrium distribution value (2 hr incubation). When a pH gradient (extravesicular acidic) created

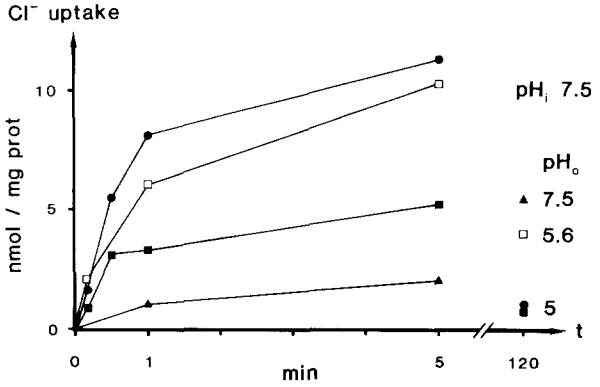

Fig. 1. Time course of Cl^- uptake by trout intestine BBM submitted to a pH gradient. Vesicles contained 40 mM HEPES and (▲, ●, ■) 40 mM citrate, 141 mM Tris base (pH_i 7.5), 79 mM sorbitol, or (□) 40 mM MES, 63 mM Tris base (pH_i 7.5), 157 mM sorbitol. SITS (0.25 mM) was added (■) to the vesicle suspension 10 min before the beginning of the incubation period. Uptake of 3.4 mM $^{36}Cl^-$ was assayed in the presence of 40 mM HEPES and (▲) 40 mM citrate, 141 mM Tris base (ph_o 7.5), 79 mM sorbitol or (●, ■) 40 mM citrate, 74 mM Tris base (pH_o 5), 163 mM sorbitol, or (□) 40 mM MES, 15 mM Tris (pH_o 5.6), 205 mM sorbitol

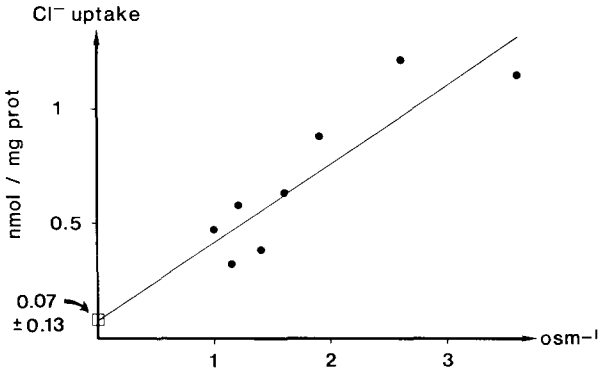

Fig. 2. Effect of external osmolarity on Cl^- uptake by trout intestine BBM. Vesicles contained 10 mM HEPES, 40 mM citrate, 150 mM Tris base (pH 7.5). Uptake of 3.4 mM $^{36}Cl^-$ was assayed during 1 hr in the presence of 10 mM HEPES, 40 mM citrate, 74 mM Tris base (pH 5) and 176, 276, 476, 576, 676, 776 and 876 mM sorbitol (osmotic pressure used in data was determined by the surfusion method). Regression equation: Cl^- uptake = 0.34 (±0.07) osm^{-1} + 0.07 (±0.13), r = 0.891

by citric acid (pH 5/7.5), or MES (pH 5.6/7.5) is imposed on BBM vesicles, the Cl^- uptake is greatly increased. Under these conditions, Cl^- ions are accumulated above the equilibrium distribution value and the "overshoot ratio" obtained is about 2. In the presence of 0.25 mM SITS, which is an inhibitor of Cl^--OH^- exchange in erythrocytes, the Cl^- uptake is markedly decreased and no concentrative Cl^- uptake is observed. These results support the existence of a Cl^--OH^- exchange (Cl^--H^+ cotransport). Actually, a part of the measured Cl^- uptake could be attributed to nonspecific binding of Cl^- on BBM themselves. To test this possibility, we measured the Cl^- accumulated by BBM vesicules submitted for 1 hr to the same pH gradient, at various osmotic pressures obtained by addition of appropriate sorbitol concentrations to the external milieu. The results presented in Fig. 2 show that the Cl^- uptake and the inverse of the extravesicular osmolarity bear a linear relationship (Cl^- uptake = 0.34 (± 0.07)osM^{-1} + 0.07 (±0.13), r = 0.891). Extrapolation of the data to an infinite osmolarity, which corresponds to a zero extravesicular space is not different from zero, indicating that nonspecific binding of Cl^- can be neglected. For this reason, no corrections have been made in expressing the data.

To test the effect of varied proton-induced driving force on Cl^- uptake, we submitted vesicles to different pH gradients ranging from 7.5/7.5 to 3.5/7.5. It can be seen from data shown in Fig. 3*A*, that Cl^- accumulation by BBM vesicles is dependent on the magnitude of the pH gradient. Clearly, Cl^- uptake by unit time increases with the increase of the pH gradient, which is in agreement with the hypothesis. Conversely, this increase does not show any saturation, which would be the case in the presence of a Cl^- : OH^- antiport. Actually, if the Cl^- uptake data obtained after 2 sec of incubation, i.e., the initial rate, are plotted against the values of the potential difference (Pd) given by the Nernst equation applied to proton concentrations, it appears that those two variables are in close linear relationship (Cl^- uptake = 0.098 (Pd) − 0.030, r = 0.974) as shown in Fig. 3*B*.

Such a result suggests that Cl^- uptake by BBM vesicles of trout intestine is energized by the potential difference created by the pH gradient through the BBM. Under these conditions, Cl^- would not be exchanged but would follow a conductive pathway through the BBM, the energy being provided by the pH gradient and a proton conductance. This suggestion is supported by the following experiment where a vesicle suspension is submitted to a pH gradient (5/7.5) with or without CCCP added in the external milieu at two concentrations (10, 50 μg/mg protein). As can be seen from Fig. 4*A* and *B*, Cl^- uptake is enhanced when CCCP is added at 10 μg/mg protein, but is inhibited when CCCP is added at a higher concentration. CCCP increases the proton conductance (Bennekou, 1984) and it seems that at low concentrations, the driving force is increased while at high concentrations the pH gradient collapses. In another experiment, the uptake of Cl^- at concentrations increasing from 3.4 to 100 mM in the presence

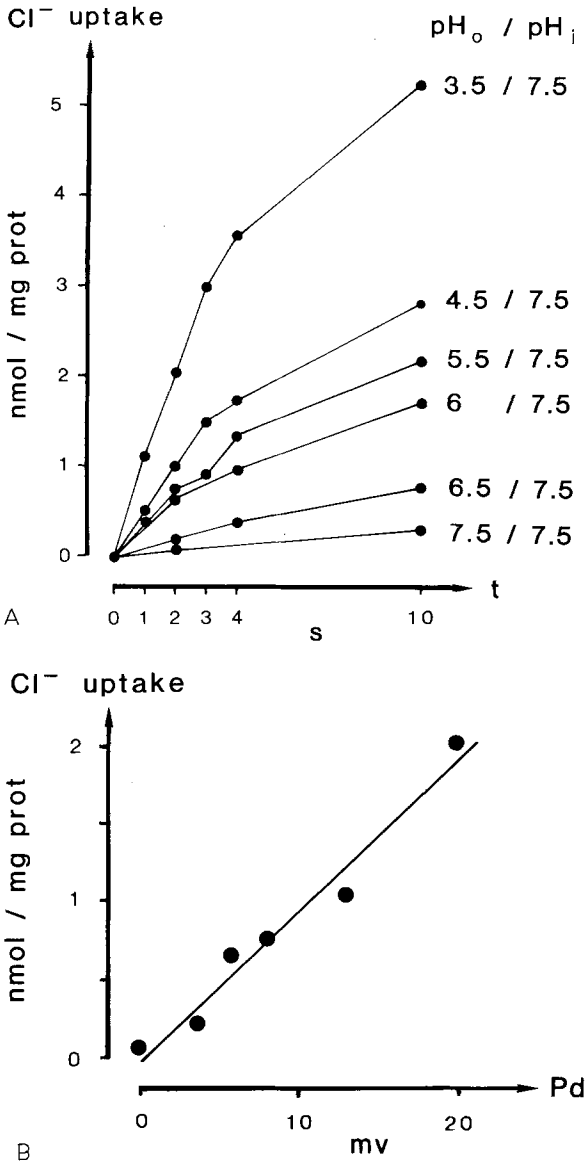

Fig. 3. Effect of increasing pH gradient on Cl^- uptake by trout intestine BBM. Vesicles contained 10 mM HEPES, 40 mM citrate, 150 mM Tris base (pH$_i$ 7.5), 20 mM sorbitol. Uptake of 3.4 mM $^{36}Cl^-$ was assayed in the presence of 10 mM HEPES, 40 mM citrate and 212 mM sorbitol, 30 mM Tris base (pH$_o$ 3.5) or 200 mM sorbitol, 41 mM Tris base (pH$_o$ 4) or 185 mM sorbitol, 57 mM Tris base (pH$_o$ 4.5) or 163 mM sorbitol, 79 mM Tris base (pH$_o$ 5) or 153 mM sorbitol, 89 mM Tris base (pH$_o$ 5.5) or 134 mM sorbitol, 108 mM Tris base (pH$_o$ 6) or 92 mM sorbitol, 150 mM Tris base (pH$_o$ 7.5). (*A*) Time course of Cl^- uptake for 10 sec at different pH values. (*B*) Cl^- uptake after 2 sec incubation plotted against the pH Nernst potential (Pd). Cl^- uptake = 0.098 (Pd) −0.030 (r = 0.974)

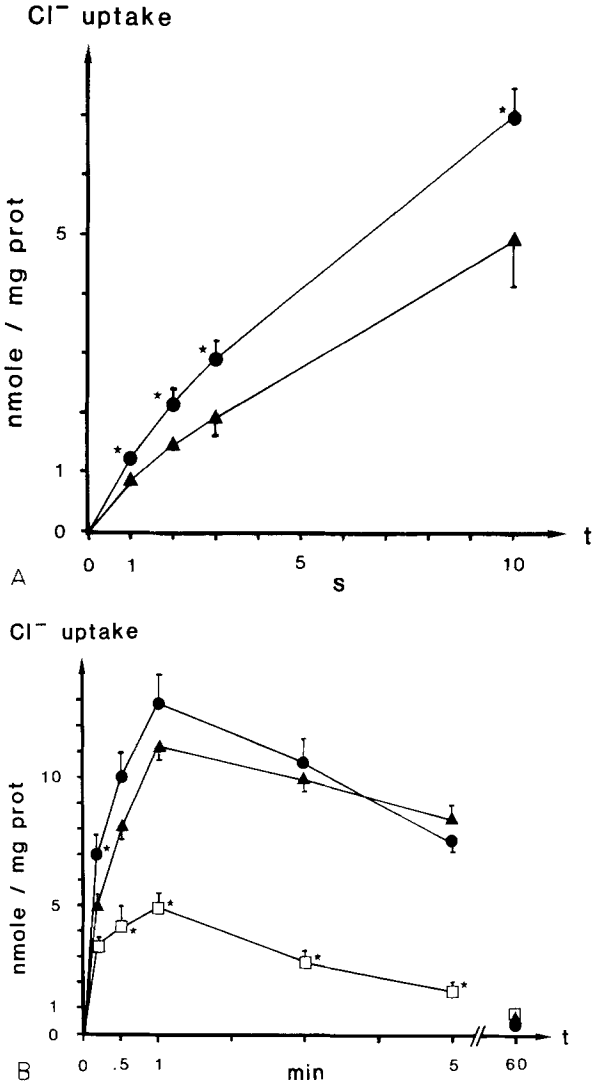

Fig. 4. (*A* and *B*) Effect of CCCP on Cl^- uptake in the presence of a pH gradient by trout intestine BBM. Vesicles contained 40 mM HEPES, 40 mM citrate, 149 mM Tris base (pH$_i$ 7.5), 21 mM sorbitol. CCCP (▲, ●, □: 0, 10, 50 μg/mg protein, respectively) was added to the vesicle suspension 10 min before the beginning of the incubation period. Uptake of 3.4 mM $^{36}Cl^-$ was assayed in the presence of 40 mM HEPES, 40 mM citrate, 53 mM Tris base (pH$_o$ 4.5), 217 mM sorbitol. Data represent the mean ± SE of four experiments done in triplicate. When error bars are absent the magnitude of SE is less than that of the symbols for the mean value. Statistical significance $P < 0.05$

or absence of CCCP (10 μg/mg protein) was measured in BBM vesicles submitted to the same pH gradient (5/7.5). The results (Fig. 5) show that (*i*) Cl^- uptake is linearly proportional to Cl^- concentration and (*ii*) Cl^- uptake is increased by CCCP at each Cl^- concentration as in the preceding experiment.

Effect of K^+ Gradients

The electrogenic nature of the Cl^- uptake has been assayed by applying K^+ gradients in the presence or absence of valinomycin to BBM vesicles submitted

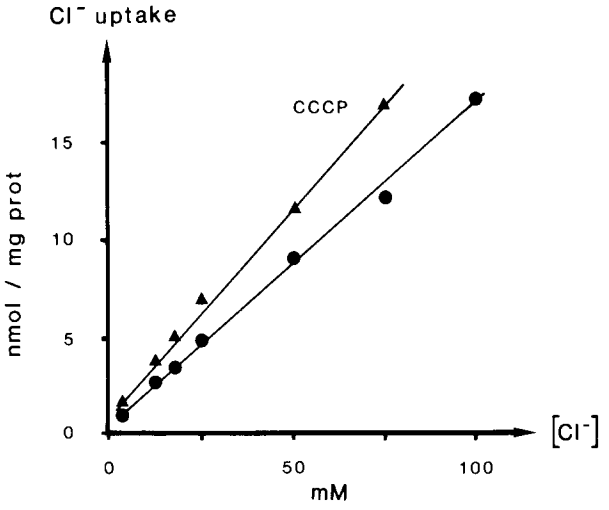

Fig. 5. Effect of increasing Cl^- concentrations and CCCP on Cl^- uptake in presence of a pH gradient by trout intestine BBM. Vesicles are loaded and CCCP added as in Fig. 4. Uptake of Cl^- was assayed as in Fig. 4, except that incubation time was 1 sec and a part of the sorbitol in the external milieu was replaced with 0, 8.6, 14.6, 21.6, 46.6, 71.6, or 96.6 mM Tris-HCl

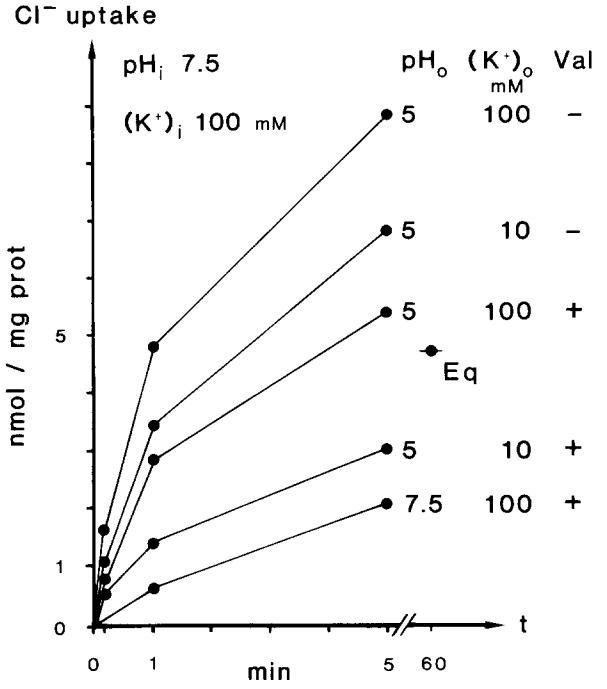

Fig. 6. Effect of potassium gradients on Cl^- uptake in the presence of a pH gradient by trout intestine BBM. Vesicles contained 40 mM HEPES, 40 mM citrate, 150 mM Tris base (pH$_i$ 7.5), 100 mM K-gluconate. (Val) valinomycin (5 μg/mg protein) was added to the vesicle suspension 10 min before the beginning of the incubation period. Uptake of 3.4 mM $^{36}Cl^-$ was assayed in the presence of 40 mM HEPES, 40 mM citrate, 40 mM Tris base (pH$_o$ 5), 70 mM sorbitol, 100 mM K-gluconate or 10 mM K-gluconate with 90 mM TMA-gluconate, or of the same milieu as the intravesicular milieu. pH$_i$, pH$_o$: intra- and extravesicular pH; (K)$_i$, (K)$_o$: internal and external concentration of K^+; Eq: equilibrium value obtained with vesicles equilibrated at pH 7.5 with and without added valinomycin

to a pH gradient (5/7.5). As shown in Fig. 6, the Cl^- uptake generated by a pH gradient is related to the K^+ gradient. As the membrane potential is decreased by the K^+ gradient, the Cl^- uptake is decreased. When the potential is considerably decreased by a K^+ gradient (10/100 mM) in presence of valinomycin, the Cl^- uptake is very close to the uptake obtained in absence of gradients. It should be noted that under these two experimental conditions the slight difference in Cl^- uptake velocities appear before 1 min incubation time. A complementary experiment done with K^+ (100/100 mM) equilibrated on both sides of the BBM has shown that the presence of valinomycin completely abolishes the increase of the Cl^- uptake provoked by a pH gradient 6/7.5 (Fig. 7).

In another experiment, the presence of a Cl^- conductance was examined by applying a K^+ gradient (100/0 mM) in presence of valinomycin to BBM vesicles equilibrated at pH 7.5 or 6. Under such conditions, the Cl^- uptake by BBM vesicles is greatly increased compared to the situation without K^+ (Fig. 8). Furthermore, at pH 6, Cl^- is accumulated against a Cl^- concentration gradient. This experiment confirms that a conductive pathway for Cl^- is present in BBM and shows that it can be increased by an acidic pH.

Discussion

The BBM vesicles purified from trout intestine used in this study are enriched in alkaline phosphatase, which is mainly located in BBM but also, to a much lesser extent, in the latero-basal membranes, by the same amount as leucine aminopeptidase, which is strictly located in the BBM of enterocytes. Thus, in all experiments, the determination of the alkaline phosphatase specific activity was used to assess the enrichment of each vesicle suspension in BBM compared to the initial homogenate. The values obtained with the purification method used in this study are higher or equivalent to those obtained for other fish intestine vesicles (Eveloff et al., 1980; Bogé & Rigal, 1981; Bogé, Rigal & Pèrés, 1982; O'Grady, Palfrey & Field, 1987). Furthermore, enrichment factors determined for enzyme markers of enterocyte membranes other than the BBM are less than unity, showing that the data reported here relate to the apical membranes.

This study examines the possibility in BBM of trout intestine of a Cl^--OH^- exchange (or Cl^--H^+ cotransport) and a Cl^- conductance. The question

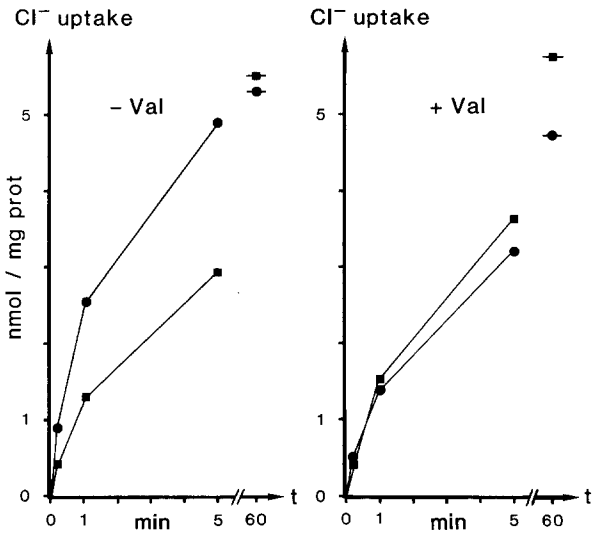

Fig. 7. Effect of the presence of valinomycin on Cl^- uptake by trout intestine BBM in the presence of a proton gradient and 100 mM K^+ on both sides of the membranes. Vesicles contained 100 mM K-gluconate, 40 mM HEPES, 40 mM MES and 53 mM Tris base (pH_i 7.5). Valinomycin was added as in Fig. 6. Uptake of 3.4 mM $^{36}Cl^-$ was assayed in the same milieu as the internal milieu (■) or (●) in the presence of 100 mM K-gluconate 40 mM HEPES, 40 mM MES, 8 mM Tris base (pH_o 6) and 45 mM sorbitol

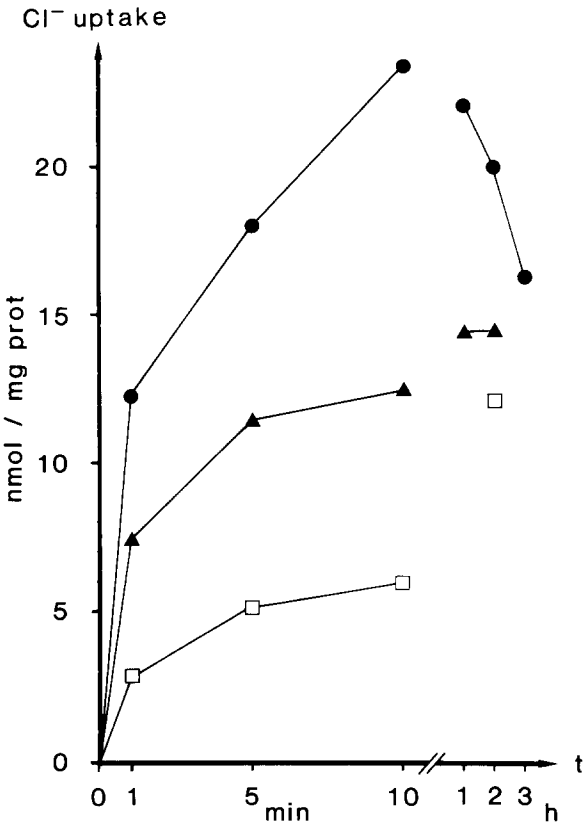

Fig. 8. Effect of equilibration pH on the time course of Cl^- uptake by trout intestine BBM in the presence of a potassium gradient with valinomycin added. Vesicles contained 100 mM TMA-gluconate, 40 mM HEPES, 40 mM MES, (□, ●) 8 mM Tris base (pH 6) and 45 mM sorbitol or (▲) 53 mM Tris base (pH 7.5). Valinomycin was added as in Fig. 6. Uptake of 3.4 mM $^{36}Cl^-$ was assayed in the same milieu as the internal milieu (□) or (●, ▲) with 100 mM K-gluconate instead of 100 mM TMA-gluconate

addressed was to determine if the Cl^- uptake obtained with pH gradients is electrogenic in nature.

pH gradients were obtained by the use of citric acid and varying concentrations of Tris base. It is unlikely that citrate exerts other effects than those of its buffering properties as there are no citrate gradients through the BBM. Furthermore, when MES is used, instead of citrate, similar results are obtained. The Cl^- uptake after 2 hr incubation time in absence of a pH gradient (7.5/7.5) is taken as a reference for equilibrium values. The "true" equilibrium value should be determined at pH 5/5. Actually, during the whole incubation period, the intravesicular pH varies from 7.5 to 5. As the uptake value after 2 hr incubation time is much smaller at pH 5 than pH 7.5 (Fig. 1), one can conclude that the uptake value determined at pH 5 is greatly underestimated after 2 hr of incubation. In the presence of the pH gradient 3.5/7.5, the Cl^- uptake velocity is very fast for 10 sec (Fig. 3), but, later, it decreases and never reaches the equilibrium value (*results not shown*). Furthermore, when the pH gradient is 5.6/7.5 in the presence of MES (Fig. 1) the Cl^- uptake value obtained after 2 hr incubation time is not very different from that obtained at pH equilibrated at 7.5. Interestingly, when vesicles are purified at pH 6, the equilibrium value of Cl^- uptake (more than 12 nmol/mg protein) and consequently the intravesicular volume, are higher than when vesicles are purified at pH 7.5 (about 5 nmol/mg protein). These observations are interpreted as an intrinsic effect of the pH which, at values smaller than 6, degrades BBM and results in a loss of intravesicular volume. A similar observation has been made by Warnock and Yee (1981) on BBM vesicles isolated from rabbit renal cortex when intravesicular and extravesicular pH are equilibrated at 4.5.

This study shows that in BBM vesicles isolated from trout intestine a Cl^- concentrative uptake can be demonstrated in the presence of either a K^+ gradient (with valinomycin, Fig. 8) or of a proton gradient (Fig. 1), whose effect is inhibited by SITS. These observations suggest that the apical membrane of the trout enterocytes is equipped with both a Cl^- conductance and a Cl^--OH^- exchange. The K^+ gradient and valinomycin changing the membrane potential could induce, via a proton conductive pathway, a pH gradient which, in turn, would stimulate Cl^- uptake by a Cl^--OH^- exchange (Cl^--

H^+ cotransport) process. Such an interpretation can be excluded because in experiments, which will be published elsewhere, it has been shown that, in the same conditions, the intravesicular pH is transiently increased by 0.1 unit, a change that cannot be responsible for the observed Cl^- uptake. An alternative explanation is that, in the presence of a proton gradient, Cl^- crosses through the BBM by the conductive pathway energized by the proton diffusion potential. If this is the case, the SITS inhibition could be explained by an inhibition of the Cl^- conductance, which has already been shown (Miller & White, 1980; Seifter, Knickelbein & Aronson, 1984). Furthermore, Ives, Chen and Verkman (1986) have demonstrated that SITS is a protonophore in BBM isolated from rabbit kidney. Thus, SITS could shunt the proton gradient as CCCP does and reduce Cl^- uptake. Results, which will be published elsewhere, obtained with a pH-sensitive fluorescent probe included in the same vesicles used in this study, show that the proton gradient is effectively collapsed by SITS. The explanation that Cl^- uptake is achieved via the conductive pathway is supported by the evidence obtained when Cl^- uptake is measured with increasing proton gradients. Indeed, under such conditions, Cl^- uptake is proportional to the potential gradient indicating that Cl^- flux is electrical in nature. The energy for the transport would be given by the proton gradient through a proton conductance. Consequently, if the proton conductance is increased, the proton motive force will be increased unless this increase leads to a collapse of the pH gradient. This was obtained when CCCP was added to the incubation medium at two different concentrations. Furthermore, in both presence and absence of CCCP, Cl^- uptake shows nonsaturable properties, whereas a Cl^--OH^- exchange (Cl^--H^+ cotransport) is a saturable mechanism as a Na^+-H^+ exchange (Aronson, Nee & Suhm, 1982; Rocco, Cragoe & Warnock, 1987). The regression lines obtained in Fig. 5 do not strictly pass through the origin; this slight deviation could be interpreted as the effect of a Cl^--OH^- exchange, a process whose dissociation constant would be very low. Indeed, it is more likely to be due to a trace of nonspecific binding, which is slightly increased in the presence of CCCP. Further evidences of the electrogenic nature of the Cl^- uptake is that (*i*) it can be modulated by potentials generated by K^+ gradients with or without valinomycin and (*ii*) in the presence of K^+ (100/100 mM) and valinomycin voltage clamp, the pH gradient 6/7.5 does not at all stimulate Cl^- uptake (Seifter et al. 1984). All these data argue against the existence of a Cl^--OH^- antiport in the trout intestine. As the BBM have been purified by a Mg^{2+} aggregation procedure, some constituents of the membrane could have been lost by such treatment. Similar unpublished results have been obtained on BBM prepared by Ca^{2+} precipitation or by the method of O'Grady et al. (1987). Furthermore, Pelletier, Duportail and Leray (1986) did not find any differences in the membrane phospholipid composition between trout intestine BBM obtained by Mg^{2+} or Ca^{2+} aggregation.

In the presence of a K^+ gradient (100/0 mM), the Cl^- uptake is considerably increased when extravesicular and intravesicular pH are equilibrated at pH 6 rather than pH 7.5. Such an observation is probably due to an increase of the Cl^- conductance, which must be activated in a protonated form, because, under the conditions of the experiment, the membrane potential, as determined by the Goldman-Katz equation is decreased by an increase in proton concentration. Internal H^+ has been shown to be an allosteric activator of the Na^+-H^+ exchanger in renal microvillus membrane vesicles (Aronson et al., 1982). Similarly, when a pH gradient (outside acidic) is imposed on BBM vesicles, the more the inside pH is decreased during the incubation, the more the Cl^- conductance is activated. It is likely that such an effect favors Cl^- uptake when the proton motive force is reduced and may be responsible for the long duration of the overshoot observed under these conditions.

As this study failed to demonstrate the presence of a Cl^--OH^- antiport in the BBM of fish intestine, this membrane behaves differently than those of rat (Liedtke & Hopfer, 1982) or guinea pig (Colin et al., 1984), which have a Cl^--OH^- antiport (Cl^--H^+ symport). Whereas the apical membrane of enterocytes from FW fish does not seem to possess a Na^+-K^+-Cl^- cotransport (Lau, 1985), the question arises of a pathway for Cl^- entry into enterocyte. The Cl^- conductance shown in this study would result in a leak of Cl^- from the enterocyte to the lumen following the potential gradient, although another possibility can be proposed. A microclimate with a low pH on the surface of the intestinal brush border, protected by mucus layer acting as a diffusion barrier (Simonneaux, Humbert & Kirsch, 1987), can create a proton gradient through the apical membrane (Ganapathy & Leibach, 1985), which could favor the absorption of Cl^- through the conductive pathway. This proposition, in addition to the fact that the presence of such a proton conductance has to be confirmed, requires the action of other membrane mechanisms to maintain an apical proton gradient.

In summary, the brush border of the enterocytes of the FW trout is devoid of a Cl^--OH^- antiport (Cl^--H^+ symport), but has a Cl^- conductance and apparently a proton conductance.

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