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Na^+-Coupled Cl^- Transport in the Gastric Mucosa of the Guinea Pig

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Abstract

The Cl^-/HCO_3^- exchange mechanism usually postulated to occur in gastric mucosa cannot account for the Na^+-dependent electrogenic serosal to mucosal Cl^- transport often observed. It was recently suggested that an additional Cl^- transport mechanism driven by the Na^+ electrochemical potential gradient may be present on the serosal side of the tissue. To verify this, we have studied Cl^- transport in guinea pig gastric mucosa. Inhibiting the (Na^+, K^+) ATPase either by serosal addition of ouabain or by establishing K^+-free mucosal and serosal conditions abolished net Cl^- transport. Depolarizing the cell membrane potential with triphenylmethylphosphonium (a lipid-soluble cation), and hence reducing both the Na^+ and Cl^- electrochemical potential gradients, resulted in inhibition of net Cl^- flux. Reduction of short-circuit current on replacing Na^+ by choline in the serosal bathing solution was shown to be due to inhibition of Cl^- transport. Serosal addition of diisothiocyanodisulfonic acid stilbene (an inhibitor of anion transport systems) abolished net Cl^- flux but not net Na^+ flux. These results are compatible with the proposed model of a Cl^-/Na^+ cotransport mechanism governing serosal Cl^- entry into the secreting cells. We suggest that the same mechanism may well facilitate both coupled Cl^-/Na^+ entry and coupled HCO_3^-/Na^+ exit on the serosal side of the tissue.

Key Words: Guinea pig; gastric mucosa; short-circuit current; chloride transport; proton transport; sodium transport; bicarbonate transport; ouabain; triphenylmethylphosphonium ($TPMP^+$); diisothiocyanodisulfonic acid stilbene (DIDS).

Introduction

Since the pioneering work of Hogben (1955), several models have been put forward to explain the mechanism of Cl^- transport in gastric mucosa. It was reported in early studies that Na^+ ions are essential for maintaining Cl^- transport in frog and guinea pig gastric mucosa (Sachs *et al.*, 1966;

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Shoemaker *et al.*, 1966). Frizzell *et al.* (1979) proposed a neutral Na^+-coupled Cl^- transport mechanism, located on the serosal membrane of the secreting cells, accounting for Cl^- entry to the parietal cells. It was also postulated that Cl^- passively diffuses out of the cells across the mucosal membrane. Machen and McLennan (1980) suggested that the "nonacidic" Cl^- (Heinz and Durbin, 1957) is transported via a similar mechanism in frog as well as in piglet gastric mucosa (McLennan *et al.*, 1980), but the "acidic Cl^-" may be transported via a different pathway, e.g., Cl^-/HCO_3^- exchange (Rehm, 1967). Using ion-selective microelectrodes, Cl^- was shown to be at equilibrium across the mucosal membrane of surface epithelial cells in frog and necturus stomachs (Machen *et al.*, 1980), giving support to the proposed model.

We have investigated the transport processes occurring in guinea pig gastric mucosa. It is shown that inhibition of the Cl^- transport processes either directly, e.g., using Cl^--free conditions, Na^+-free conditions, or diisothiocyanodisulfonic acid stilbene (DIDS), or indirectly using ouabain, K^+-free conditions, or triphenylmethylphosphonium ($TPMP^+$), is accompanied by a concomitant inhibition of net proton transport. On the basis of our findings we propose that transport of Cl^- ions is carried out via a Na^+–Cl^- cotransport mechanism, facilitating entry of Cl^- from the serosal bathing solution to the secreting cells, while exit of Cl^- to the mucosal solution is a passive diffusion process driven by the electrochemical potential gradient of Cl^- across the mucosal membrane. We further suggest that the same mechanism serves as a Na^+–HCO_3^- transporter from the cell interior to the serosal solution.

Materials and Methods

The stomachs of albino guinea pigs weighing 300–500 g were removed from anesthetized animals. The mucosa were scraped, divided into two halves for matched experiments, and mounted in Ussing chambers (1.5 cm diameter). A voltage clamp device enabled us to short-circuit the tissue and measure, continuously, the short-circuit current (I_{sc}). The conductance of the tissue was measured by applying ±5-mV pulses periodically for 10 sec and recording the resultant changes in current.

The gastric mucosa, bathed in HCO_3^--free solution (composed of NaCl 124, KCl 5, $MgCl_2$ 2, $CaCl_2$ 1, and glucose 20 mM), with 100% oxygen supply at 37°C, reached a steady state after 1–2 hr and was stable for at least 5 hr. Under normal conditions we measured a potential difference of 26 ± 0.8 mV, an I_{sc} of 247 ± 11.1 $\mu A \cdot cm^{-2}$, and an acid secretion rate of 3.3 ± 0.26 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$. This was recorded continuously using two radiometer pH-stat

systems (pH meter 26 equipped with titrator II, automatic burette unit AB412, and titrigraph SBR2c). The end point of the mucosal-solution titration was set to pH 5.0, while the serosal solution was kept at pH 7.0 by means of 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES). The rate of H^+ transport was obtained by reading from the titrigraph chart the number of moles of OH^- ions delivered to the mucosal solution over any given period of time.

In ion-substitution experiments, SO_4^{-2}, Na^+, and choline replaced Cl^-, K^+, and Na^+ respectively. To establish ion-free conditions, the procedure we used involved several successive washings of both sides of the tissue. The osmolarity of all solutions, measured by an Osmett (Precision System Inc.), was adjusted to 280 mOsm/kg H_2O by means of mannitol.

The unidirectional fluxes of Na^+ and Cl^- were measured by adding tracer to either side of the tissue (0.5 μCi · ml^{-1} ^{22}Na or ^{36}Cl). Every 15–20 min samples were removed from both sides, starting 15–20 min after tracer addition. The volume of the bathing solution was restored by adding the proper unlabeled solution to both sides of the tissue. ^{22}Na was assayed in a gamma spectrometer and ^{36}Cl in a liquid scintillation counter. The rates of appearance of tracer on the unlabeled sides were calculated by linear regression. Unidirectional fluxes were obtained from the ratio of the slope to the specific activity in the labeled compartment. Net fluxes were determined in paired experiments as the difference between unidirectional fluxes, and the mean and standard error of the mean were calculated. In all cases the Student's *t*-test was used as a measure of significance.

All chemicals were analytically pure. Triphenylmethylphosphonium bromide was purchased from ICN Pharmaceuticals, Inc. 4,4' Diisothiocyano-2,2'-disulfonate stilbene was a kind gift from Dr. Z. I. Cabantchik.

Results

Inhibition of Cl^- Transport by Ouabain and by K^+-Free Conditions

Ouabain, a specific inhibitor of (Na^+, K^+) ATPase, has been shown to inhibit acid secretion in frog gastric mucosa in a concentration-dependent manner (Davenport, 1962). Applying ouabain (10^{-5} M) to the serosal side of guinea pig gastric mucosa reduced I_{sc} to a value of 13 ± 3.9 μA · cm^{-2} within 30 min, while the control was practically steady (Fig. 1A). The net proton transport rate ($J_{H^+}^{net}$) decreased from 3.3 ± 0.26 to 0.87 μeq · cm^{-2} · hr^{-1} (Fig. 1C). No further decrease in I_{sc} or acid secretion was obtained by higher doses of ouabain (not shown). Both I_{sc} and acid secretion were fully abolished by saturating the bathing solutions with nitrogen instead of oxygen, before or after ouabain addition (not shown).

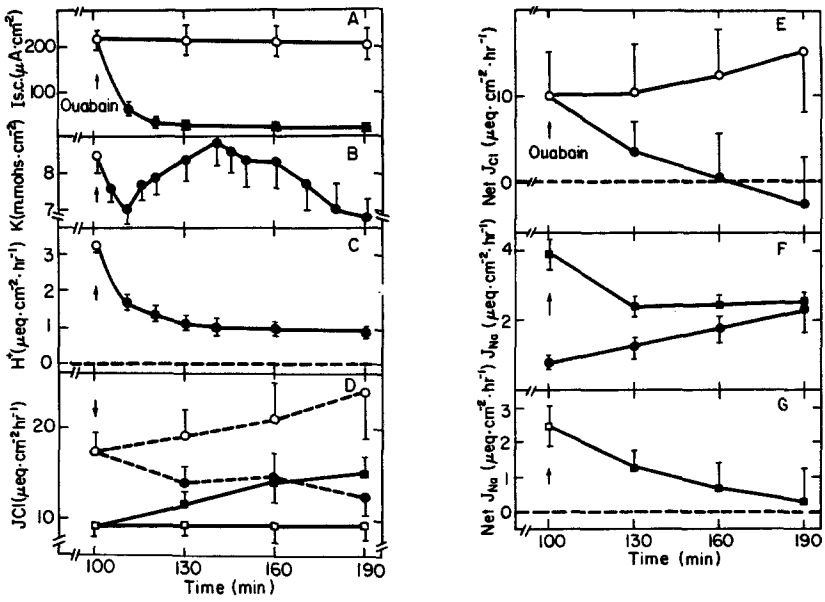

Fig. 1. Effect of 10^{-5} M ouabain on: (A) short-circuit current (I_{sc}): (O) control, (●) treatment ($n = 32$); (B) conductance; (C) rate of net proton secretion to the mucosal solution ($J_{H^+}^{net}$); (D) unidirectional fluxes of Cl^-: (O, ●; □, ■) values of $J_{Cl^-}^{S\to M}$ and $J_{Cl^-}^{M\to S}$ respectively, ($n = 10$); (E) $J_{Cl^-}^{net}$; (F) unidirectional fluxes of Na^+: (●, ■) values of $J_{Na^+}^{S\to M}$ and $J_{Na^+}^{M\to S}$ respectively, ($n = 7$); (G) $J_{Na^+}^{net}$. Open and closed symbols are control and experimental values, respectively. Ouabain (10^{-5} M) was added to the serosal bathing solution. Values are mean ± SEM in n experiments.

Since 90% of the I_{sc} is carried by net Cl^- ion transport (Ayalon *et al.*, 1980), abolition of $J_{Cl^-}^{net}$ should account for the ouabain effect. Figures 1D and E show that this is the case. The unidirectional fluxes of Cl^- from serosa to mucosa ($J_{Cl^-}^{S\to M}$) decreased from 16.67 ± 1.71 to 12.8 ± 3.4 μeq · cm^{-2} · hr^{-1} while the $J_{Cl^-}^{M\to S}$ increased monotonically (Fig. 1D) from 8.5 ± 0.8 to 15.3 ± 2.0 μeq · cm^{-2} · hr^{-1}. The increase in the latter may reflect an increase in the paracellular pathway conductance as was shown to occur in frog skin (Corcia *et al.*, 1980). This interpretation is supported by the fact that the back flux of Na^+, $J_{Na^+}^{S\to M}$, also increased gradually after addition of ouabain (Fig. 1F). The interpretation of Fig. 1B follows readily. In the first period a decrease in the conductance occurs, probably as a result of a reduction in both $J_{Cl^-}^{net}$ (Fig. 1E) and $J_{Na^+}^{net}$ (Fig. 1G). A concomitant increase in the passive conductance should cause an increase in total conductance. The value of $J_{Cl^-}^{net}$ after ouabain treatment was -2.5 ± 5.4 μeq · cm^{-2} · hr^{-1}, which does not differ significantly from zero ($P > 0.5$). As was expected, $J_{Na^+}^{net}$ was abolished ($P > 0.2$) after 60 min treatment with ouabain (Fig. 1G).

Establishing K^+-free conditions is known to cause (Na^+, K^+) ATPase to

operate in the 1:1 Na^+–Na^+ exchange mode (Glynn and Karlish, 1975). Accordingly, in the intact tissue this treatment should result in inhibition of electrogenic Na^+ transport, mimicking the effect of ouabain. Therefore, in order to show that inhibition of Cl^- transport by ouabain is not due to direct inhibition of a Cl^- transporting device, but rather an indirect consequence of (Na^+, K^+) ATPase inhibition, we replaced K^+ by Na^+ on both sides of the tissue. The results are depicted in Fig. 2A–G. The value of I_{sc} (Fig. 2A) is markedly decreased by successive washings of both sides by K^+-free solutions to 11.6 ± 1.1 $\mu A \cdot cm^{-2}$. The Na^+ unidirectional fluxes were both increased: $J_{Na^+}^{M\to S}$ to 6.8 ± 2.0 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$, and $J_{Na^+}^{S\to M}$ to 6.2 ± 1.4 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$, leading to a net flux of Na^+ of 0.56 ± 1.60 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$. This did not differ significantly from zero ($P > 0.01$), as predicted (Fig. 2F, G). The value of $J_{Cl^-}^{S\to M}$ decreased to 11.5 ± 1.8 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$ while $J_{Cl^-}^{M\to S}$ increased to 13.2 ± 1.7 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$ after 80 min of treatment (Fig. 2D), leaving a $J_{Cl^-}^{net}$ of −1.7 ± 1.8 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$ (Fig. 2E). The changes in the total conductance again show a decrease followed by an increase to a level higher than the control value (Fig. 2B). The former is attributed to the decrease in $J_{Cl^-}^{net}$ and $J_{Na^+}^{net}$, the latter to the increase in passive conductance reflected by the increase in $J_{Cl^-}^{M\to S}$ and $J_{Na^+}^{S\to M}$. The increase in $J_{Na^+}^{S\to M}$ may

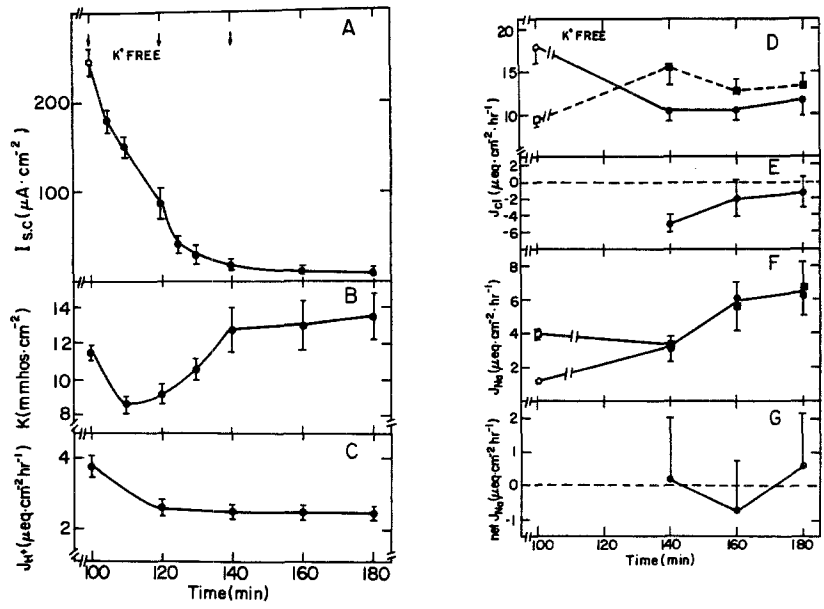

Fig. 2. Effect of K^+ ion removal from the bathing solutions (replaced by Na^+) on: (A) I_{sc} ($n = 20$); (B) conductance; (C) J_{H^+}; (D) $J_{Cl^-}^{S\to M}$, $J_{Cl^-}^{M\to S}$ ($n = 10$); (E) $J_{Cl^-}^{net}$; (F) $J_{Na^+}^{S\to M}$, $J_{Na^+}^{M\to S}$ ($n = 7$); (G) $J_{Na^+}^{net}$. Symbols are the same as in Fig. 1.

also explain the increase in $J_{Na^+}^{M\to S}$ since the net flux of Na^+ is zero. The rate of proton transport, shown in Fig. 2C, was reduced to 2.65 ± 0.35 μeq · cm^{-2} · hr^{-1} under these experimental conditions. The fact that $J_{H^+}^{net}$ was not reduced to zero is in contrast to what has been found by other investigators (e.g., Davis *et al.*, 1965) for different gastric mucosal preparations. A possible explanation for this discrepancy is given in the discussion.

Effect of Ouabain on Na^+ and H^+ Transport under Cl^--Free Conditions

The same phenomenon of near-abolition of I_{sc} accompanied by a reduction in the rate of acid secretion was obtained by removal of Cl^- from both bathing solutions as shown in Figs. 3A and C. The value of I_{sc} decreased to 31.65 ± 3.33 μA · cm^{-2} within 40 min while net transport of protons decreased to 1.87 ± 0.21 μeq · cm^{-2} · hr^{-1}. Figure 3D shows the effect of replacing Cl^- by SO_4^{-2} on the unidirectional fluxes of Na^+. The value of $J_{Na^+}^{M\to S}$ decreased to 2.51 ± 0.14 μeq · cm^{-2} · hr^{-1} within 80 min while $J_{Na^+}^{S\to M}$, which is considered to occur primarily via the paracellular pathway, remained unchanged for 60 min and then increased monotonically to 1.96 ± 0.13 μeq · cm^{-2} · hr^{-1} at 80 min, corresponding to the increase in the conductance of the tissue (Fig. 3B). The resultant net Na^+ transport was decreased to 0.97 ± 0.11 μeq · cm^{-2} · hr^{-1}, as seen in Fig. 3E. Addition of

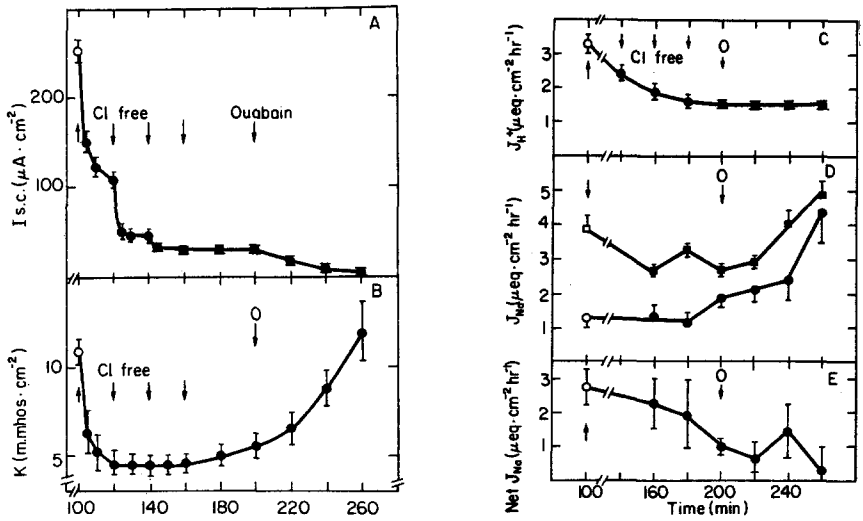

Fig. 3. Effect of removal of Cl^- ions (replaced by SO_4^{-2}) from the bathing solutions (successive washings are indicated by arrows) followed by addition of 10^{-5} M ouabain to the serosal side (t = 200 min) on: (A) I_{sc} (n = 18); (B) conductance; (C) $J_{H^+}^{net}$; (D) $J_{Na^+}^{M\to S}$, $J_{Na^+}^{S\to M}$ (n = 6); (E) $J_{Na^+}^{net}$. Symbols are the same as in Fig. 1. The letter O indicates application of ouabain.

ouabain to the serosal side resulted in a further reduction of I_{sc}, as shown in Fig. 3A, to 6.13 ± 2.21 $\mu A \cdot cm^{-2}$. Measuring $J_{Na^+}^{S \to M}$ and $J_{Na^+}^{M \to S}$ after ouabain application (Fig. 3D) revealed that both unidirectional fluxes were increased, but the resultant $J_{Na^+}^{net}$ (Fig. 3E) was abolished ($P > 0.7$), strongly suggesting that the reduction in I_{sc} was due to a cessation of net Na^+ transport. It is well established that $J_{H^+}^{net}$ is reduced but not abolished in gastric mucosal preparations bathed under Cl^--free solutions. Figure 3G shows that this is also the case in guinea pig gastric mucosa. More important is that administration of ouabain to the serosal bathing solution did not produce any further effect on the rate of proton transport. This suggests that there is no direct inhibition of the (H^+, K^+) ATPase by ouabain, and the $J_{H^+}^{net}$ inhibition by this inhibitor seen in Cl^--containing solutions is probably a secondary phenomenon. However, Carrasquer *et al.* (1981) consider that ouabain may inhibit the H^+ transport directly, since it was shown to inhibit J_{H^+} in frog gastric mucosa bathed in Cl^--free solutions. We believe the difference between the results in frog and guinea pig gastric mucosal preparations may well be attributable to the different species involved, or to the difference of two orders of magnitude in the ouabain concentrations (the concentration used in frog gastric mucosa was 10^{-3} M).

Inhibition of Cl^- Transport by Omission of Na^+ from the Bathing Solutions

Introducing a Na^+-free solution on the serosal side alone resulted in a reduction in the I_{sc} (Fig. 4) corresponding to net Cl^- transport (*vide infra*). On the other hand, Na^+-free conditions on the mucosal side lead to a minor change in I_{sc}. This resembles inhibition of the electrogenic mucosal to serosal Na^+ transport via the (Na^+, K^+) ATPase. Omission of Na^+ from both bathing solutions resulted in a reduction in I_{sc} (Fig. 5A) to 30.9 ± 1.71 $\mu A \cdot cm^{-2}$. The remaining I_{sc} was insensitive to further washing with Na^+-free solutions and to 10^{-5} M ouabain. The net Cl^- transport decreased to zero ($P > 0.2$) with no significant change in the passive permeability to Cl^-, as shown in Figs. 5D and E. Figure 5C shows that the acid secretion rate also decreased (from 3.84 ± 0.17 to 0.86 ± 0.12 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$) and then remained unchanged in the presence of ouabain, again probably due to the inhibition of $J_{Cl^-}^{net}$.

Inhibition of Cl^- Transport by DIDS

DIDS is known as an anion transport inhibitor (Cabantchik *et al.*, 1978). Shoemaker (1980) showed that it also inhibits both Cl^- transport and H^+ transport in frog gastric mucosa. The effects of DIDS (10^{-3} M) on the electrical parameters and unidirectional fluxes of $^{22}Na^+$ and $^{36}Cl^-$ in guinea

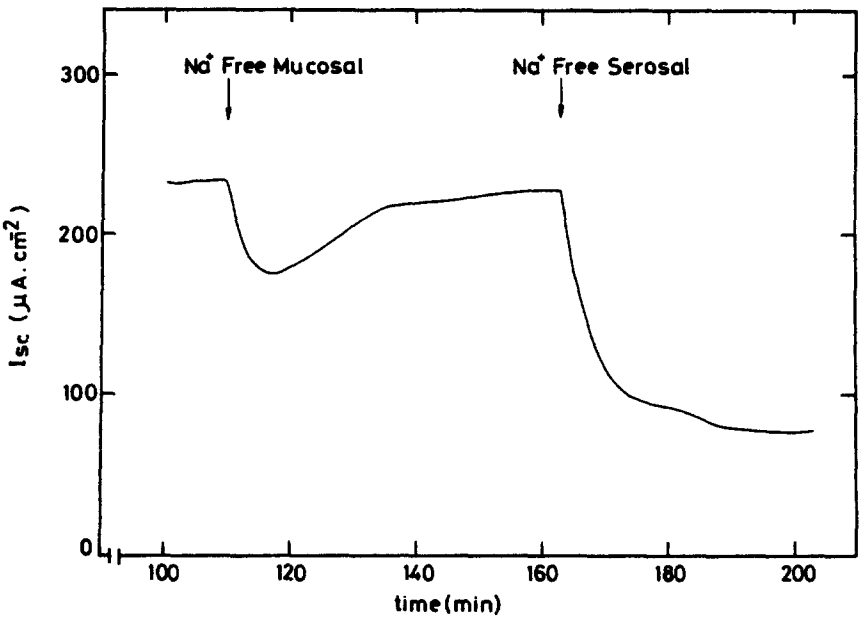

Fig. 4. A representative experiment showing the effect of Na^+-free conditions on either the serosal or mucosal side on the short-circuit current.

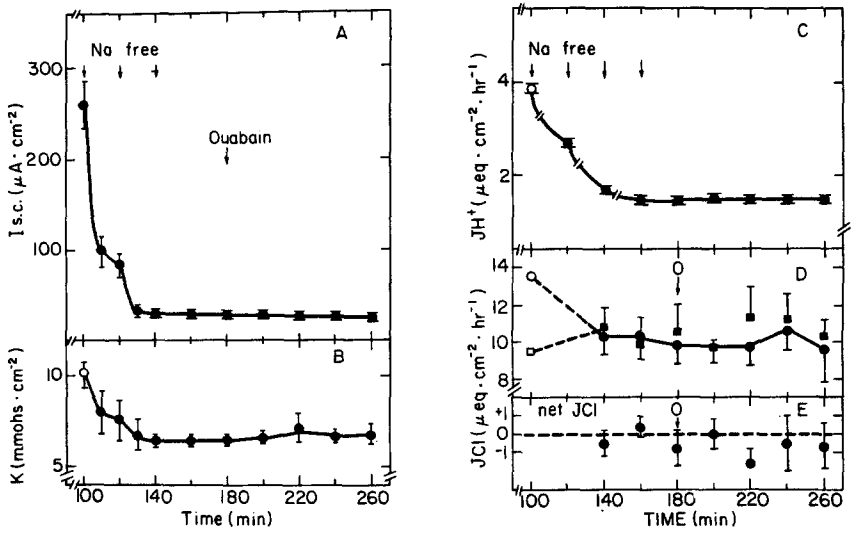

Fig. 5. Effect of removal of Na^+ ions from the bathing solutions (replaced by choline$^+$). (A) I_{sc} ($n = 15$); (B) conductance; (C) $J_{H^+}^{net}$; (D) $J_{Cl^-}^{S \to M}$, $J_{Cl^-}^{M \to S}$ ($n = 5$); (E) $J_{Cl^-}^{net}$. (Different successive washings are indicated by arrows in A.) Ouabain (10^{-5} M) was added to the serosal side (indicated by an arrow and the letter O at $t = 180$ min). Symbols are the same as in Fig. 1.

Table I. Effects of DIDS (10^{-3} M) Applied to the Serosal Side of Guinea Pig Gastric Mucosa (after 90 min)[a]

	Control	Experimental
$I_{sc}(\mu A \cdot cm^{-2})$	237.4 ± 10.1	12.83 ± 1.81
$K(mmhos \cdot cm^{-2})$	9.42 ± 0.65	12.55 ± 1.30
PD (mV)	25.4 ± 1.24	2.11 ± 0.30
$J_{H^+}^{net}$	3.09 ± 0.36	0.24 ± 0.09
$J_{Na^+}^{M\to S}$	5.54 ± 0.59	4.45 ± 0.51
$J_{Na^+}^{S\to M}$	1.90 ± 0.21	3.28 ± 0.68
$J_{Na^+}^{net}$	3.54 ± 0.59	1.28 ± 0.82
$J_{Cl^-}^{S\to M}$	17.86 ± 1.33	6.97 ± 1.28
$J_{Cl^-}^{M\to S}$	12.11 ± 1.06	7.74 ± 1.20
$J_{Cl^-}^{net}$	5.94 ± 1.85	0.77 ± 2.48

[a] All values of the fluxes are given in $\mu eq \cdot cm^{-2} \cdot hr^{-1}$. ($n = 30, 6$ for electrical, flux parameters respectively.)

pig gastric mucosa are summarized in Table I. As expected, net Cl^- transport is reduced by DIDS, and the decrease corresponds to the decrease in the I_{sc}. The passive conductance increases, as seen in the elevated $J_{Na^+}^{S\to M}$ and total conductance. The value of $J_{Na^+}^{M\to S}$ decreased as indicated. As a result net active Na^+ transport was also inhibited, but not completely, indicating that the (Na^+, K^+) ATPase was not affected directly. Reduction of $J_{H^+}^{net}$ was also observed as a result of DIDS, most likely as a consequence of the abolition of net Cl^- transport.

Inhibition of Cl^- Transport by $TPMP^+$

According to our model, Cl^- exit from the cell to the mucosal solution is a passive process. On this assumption it is suggested that the abolition of Cl^- transport brought about by inhibition of the (Na^+, K^+) ATPase is due to depolarization of the cell potential. This results in a reduced driving force for Cl^- diffusion across the mucosal membrane. A direct implication of this argument is that inhibition of net Cl^- transport is expected upon depolarizing the cell potential. We used $TPMP^+$, a lipid-soluble cation (Grinius *et al.*, 1970), to produce this effect.

Table II summarizes the results obtained 60 min after applying $TPMP^+$ (10^{-3} M) to the serosal solution. As expected, $J_{Cl^-}^{net}$ is reduced with a concomitant reduction in J_{H^+}. The value of $J_{Na^+}^{net}$ was not significantly different from zero ($P > 0.1$) after $TPMP^+$ treatment. The total conductance decreased as a result of the decrease in net ion transport.

Discussion

Our results confirm that in guinea pig gastric mucosa Cl^- transport across the serosal membrane of the acid-secreting cells is coupled to Na^+

Table II. Effects of $TPMP^+$ (10^{-3} M) Applied to the Serosal Side of Guinea Pig Gastric Mucosa[a]

	Control	Experimental
I_{sc} ($\mu A \cdot cm^{-2}$)	232 ± 23.9 (21)	−1.81 ± 0.65 (21)
K (mmhos · cm^{-2})	12.08 ± 1.76 (21)	4.72 ± 0.44 (21)
PD (mV)	19.4 ± 1.16 (21)	−0.39 ± 0.12 (21)
$J_{H^+}^{net}$	5.81 ± 0.59 (21)	0.27 ± 0.09 (21)
$J_{Na^+}^{S\to M}$	2.32 ± 0.43 (6)	3.21 ± 0.69 (6)
$J_{Na^+}^{M\to S}$	3.86 ± 0.46 (6)	2.86 ± 0.72 (6)
$J_{Na^+}^{net}$	1.41 ± 0.20	−1.22 ± 0.75
$J_{Cl^-}^{S\to M}$	19.79 ± 1.08 (5)	4.53 ± 1.17 (5)
$J_{Cl^-}^{M\to S}$	11.18 ± 0.76 (5)	3.59 ± 0.55 (5)
$J_{Cl^-}^{net}$	8.87 ± 0.93	2.35 ± 1.23

[a] All fluxes are given in $\mu eq \cdot cm^{-2} \cdot hr^{-1}$.

transport. This conclusion is based on the observation that serosal Na^+-free conditions are inhibitory to Cl^- transport, in agreement with results obtained by other investigators (Machen and McLennan, 1980). It is generally accepted, as a consequence of the effect of ouabain, that (Na^+, K^+) ATPase is present in the acid-secreting cells of gastric mucosa. Its activity is considered essential for the maintenance of Cl^- and H^+ transport (Forte and Machen, 1975; Hansen *et al.*, 1975; Machen *et al.*, 1978), in spite of the fact that it is found in relatively low amounts in the stomach (Hansen *et al.*, 1972). Since we have shown that inhibition of Cl^- transport occurs upon depolarization of the cell potential by $TPMP^+$, we suggest that depolarization is also associated with inhibition of the (Na^+, K^+) ATPase. This hypothesis is supported by our observation that ouabain caused depolarization in isolated gastric glands (unpublished results). It is important to note that $TPMP^+$ might depolarize membrane potentials in an intracellular compartment (e.g., mitochondria), leading to a nonspecific effect on metabolism. On the other hand, the inhibition of net Cl^- transport obtained with $TPMP^+$ is in excellent agreement with the effect of high K^+ on the serosal side of frog gastric mucosa (Harris and Edelman, 1964; Hogben, 1955) and piglet gastric mucosa (McLennan *et al.*, 1980).

Since we showed that K^+-free conditions are also inhibitory to (Na^+, K^+) ATPase, we suggest that Cl^- transport is inhibited indirectly as in the case of the ouabain effect. Nevertheless, the possibility of a direct effect on the Cl^- transport mechanism cannot be excluded. For example, recently a $Na^+ : K^+ : Cl^-$ coupled transport was suggested for the thick ascending limb of Henle's loop (Greger and Schlatter, 1981). This mechanism, however, predicts increased Cl^- transport in the presence of high K^+.

Our observations are compatible with our proposed model, which assumes that Cl^- is essentially at equilibrium across the mucosal membrane of the parietal cells. Depolarization of the cell membrane potential establishes a

driving force for Cl^- favoring its entry from the mucosal side, bringing about an inhibition of the net serosal to mucosal Cl^- transport. This assumption is supported by the fact that the mucosal membrane of frog gastric mucosa is highly permeable to Cl^- (Durbin, 1977) and by direct measurements of Cl^- activity in surface epithelial cells (Machen *et al.*, 1980).

We have shown that there is no direct inhibition of J_{H^+} under Cl^--free conditions, which indicates that in our tissue there is no direct inhibition of a proton transporter by ouabain. It was suggested that compartmentalized K^+ is important to maintain proton transport (Davenport, 1962; Davis *et al.*, 1965; Takeguchi *et al.*, 1979; Koelz *et al.*, 1981; France and Durbin, 1981), and this may explain the inhibition of proton transport produced by ouabain and by K^+-free conditions. On the other hand, it is not compatible with the measured intracellular free K^+ activity (Schettino and Curci, 1980).

It is apparent from our results that under all experimental conditions where inhibition of Cl^- transport was observed, a concomitant reduction in J_{H^+} was detected. The influence of Cl^--free and Na^+-free conditions, as well as the inhibition by DIDS, is in agreement with earlier findings (Rehm *et al.*, 1963; Sachs *et al.*, 1966; Shoemaker, 1980) and support our contention that Cl^- transport is in each case affected directly. Indirect effects, as discussed earlier, were established using either ouabain, K^+-free conditions, or $TPMP^+$. Our results are in excellent agreement with the recognized linear relation between the rate of acid secretion and the rate of Cl^- transport reported for frog gastric mucosa and other preparations (Heinz and Durbin, 1957; Forte, 1969; Kuo and Shanbour, 1979).

It is well established that no direct coupling exists between the transport of H^+ and that of Cl^- in gastric mucosa, since the former is transported via the (H^+, K^+) ATPase. The origin of the correlation between them must therefore have a different explanation. A Na^+–Cl^- cotransporter located on the serosal membrane cannot by itself account for the observed inhibition of J_{H^+}. Postulating an additional Cl^-/HCO_3^- exchange mechanism (Rehm, 1967) does not necessarily help, since the inhibition of H^+ transport observed under Na^+-free conditions, where inhibition of the former mechanism alone would be predicted, is hard to explain. A well-documented fact is the constant 1:1 ratio of H^+ transported (to the mucosal bathing solution) to HCO_3^- released (to the serosal side of the tissue) (Teorell, 1951). Obviously, inhibition of the latter should result in inhibition of the former, perhaps by raising the intracellular pH, i.e., reducing the concentration of a substrate of the (H^+, K^+) ATPase. In the light of this assumption we suggest the transport model in Fig. 6 as most economical. Here the self-same mechanism both cotransports Na^+–Cl^- from the serosal bathing solution to the cell and facilitates the coexit of Na^+–HCO_3^- from the cell to the serosal solution. No fixed stoichiometry is postulated between Cl^- and HCO_3^- since this quantity

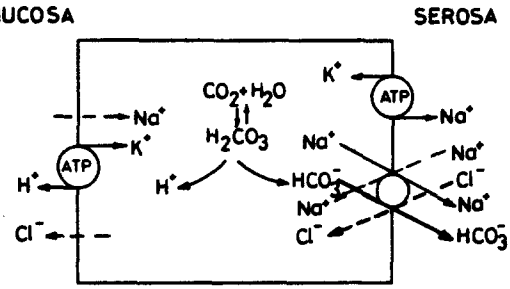

Fig. 6. A model for ion transport processes occurring in the secreting cells of gastric mucosa. (1) A (Na^+, K^+) ATPase located on the serosal membrane of the secreting cell drives electrogenic Na^+ transport. (2) Electroneutral H^+ transport (in exchange for K^+) across the mucosal membrane takes place via a (H^+, K^+) ATPase. (3) Na^+–Cl^- cotransport facilitates Cl^- entry from the serosal side to the cell interior. It is proposed that the same carrier serves as a Na^+–HCO_3^- cotransporter, giving rise to HCO_3^- exit from the cell to the serosal side of the tissue. (4) Cl^- exit from the cell to the mucosal bathing solution is postulated to be a passive process.

was shown to be variable (the Cl^-/HCO_3^- ratio is 1:1 under open-circuit conditions, while under short-circuit conditions it increases) (Forte and Machen, 1975). In the light of this model, the inhibition of proton transport may only be apparent and would depend on the relative permeabilities of the mucosal and serosal membranes of the secreting cells to HCO_3^-. If both membranes were impermeable, and there were no leaks through the transporting mechanism, a complete and real inhibition of the H^+ pump would be expected. On the other hand, a leak pathway in the mucosal membrane would result in an apparent inhibition of the net process, while the H^+ pump would be working under "level flow" conditions. Incomplete abolition of net proton transport would involve a considerable leak for HCO_3^- across the serosal membrane, either passive or through the proposed mechanism. The latter argument may also account for the variability in the extent of inhibition of J_{H^+} under similar experimental conditions in different gastric mucosal preparations (e.g., K^+-free conditions).

It should be noted that our model does not rule out the possibility that an additional mechanism for Cl^- transport located on the mucosal membrane exists, as was recently postulated in "stimulation-associated" membrane vesicles (isolated from piglet gastric mucosa stimulated by histamine) (Wolosin and Forte, 1981).

We still lack the most crucial experimental evidence, namely the profile of the electrochemical potential gradient of Cl^- across the gastric mucosa, in order to test the predictions of our model properly. This test would involve

measurements of ionic activities as well as electrical potentials within the parietal cells. An important point which merits further investigation is the correlation between the Na^+ transport and other transport processes. It is significant that a reduction in $J_{Na^+}^{net}$ was detected in tissues exposed to DIDS, $TPMP^+$, and Cl^--free conditions.

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