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Effect of current direction and K^+ on polarization of the frog gastric mucosa proton pump

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Abstract

When current was sent from serosa (S) to mucosa (M) across the frog stomach, there was a polarization (POL) of the open circuit potential (OCPD). POL was not affected by NaCl-free solutions, but was decreased by inhibitors of the H^+ pump. In present experiments, current was sent to clamp the PD (VC) across the mucosa in steps of 20 mV up to 100 mV below the control OCPD, that is, current was sent from M to S. All experiments were performed in NaCl-free solutions. The POL was expressed as a % of the difference between the VC PD and the control OCPD. In 4 mM K^+ control solutions, the POL was 11.8%; with 10^{-3} M omeprazole (H^+/K^+ pump inhibitor), 1.1; with 10^{-5} M SCH 28080 (H^+/K^+ pump inhibitor), 3.6; with 10^{-3} M famotidine (H_2 blocker), 1.6; and with 10^{-2} M SCN^-, 25.4 (inhibition of H^+ sec, but not of the pump); in 79 mM K^+ control solutions, 26.2; with 10^{-3} M omeprazole, 4.2; with 10^{-5} M SCH 28080, 15.9; with 10^{-3} M famotidine, 5.6; and with 10^{-2} M SCN^-, 29.9. POL was higher in high K^+ than in low K^+ solutions contrary to what was observed in previous experiments with current sent from S to M. Results are explained on the basis of an electrogenic H^+/K^+-ATPase pump which includes a H^+ channel, permeable to K^+. With high K^+ solutions, K^+ is driven through the H^+ channel onto the antiporter (ATPase) when current is sent from M to S, resulting in a greater POL of the pump.

Keywords: Membrane potential; Voltage-clamp technique; Thiocyanate; Famotidine; Omeprazole; SCH 28080; (*R. pipiens*); (Gastric mucosa)

1. Introduction

There are two schools of thought about the electrical nature of the hydrogen ion transport mechanism in the gastric mucosa. On the basis of data gathered from experiments on vesicles formed from the apical membrane of the oxyntic cells, some have concluded that the mechanism is electroneutral [1–4]. On the basis of data gathered from experiments on the intact mucosa, others have concluded that the mechanism is electrogenic [5–8]. The lack of electrogenicity in the vesicles may be a result of their preparation as stated by Forte et al. [4].

In experiments in which current was sent across the frog gastric mucosa, there was a polarization of the open circuit voltage which was shown to be independent of the capacitance of the tissue [9,10]. Recently, we have induced polarization by the method of voltage clamping [11–13]. We showed by such experiments that the observed polarization was principally due to polarization of the proton pump and could not be explained by the redistribution of ions, that is, we presented further support for the notion that the pump is electrogenic. The reasons for this conclusion were: (1) polarization could be achieved in high K^+/NaCl-free solutions [11–13]; (2) polarization was markedly reduced by inhibitors of the H^+/K^+-ATPase [12,13]; (3) polarization was not affected by SCN^- [13], an inhibitor of H^+ secretion which does not directly act on the pump [14–16].

In the experiments cited above [11–13], voltage clamping was achieved by sending current from serosa to mucosa. In the present paper we will show that the voltage clamp polarization is asymmetrical, i.e., it depends on the direction of the external current. While the polarization in NaCl-free solutions was greater in 4 mM than in 79 mM K^+ when current was sent from serosa to mucosa, present experiments will show that the opposite is true when current is sent from mucosa to serosa. Not only is the polarization greater in 79 mM than in 4 mM K^+ but the effect of inhibitors of the proton pump on the polarization

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is diminished in high K^+ when current is sent from mucosa to serosa.

2. Methods

Experiments were performed on fundi of stomachs of *Rana pipiens* by an in vitro method in which the stomachs were mounted between a pair of cylindrical chambers [17]. All experiments began with standard Cl^- solutions on both sides of the mucosa to check the viability of the mucosa. The Cl^- nutrient (serosal) solution contained (in mM): Na^+, 102; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 81; SO_4^{2-}, 0.8; HCO_3^-, 25; phosphate, 1; and glucose, 10; and the Cl^- secretory (mucosal) solution which is hypertonic [18] contained Na^+, 156; K^+, 4; and Cl^-, 160. For increases to 79 mM K^+ concentrations, K^+ replaced Na^+ and, for Na^+-free solutions, choline replaced Na^+. For Cl^--free solutions, SO_4^{2-} replaced Cl^- and sucrose was added to make up any osmotic deficit. Famotidine, omeprazole, Sch 28080 or SCN^- were added to the nutrient (serosal) solution to a concentration of 10^{-3} M, 10^{-3} M, 10^{-5} M and 10^{-2} M respectively to reduce the H^+ secretion.

The H^+ secretory rate before addition of the inhibitors, the transmucosal resistance and the transmucosal potential difference (PD) were measured. Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The PD is considered positive when the nutrient side is positive relative to the secretory side of the stomach. The resistance was determined as the change in PD per unit of applied current. Current (20 μA per 1.3 cm^2 of tissue area) was applied for 1 or 2 s, first in one direction and 2 or 3 s later, in the other direction. For voltage clamping, the voltage was clamped in steps of 20 mV up to 80 or 100 mV below the open circuit PD. During the voltage clamp period, the current was interrupted periodically for about 2 s in order to obtain the open-circuit voltage. The H^+ secretory rate was determined by the pH stat method of Heinz and Durbin [6]. The pH of the secretory solution was generally maintained between 4.7 and 5.0 and the pH of the nutrient solution was about 7.2–7.3. Both sides of the gastric mucosa were gassed with 95% O_2/5% CO_2 throughout these experiments and 0.1 mM histamine in the nutrient solution was used to stimulate secretion. Histamine was not present when omeprazole, famotidine, SCH 28080 or SCN^- were added to the solution. Linear regression analysis (Indicator Variables and a Full Model Reduced Model Test [19]) was used for statistical analysis of the regression lines. For other purposes, Student's *t*-test with paired observations was used.

3. Results

Although we have shown that polarization of the frog gastric mucosa was mostly due to polarization of the proton pump and not to ion redistribution of Na^+ or Cl^-, all experiments presented in this paper will be in NaCl-free solutions to exclude the ion redistribution of Na^+ or Cl^- or the Na^+/K^+-ATPase [20] as possible contributors to the observed polarization [11–13]. Data will be presented such that the first 4 figures will show results from representative experiments. The bulk of the experiments will be presented in tabular form and in Figs. 5 and 6.

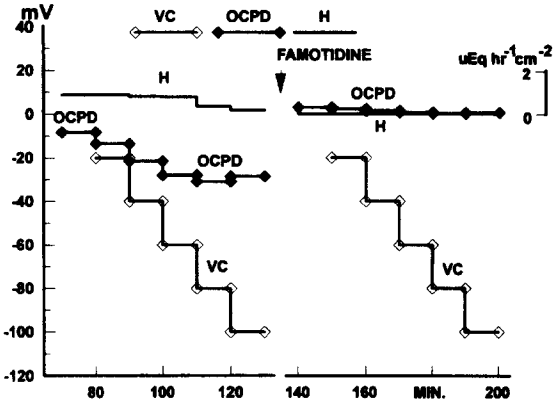

Fig. 1. Voltage-clamp potential (VC), open circuit PD (OCPD) and H^+ secretion rate (H) are plotted versus time with NaCl-free/4 mM K^+ solns, left panel. NaCl-free/4 mM K^+ solns plus famotidine (10^{-3} M), right panel (one expt.). Voltage clamp was obtained manually and its plot is factual. OCPD was continuously plotted during the pre-clamp period. During VC, OCPD was obtained by opening the circuit for about 2 s, every 1–2 min. Values for OCPD and H are average values for the 10-min periods (< 5% dev. from mean). Values of OCPD were not recorded during the first 30–60 s of VC (see text).

3.1. Effect of negative voltage clamping (VC) on open circuit PD (OCPD) in Na^+-free / Cl^--free / 4 mM K^+ with and without 10^{-3} M famotidine

Fig. 1 shows data from one representative experiment in which the voltage was clamped manually in steps of 20 mV from −20 to −100 mV. The figure presents the VC PD, the OCPD and the H^+ secretion (H) versus time. The OCPD was recorded continuously before voltage clamping. Although the lines representing OCPD are plotted as a continuous line, they were recorded every 1–2 min for the OCPD. During voltage clamping, the OCPD was recorded by releasing the clamp for 1–2 s. The OCPD values were practically constant during the 10-min periods. The values used were the mean values for the period. No attempt was made to record the first 30–60 s for OCPD, since the early effects were well studied and documented previously [9,10].

The left panel shows data in the absence of famotidine. The OCPD decreased from about −8 mV before voltage clamp to a minimum of about −30 mV when the voltage was clamped at −80 or −100 mV. A VC of about −72 mV below the pre-clamp level (i.e., voltage clamp of −80 mV minus the pre-clamp OCPD of −8 mV) decreased the OCPD by about 22 mV.

In this particular experiment, the H^+ secretion de-

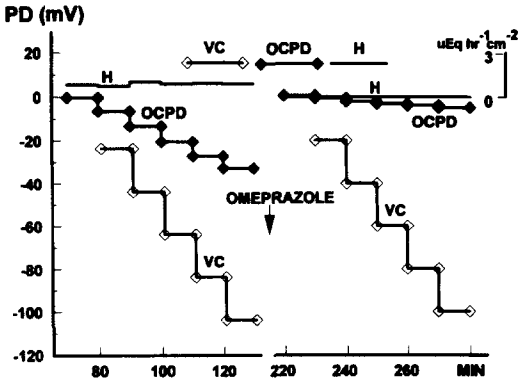

Fig. 2. Voltage clamp potential (VC), open circuit PD (OCPD) and H^+ secretion rate (H) are plotted versus time with NaCl-free/79 mM K^+ solns, left panel. NaCl-free/79 mM K^+ solns plus omeprazole (10^{-3} M), right panel (one expt.). See legend for Fig. 1.

creased during voltage clamping. The post-clamp values of H^+ secretion were significantly ($P < 0.01$) lower than pre-clamp values in 5 experiments (pre-famotidine) with means of 2.2 (S.E. ± 0.3) and 0.3 (S.E. ± 0.3) μEq h^{-1} cm^{-2}, respectively.

Voltage clamp did not affect the transepithelial resistance which, in 12 experiments, was 195 (S.E. ± 14) before and 203 (S.E. ± 27) ohm cm^2 after voltage clamp.

The right panel shows data in the presence of famotidine. The OCPD decreased minimally from 3 mV before voltage clamp to about 1 mV when the voltage was clamped at −100 mV. A VC of about −103 mV below the pre-clamp level decreased the OCPD by about 2 mV. There was no H^+ secretion in the presence of famotidine.

The transepithelial resistance decreased significantly during voltage clamp ($P < 0.05$) in Na^+-free/Cl^--free/4 mM K^+ with 10^{-3} M famotidine, in 5 experiments, from 263 (S.E. ± 58) before to 137 (S.E. ± 38) ohm cm^2 after voltage clamp.

3.2. Effect of negative voltage clamping (VC) on open circuit PD (OCPD) in Na^+-free, Cl^--free and high K^+ (79 mM), with and without 10^{-3} M omeprazole

Fig. 2 shows data from a representative experiment in which the voltage was clamped in steps of 20 mV from −20 to −100 mV. (See paragraph above referring to Fig. 1.)

The left panel shows data in the absence of omeprazole. The OCPD decreased from about 0 mV before voltage clamp to a minimum of about −33 mV when the voltage was clamped at −100 mV. A VC of −100 mV below the pre-clamp level decreased the OCPD by 33 mV.

In this particular experiment, the H^+ secretion did not change significantly during voltage clamping.

The post-clamp values of H^+ secretion were not significantly different from the pre-clamp values; in 11 experiments the means were 1.0 (S.E. ± 0.3) and 1.3 (S.E. ± 0.2) μEq h^{-1} cm^{-2}, respectively. The transepithelial resistance increased during voltage clamp ($P < 0.01$), in 13 experiments, from 114 (S.E. ± 9) before to 212 (S.E. ± 19) ohm cm^2 after voltage clamp.

The right panel shows data in the presence of omeprazole. The OCPD decreased minimally from 2 mV before voltage clamp to about −7 mV when the voltage was clamped at −100 mV. A VC of about −102 mV below the pre-clamp level decreased the OCPD by about 9 mV. There was no H^+ secretion in the presence of omeprazole.

Voltage clamp did not affect the transepithelial resistance which, in 5 experiments in Na^+-free, Cl^--free and high K^+ (79 mM), with 10^{-3} M omeprazole, was 195 (S.E. ± 31) before and 161 (S.E. ± 25) ohm cm^2 after voltage clamp.

3.3. Effect of negative voltage clamping (VC) on open circuit PD (OCPD) in the presence of 10^{-2} M SCN^-, in Na^+-free, Cl^--free and in low or high K^+ (4 or 79 mM)

Fig. 3 shows data from a representative experiment in which the voltage was clamped in steps of 20 mV from −20 to −100 mV. (See paragraph above referring to Fig. 1.)

The left panel shows data in 4 mM K^+. The OCPD decreased from about 7 mV before voltage clamp to a minimum of about −17 mV when the voltage was clamped at −100 mV. A VC of about −107 mV below the pre-clamp level decreased the OCPD by about 24 mV.

There was no H^+ secretion in the presence of SCN^-.

Voltage clamp did not affect the transepithelial resistance which, in 6 experiments in the presence of 10^{-2} M SCN^-, in NaCl-free/4 mM K^+, was 312 (S.E. ± 73) before and 313 (S.E. ± 46) ohm cm^2 after voltage clamp.

The right panel shows data in 79 mM K^+. The OCPD decreased from 5 mV before voltage clamp to about −32 mV when the voltage was clamped at −100 mV. A VC of about −105 mV below the pre-clamp level decreased the OCPD by about 37 mV.

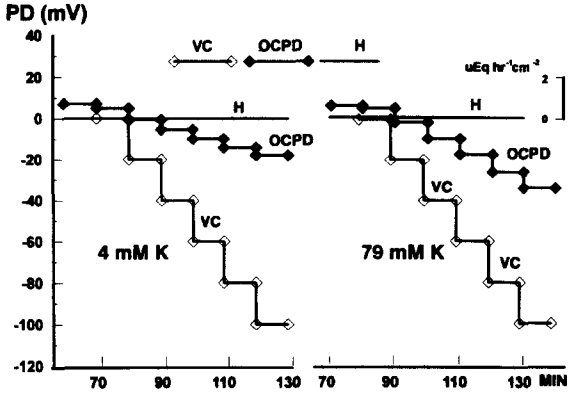

Fig. 3. Voltage clamp potential (VC), open circuit PD (OCPD) and H^+ secretion rate (H) are plotted versus time with NaCl-free/4 mM K^+ solns, plus SCN^- (10^{-2} M), left panel. NaCl-free/79 mM K^+ solns plus SCN^- (10^{-2} M), right panel (one expt.). See legend for Fig. 1.

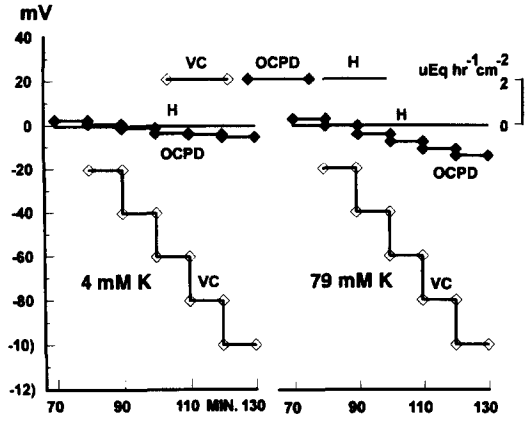

Fig. 4. Voltage clamp potential (VC), open circuit PD (OCPD) and H^+ secretion rate (H) are plotted versus time with NaCl-free/4 mM K^+ solns, plus SCH 28080 (10^{-5} M), left panel. NaCl-free/79 mM K^+ solns plus SCH 28080 (10^{-5} M), right panel (one expt.). See legend for Fig. 1.

The transepithelial resistance increased during voltage clamp ($P < 0.05$) which, in 5 experiments, in the presence of 10^{-2} M SCN^-, in NaCl-free/79 mM K^+, was 154 (S.E. ± 26) before and 216 (S.E. ± 40) ohm cm^2 after voltage clamp.

3.4. Effect of negative voltage clamping (VC) on open circuit PD (OCPD) in the presence of 10^{-5} M SCH 28080, in Na^+-free, Cl^--free and in low or high K^+ (4 or 79 mM)

Fig. 4 shows data from a representative experiment in which the voltage was clamped in steps of 20 mV from −20 to −100 mV. Please refer to paragraph above on Fig. 1.

The left panel shows data in 4 mM K^+. The OCPD decreased from about 3 mV before voltage clamp to a minimum of about −5 mV when the voltage was clamped at −100 mV. A VC of about −103 mV below the pre-clamp level decreased the OCPD by about 8 mV.

There was no H^+ secretion in the presence of SCH 28080.

Voltage clamp did not affect the transepithelial resistance which, in 6 experiments in the presence of 10^{-5} M SCH 28080, in NaCl-free/4 mM K^+, was 243 (S.E. ± 37) before and 221 (S.E. ± 38) ohm cm^2 after voltage clamp.

The right panel shows data in 79 mM K^+. The OCPD decreased from 3 mV before voltage clamp to about −14 mV when the voltage was clamped at −100 mV. A VC of about −103 mV below the pre-clamp level decreased the OCPD by about 17 mV.

Voltage clamp did not affect the transepithelial resistance which, in 6 experiments in the presence of 10^{-5} M SCH 28080, in NaCl-free/79 mM K^+, was 241 (S.E. ± 34) before and 222 (S.E. ± 41) ohm cm^2 after voltage clamp.

3.5. Summary of the data

Graphic representations of the experiments presented in Table 1 are shown in Figs. 5 and 6. In Fig. 5 the solutions were NaCl-free/4 mM K^+ whereas in Fig. 6 the solutions were NaCl-free/79 mM K^+. In both figures, the decrease in OCPD (polarization) induced by voltage clamping (OCPD(vc) − OCPD) is plotted versus the difference between the voltage clamp PD and the control OCPD (VCPD − OCPD). The regression parameters of the decrease in OCPD versus the decrease in transepithelial PD by voltage clamping are presented in Table 1. The slopes of the lines represent the polarization of the PD as a decrease in the

Table 1
Effect of voltage clamping on open circuit potential (regression line parameters)

	(*n*)	Slope	Intercept	*r*
Na^+-free/Cl^--free/4 mM K^+	(16)	11.8	−3.70	0.32
+ omeprazole	(6)	1.1 [a,f]	−0.89	0.32
+ famotidine	(5)	1.6 [a,f]	−0.46	0.19
+ SCH 28080	(6)	3.6 [a,f]	−1.35	0.32
+ SCN^-	(7)	25.4 [a,c]	0.94	0.39
Na^+-free/Cl^--free/79 mM K^+	(15)	26.2 [a]	−0.34	0.56
+ omeprazole	(5)	4.2 [b,f]	−1.14	0.46
+ famotidine	(5)	5.8 [b,f]	−0.23	0.38
+ SCH 28080	(6)	15.9 [b,d,f]	−0.57	0.43
+ SCN^-	(6)	29.9 [e]	0.99	0.65

Current sent from mucosa to serosa.
n = number of experiments; (significant differences which follow, refer to slopes) a, sign. diff. from Na^+-free/Cl^--free/4 mM K^+ (without inhib.) ($P < 0.01$); b, sign. diff. from Na^+-free/Cl^--free/79 mM K^+ (without inhib.) ($P < 0.01$); c, sign. diff. from other inhibitors in Na^+-free/Cl^--free/4 mM K^+ solns. ($P < 0.01$); d, sign. diff. from other inhibitors in Na^+-free/Cl^--free/79 mM K^+ solns. ($P < 0.01$); e, sign. diff. from other inhibitors in Na^+-free/Cl^--free/79 mM K^+ solns. ($P < 0.01$); f, sign. diff. from the same inhibitor in Na^+-free/Cl^--free/4 mM K^+ solns. versus in Na^+-free/Cl^--free/79 mM K^+ solns. ($P < 0.01$).

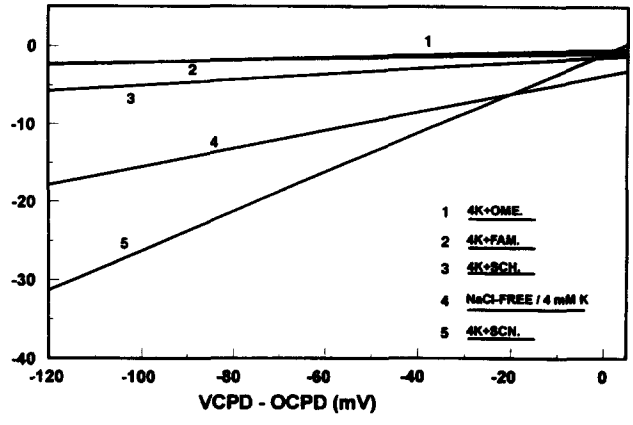

Fig. 5. Effect of voltage clamp on open circuit PD in NaCl-free/4 mM K^+ solns. Increase in OCPD during voltage clamp ($OCPD_{VC}$ − OCPD) plotted vs. increase in voltage by voltage clamp over the pre-clamp PD (VCPD − OCPD). The slope represents the increment in OCPD (polarization) induced by voltage clamp. Values of regression parameters resented in Table 1.

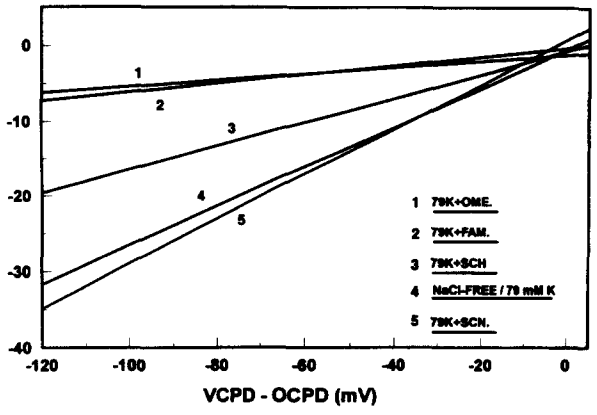

Fig. 6. Effect of voltage clamp on open circuit PD in NaCl-free/79 mM K^+ solns. See Fig. 5 for legend.

OCPD per 100 mV decrease in PD by voltage clamp. We first note that, in the absence of inhibitors, the magnitude of the polarization was greater in 79 than in 4 mM K^+ ($P < 0.01$). In the presence of inhibitors of the pump, the magnitude of the polarization was also greater in 79 than in 4 mM K^+ ($P < 0.01$). On the other hand, with SCN^-, which inhibits H^+ secretion but does not inhibit the pump, the polarization was not significantly different in 79 from 4 mM K^+.

In 4 mM K^+, the polarization was greater in the absence of inhibitors than in the presence of omeprazole, famotidine or SCH 28080 ($P < 0.01$). Moreover, there was no significant difference in polarization among the three inhibitors. In the case of SCN^-, its presence increased the polarization over control ($P < 0.01$).

In 79 mM K^+, the polarization followed the same pattern as in 4 mM K^+ the inhibitors also decreasing the polarization. However, with 79 mM K^+, omeprazole and famotidine inhibited the polarization to a greater extent than SCH 28080. In 79 mM K^+, as in 4 mM K^+, the polarization with SCN^- was the same as in control solutions. Also with both concentrations of K^+, the polarization with SCN^- was greater than the polarization with any of the three inhibitors of the pump.

4. Discussion

There is incontrovertible evidence for the existence of a neutral H^+/K^+-ATPase pump from the work in vesicles from the gastric mucosa secretory membrane [1–4]. From work on intact tissue, on the other hand, there is ample evidence that H^+ secretion is electrogenic [5–8]. In recent publications [11–13], we have provided further evidence of the electrogenicity of the proton pump in the intact stomach and presented a phenomenological construct, in which the two mechanisms were put together. In the most recent publications [12,13], we showed that the polarization induced by producing a voltage clamp across the gastric mucosa was markedly reduced by H_2-blockers [21,22], famotidine [12] and cimetidine [13], and by omeprazole [12], which in the active form is a sulfonamide that inhibits the H^+/K^+-ATPase by reacting with its sulfhydryl groups [23,24] and by SCH 28080 [13], a reversible competitive inhibitor of the K^+-induced hydrolysis of the H^+/K^+-ATPase [25].

While the inhibitors mentioned above interfered with the voltage clamp polarization, SCN^-, a known inhibitor of H^+ secretion [14–16,26–30], which does not affect the pump but induces back diffusion of HSCN, did not interfere with the polarization [13]. These findings support the concept that, in order to inhibit the voltage clamp polarization, one must inhibit the proton pump per se. This notion was given additional support by the finding that there was no voltage clamp polarization when K^+ was removed from the bathing solutions [12].

With control solutions, the transepithelial potential is such that the serosal side is positive to the mucosal side. In the voltage clamp experiments quoted above, the serosal side was made more positive by sending current from serosa to mucosa. Therefore, we will refer to these experiments as positive voltage clamp (positive VC) experiments.

Results reported in this paper were obtained by sending current from mucosa to serosa and we will refer to these as negative voltage clamp (negative VC) experiments, that is, in present experiments the serosa was made negative to the mucosa. A comparison of the data in positive versus negative VC is as follows:

(1) As in positive VC, in negative VC there was a polarization in the direction of the VC. This polarization could not be attributed to ion redistribution of Cl^- or Na^+ across the plasma membranes since all experiments reported here were performed in NaCl-free solutions. The absence of Na^+, also ruled out the Na^+/K^+-ATPase as a contributor to the polarization.

(2) Similarly, inhibitors of the proton pump (famotidine, omeprazole and SCH 28080), inhibited the negative VC

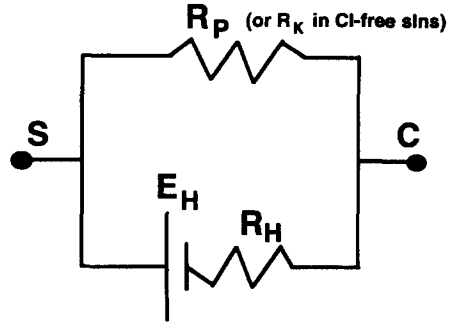

Fig. 7. Equivalent circuit across the secretory membrane of gastric mucosa. E_H is the proton pump emf and R_H the pump resistance. R_P is the resistance of the parallel pathway. In Cl^--containing solutions, the parallel resistance represents the passive K^+ and Cl^- pathways while, in Cl^--free solutions, the parallel pathway is the K^+ pathway.

polarization, as they did in the positive VC experiments, particularly in 4 mM K^+ solutions, while SCN^- did not interfere with the polarization.

(3) A major difference between positive and negative VC experiments is the effect on polarization of the K^+ concentration. With positive VC, the polarization in 4 mM K^+ (35 mV%) was greater than in 79 mM K^+ (19 mV%) [12,13], while, with negative VC, the polarization in 79 mM K^+ (26.2 mV%) was greater than in 4 mM K^+ (11.8 mV%) (see Table 1). A working model that explained the effect of K^+ on the polarization in positive VC experiments is shown as an equivalent circuit located in the secretory membrane and presented in Fig. 7. The emf is the proton pump emf and the parallel resistance represents the passive K^+ pathway. The PD across this circuit is given by

$$V_{CS} = E_H R_P/(R_P + R_H)$$

where V_{CS} is the PD across the secretory membrane, E_H is the pump emf, R_P is the resistance of the parallel pathway and R_H the pump resistance. In Cl^--containing solutions, the parallel resistance represents the passive K^+ and Cl^- pathways while, in Cl^--free solutions, the parallel pathway is the K^+ pathway.

We note that the gastric mucosa is a tight epithelium, i.e., the resistance via the lumina and tubular cells is low and that via the surface cells and transintercellular pathways is high [31]. For example, the lumina are open during secretion even though exposed to a hypotonic secretory solution (29 mM Cl^-). Replacement of 25 mM Cl^- with 25 mM SCN^- markedly increased the resistance from about 200 to about 1000 ohm cm^2. Addition of mannitol in increasing molarity brought the resistance down to control levels. Also, addition of 2 mM Ba^{2+} to the nutrient fluid increased the resistance from about 200 to about 800 ohm cm^2. These data show that under normal circumstances the resistance across the lumina and tubular cells is low and the resistance across the surface cells and the paracellular (transintercellular) pathways is normally high (see Ref. [31] for further experiments and details). Therefore, it follows that most of the changes observed in the present experiments are the result of changes in the cellular pathways located in the secretory (luminal) and nutrient (serosal) membranes.

If the effects on the trans-epithelial resistance and PD are mostly due to effects on the secretory membrane, changes in the trans-epithelial PD should be a reflection of the changes in V_{CS}. Polarization of the trans-epithelial PD as a result of the polarization of the pump emf (E_H) will depend also on the relative values of the resistances at the time of the voltage clamp. For example, in Na^+-free/Cl^--free/79 mM K^+ the parallel resistance, R_K, should be lower than in Na^+-free/Cl^--free/4 mM K^+ solutions and an equal change in polarization of E_H should result in a smaller change in polarization of V_{CS} in 79 mM K^+. Therefore, in 79 mM K^+ one should expect a smaller polarization of the trans-epithelial PD than in 4 mM K^+, which was the finding with positive VC. On the other hand, the finding was the opposite with negative VC.

The passive K^+ pathway cannot account for the difference in polarization behavior between high and low K^+ and between positive and negative VC. These differences in behavior may be explained on the basis of the model previously published [12,13] which is a modification of the model reported earlier by Forte et al. [4]. Our model represents the proton pump in the secretory (luminal) membrane with the parallel K^+ and Cl^- pathways. The proton pump is made up of the H^+/K^+-ATPase with K^+ and H^+ channels in series, which permit the flow of current through the pump and render the pump electrogenic [12,13]. In the model by Forte et al. the transmembrane K^+ pathway and the K^+ pump pathway are the same while in our modified model the two K^+ pathways are independent.

Data in 4 and in 79 mM K^+, in negative VC experiments, may be explained if the H^+ channel were also permeable to K^+ as long as the affinity for H^+ is much higher than for K^+. It is with high K^+ in the bathing solutions and current sent from mucosa to serosa that K^+ will penetrate the H^+ channel and access the binding site of the H^+/K^+-ATPase antiport. Therefore, the high polarization observed in experiments with 79 mM K^+ and negative VC may be explained by the penetration of K^+ from the lumen side inducing an increase in E_H (see the circuit diagram – Fig. 7). This increase in E_H overcomes the shunting effect of the parallel pathway R_K.

The modified model described above is consistent with the fact that the effect of the pump inhibitors is significantly reduced in 79 mM K^+ in negative VC (see Table 1) and not in positive VC [12,13]. It should be noted that SCH 28080 acts by competing with K^+ as it reacts with the H^+/K^+-ATPase antiport [25] and SCH 28080 did not inhibit the negative VC polarization in the presence of 79 mM K^+ (see Table 1), that is, it looks like external current from mucosa to serosa, with 79 mM K^+ displacing SCH 28080 from H^+/K^+-ATPase binding site. We should note that this working model has explained data in previous studies [12,13,30].

In summary, further evidence is presented for the electrogenicity of the gastric mucosa proton pump, the main constituent of which is the H^+/K^+-ATPase antiport, plus K^+ and H^+ channels in series. The latter channels render the pump electrogenic. The H^+ channel, located between the enzyme and the lumen, appears to have some permeability to K^+.

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