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Electrogenicity of the frog gastric mucosa proton pump based on polarization responses in the presence of H^+-secretion inhibitors

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Recently, we have shown that polarization of an electrogenic H^+/K^+-ATPase pump located in the secretory (luminal) membrane of the frog gastric mucosa is the major factor contributing to the increase in open circuit potential difference (OCPD) induced by voltage clamping. While this transmucosal polarization was not affected by removal of Cl^- and Na^+ and minimally affected by increasing the K^+ concentration to 79 mM in both nutrient and secretory solutions, it was markedly reduced by 10^{-3} M famotidine (beta blocker) or 10^{-4} M omeprazole (H^+/K^+-ATPase inhibitor) in the nutrient solution. In present experiments, the effects of three other inhibitors of H^+ secretion were examined, namely, cimetidine (beta blocker), SCH 28080 (H^+/K^+-ATPase inhibitor) and SCN^- (non-specific inhibitor). While cimetidine and SCH 28080 markedly reduced the polarization induced by voltage clamp, SCN^- affected the polarization to a lesser extent. These data further support the electrogenicity of the frog gastric mucosa proton pump and the lack of a direct effect of SCN^- on the pump.

Introduction

One of the problems in the understanding of the mechanism of HCl acid secretion in the gastric mucosa is whether H^+ is secreted via an electrogenic or a neutral pump. The discovery of the gastric H^+/K^+-ATPase [1] and work done on vesicles extracted from the luminal membrane of the oxyntic cells [2–4] have provided strong support for a neutral H^+/K^+ pump. On the other hand, data obtained from experiments performed on the intact frog gastric mucosa in Cl^--free solutions are difficult to explain with a neutral model and are readily explained with an electrogenic H^+ pump model [5–8]. In this regard, we consider the effect of polarization.

When current is sent across the frog gastric mucosa, there is a polarization of the open circuit voltage which has been shown to be independent of the capacitance of the tissue [9,10]. Moreover, a non-linear resistance was ruled out as the cause of the polarization [9,10]. The observed polarization could be due to ion redistribution with change in diffusion EMFs and/or a change in the EMF of the active pumps during current sending. We have shown that polarization still is present despite Na^+-free, Cl^--free and high K^+ (79 mM) bathing solutions [11,12]. These data suggested that a mechanism other than the K^+ and Cl^- diffusion EMFs or the electrogenic Na^+/K^+-ATPase must be responsible, at least in part, for the polarization effect. Recently [12], we have shown that the polarization was inhibited by two inhibitors of H^+ secretion, the H_2-blocker famotidine and by omeprazole, an inhibitor of the H^+/K^+-ATPase enzyme. Furthermore, voltage-clamp polarization did not occur in K^+-free solutions [12]. These data showed that the proton pump, in which K^+ is involved, is mostly responsible for the voltage-clamp polarization and, thus, support the concept that the proton pump is electrogenic.

In present experiments, voltage clamping was performed in the presence and absence of three different type of inhibitors of H^+ secretion: Cimetidine, an H_2 blocker [13,14]; SCH 28080, a reversible inhibitor of the H^+/K^+-ATPase enzyme [15] and SCN^-, an in-

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hibitor of H^+ secretion that does not affect the pump but causes a dissipation of the proton gradient by back diffusion of HSCN [16–18]

We will show that polarization is markedly decreased or abolished by cimetidine and SCH 28080, but only moderately affected by SCN^-. These experiments further support the electrogenicity of the proton pump and that SCN^- inhibits H^+ secretion without affecting the pump.

Materials and 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 [19]. All experiments began with standard Cl^- solutions on both sides 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 [20] contained Na^+, 156; K^+, 4 and Cl^-, 160. For increases in 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. Cimetidine, SCH 28080 or SCN^- were added to the nutrient (serosal) solution to a concentration of 10^{-3} M, $5 \cdot 10^{-5}$ M and 10^{-2} M, respectively.

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 80–120 mV above and 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 cimetidine, SCH 28080 or SCN^- were added. Linear regression analysis (Indicator Variables and a Full Model Reduced Model Test [21]) was used for statistical analysis of the regression lines. For other purposes, Student's *t*-test with paired observations was used.

Results

Data will be presented such that Figs. 1–3 will show results from one experiment each in regular, in Na^+-free/Cl^--free/4 mM K^+ and in Na^+-free/Cl^--free/79 mM K^+ solutions. NaCl-free and NaCl/high K^+ solutions are used to eliminate the effect of ion-diffusion gradients during polarization with each one of the inhibitors. One of the three inhibitors used will be added to the nutrient solution in each of the experiments presented. Fig. 4 and Table I will present the summary of all the data (12 experimental groups).

Effect of voltage clamping (VC) on open circuit PD (OCPD) with control solutions and 10^{-3} M cimetidine

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, nutrient side positive. The figure presents the VC, the current (I) necessary to clamp the voltage and the OCPD versus time. The OCPD was recorded continuously before voltage clamping. Although the lines representing I and OCPD are plotted as a continuous line, they were recorded periodically, every 1–2 min for the OCPD and more frequently for I. During voltage clamping, the OCPD was recorded by releasing the clamp for 1–2 s. The I and 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 either current or OCPD,

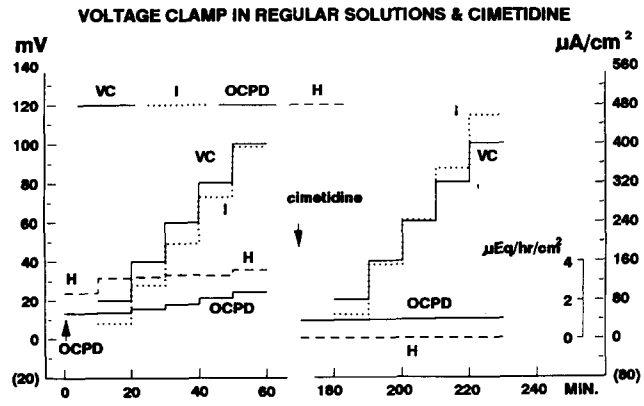

Fig. 1. Left panel: Voltage-clamp potential (VC), current (I), open circuit PD (OCPD) and H^+ secretion rate (H) are plotted versus time with regular solutions. Right panel: Regular solutions plus cimetidine (10^{-3} M; 1 experiment). Voltage clamp was obtained manually and its plot is factual. OCPD 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 I, OCPD and H are average values for the 10-min periods (< 5% deviation from mean). Values of I and OCPD were not recorded during the first 30–60 s of VC (see text).

since the early effects were well-studied and documented previously [9,10].

The left panel of Fig. 1 shows data obtained during the control period, in the absence of cimetidine. The OCPD increased from about 13 mV pre-clamping to a maximum of about 26 mV when the voltage was clamped at 100 mV. A VC of about 80 mV above the pre-clamp level increased the OCPD by about 13 mV.

In this particular experiment, the H^+ secretion increased slightly during voltage clamping. The mean value of H^+ secretion for seven experiments during the control period was (mean ± S.E.) 2.9 ± 0.4 μA/h per cm^2. The H^+ secretion increased to 3.4 ± 0.3 during voltage clamp, and returned to 2.8 ± 0.4 μA/h per cm^2 following voltage clamp. The increase during voltage clamp was not significant ($0.1 > P > 0.05$) but the secretion increased in six out of seven experiments.

Voltage clamp did not significantly affect the transepithelial resistance which, in seven experiments, was 102 ± 9 before and 98 ± 9 ohm cm^2 after voltage clamp (mean ± S.E.).

The right panel of Fig. 1 presents data in the same experiment after addition of cimetidine. In the presence of cimetidine, the OCPD did not appreciably change, that is, the H^+ secretion inhibitor abolished the voltage-clamp polarization.

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

In regular solutions (NaCl/4 mM K^+), addition of cimetidine increased the resistance from 120 ± 11 to 160 ± 13 ohm cm^2 (5 experiments). In Na^+-free/Cl^--free/4 mM K^+ solutions (5 experiments), cimetidine did not significantly ($P > 0.5$) change the resistance, that is, respectively, the values were 227 ± 61 and 190 ± 38 ohm cm^2 (mean ± S.E.). In Na^+-free/Cl^--free/79 mM K^+ solutions (5 experiments), cimetidine did not change ($P > 0.05$) the resistance with values of 206 ± 23 before and 207 ± 24 ohm cm^2 after addition of the inhibitor.

With cimetidine (same experiments as in previous paragraph), voltage clamp did not affect the resistance in NaCl/4 mM K^+ solutions, with values of 160 ± 22 before and 154 ± 26 ohm cm^2 after voltage clamp. No significant effect was found in Na^+-free Cl^--free/4 mM K^+ solutions, with values of 190 ± 30 before and 154 ± 11 ohm cm^2 after voltage clamp ($P > 0.2$). No significant change in resistance was found either in Na^+-free/Cl^--free/79 mM K^+ solutions, with values of 207 ± 24 before and 189 ± 28 ohm cm^2 after voltage clamp.

Effect of voltage clamping (VC) on open circuit PD (OCPD) in Na^+-free, Cl^--free and 4 mM K^+ solutions, without and with 10^{-2} M SCN^-

Fig. 2 shows data from a representative experiment in which the voltage was clamped in steps of 20 mV

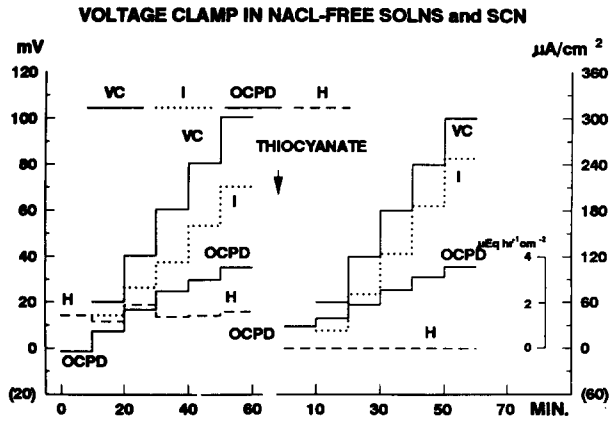

Fig. 2. Left panel: Voltage-clamp potential (VC), current (I), open circuit PD (OCPD) and H^+ secretion rate (H) are plotted versus time with NaCl-free/4 mM K^+ solutions. Right panel: NaCl-free/4 mM K^+ solutions plus SCN^- (10^{-2} M; 1 experiment). For more details, see legend for Fig. 1.

from 20 to 100 mV, nutrient side positive. Refer to paragraph above on Fig. 1. The left panel of Fig. 2 shows data obtained during the control period. As shown previously [2,22], removal of Na^+ and Cl^- from the solutions did not decrease the response of the OCPD, that is, the OCPD increased from about −2 mV during the pre-clamp period to a maximum of about 38 mV when the voltage was clamped at 100 mV. Thus, a VC of about 100 mV above the pre-clamp level increased the OCPD by about 40 mV. This polarization of 40 mV in Na^+-free/Cl^--free solutions was not smaller than the polarization of 13 mV observed in control solutions (Fig. 1).

The H^+ secretion did not significantly change during voltage clamp in this particular experiment or in 12 experiments, with values of 1.7 ± 0.2 and 1.6 ± 0.3 μA/h per cm^2 during control and voltage-clamp periods, respectively. The H^+ secretion decreased ($P < 0.01$) to 1.1 ± 0.3 μA/h per cm^2 following release of the voltage clamp.

Voltage clamp did not affect the transepithelial resistance which, in 12 experiments, was 195 ± 14 before and 192 ± 15 ohm cm^2 following voltage clamp.

The right panel of Fig. 2 presents data in the same experiment after addition of SCN^-. Contrary to what was observed with cimetidine, in the presence of SCN^-, the OCPD increased during voltage clamp from about 10 mV pre-clamp level to about 38 mV when VC was 100 mV. Thus, a VC of about 90 mV above the pre-clamp level increased the OCPD by about 28 mV.

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

In regular solutions (NaCl/4 mM K^+), addition of SCN^- increased the resistance from 125 ± 14 to 153 ± 14 ohm cm^2 (6 experiments) ($P < 0.05$). In Na^+-free/Cl^--free/4 mM K^+ solutions (6 experiments), SCN^- did not significantly ($P > 0.5$) change the resistance,

that is, respectively, the values were 321 ± 83 and 312 ± 73 ohm cm^2. In Na^+-free/Cl^--free/79 mM K^+ solutions (6 experiments), SCN^- did not change ($P > 0.8$) the resistance with values of 152 ± 25 before and 154 ± 26 ohm cm^2 after addition of the inhibitor.

With SCN^- (same experiments as in previous paragraph), voltage clamp decreased the resistance in NaCl/4 mM K^+ solutions, with values of 153 ± 14 before and 119 ± 11 ohm cm^2 after voltage clamp ($P < 0.05$). No effect was found in Na^+-free Cl^--free/4 mM K^+ solutions, with values of 312 ± 73 before and 275 ± 57 ohm cm^2 after voltage clamp ($P > 0.2$). No change in resistance was found either in Na^+-free/Cl^--free/79 mM K^+ solutions, with values of 154 ± 26 before and 157 ± 23 ohm cm^2 after voltage clamp.

Effect of voltage clamping (VC) on open circuit PD (OCPD) in Na^+-free / Cl^--free, 79 mM K^+ solutions, without and with $5 \cdot 10^{-5}$ M SCH 28080

Fig. 3 shows data from a representative experiment in which the voltage was clamped in steps of 20 mV from 20 to 100 mV, nutrient side positive. Refer to paragraph describing Fig. 1. The left panel of Fig. 3 shows data obtained during the control period. Previously, it was found that the increase in OCPD by VC was lower in 79 mM K^+ than in 4 mM K^+ in NaCl-free solutions. In this experiment the OCPD increased from about 2 mV during the pre-clamp period to a maximum of about 11 mV when the voltage was clamped at 100 mV. Here, a VC of about 100 mV above the pre-clamp level increased the OCPD by about 9 mV.

In this particular experiment, the H^+ secretion decreased slightly during voltage clamping. The mean value of H^+ secretion for 20 experiments during the control period was 1.8 ± 0.3 μA/h per cm^2. The H^+ secretion, measured in 15 experiments during the period of voltage clamp, decreased ($P < 0.01$) to 1.3 ± 0.3 μA/h per cm^2 during this period, with a further significant decrease ($P < 0.01$) to 0.8 ± 0.2 μA/h per cm^2 after release of the voltage clamp.

The transepithelial resistance, measured in 13 experiments, increased significantly ($P < 0.01$) from 114 ± 9 before to 163 ± 10 ohm cm^2 after voltage clamp.

The right panel of Fig. 3 presents data in the same experiment after addition of SCH 28080. In the presence of the H^+ inhibitor SCH 28080, the OCPD did not appreciably change, that is, the H^+ secretion inhibitor abolished the voltage-clamp polarization.

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

In regular solutions (NaCl/4 mM K^+), addition of SCH 28080 increased the resistance from 124 ± 18 to 209 ± 16 ohm cm^2 (6 experiments) ($P < 0.05$). In Na^+-free/Cl^--free/4 mM K^+ solutions (6 experiments), SCH 28080 did not significantly ($P > 0.5$) change the resistance, that is, respectively, the values were 262 ± 30 and 243 ± 37 ohm cm^2. In Na^+-free/Cl^--free/79 mM K^+ solutions (6 experiments), SCH 28080 did not change ($P > 0.05$) the resistance with values of 227 ± 26 before and 241 ± 34 ohm cm^2 after addition of the inhibitor.

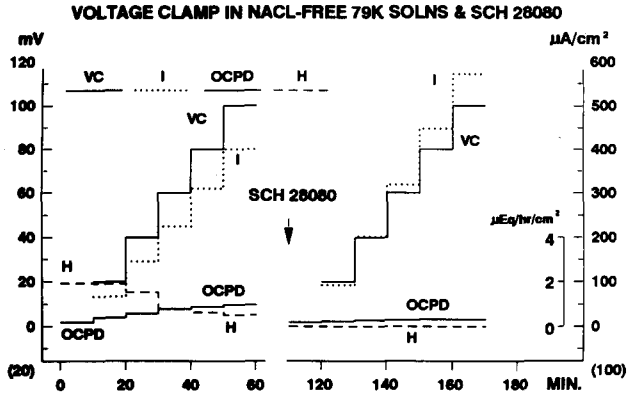

Fig. 3. Left panel: Voltage-clamp potential (VC), current (I), open circuit PD (OCPD) and H^+ secretion rate (H) are plotted versus time with NaCl-free/79 mM K^+ solutions. Right panel: NaCl-free/79 mM K^+ solutions plus SCH 28080 ($5 \cdot 10^{-5}$ M; 1 experiment). For more details, see legend for Fig. 1.

With SCH 28080 (same experiments as in previous paragraph), voltage clamp decreased the resistance in NaCl/4 mM K^+ solutions, with values of 209 ± 16) before and 181 (S.E. ± 16) ohm cm^2 after voltage clamp ($P < 0.05$). No effect was found in Na^+-free Cl^--free/4 mM K^+ solutions, with values of 243 ± 37 before and 225 ± 36 ohm cm^2 after voltage clamp ($P > 0.2$). No change in resistance was found either in Na^+-free/Cl^--free/79 mM K^+ solutions, with values of 241 ± 34 before and 205 ± 30 ohm cm^2 after voltage clamp.

A graphic representation of the experiments summarized in Table I is shown in Fig. 4. The increase 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 increase in OCPD (polarization) versus the increase in transepithelial PD by voltage clamping (OCPD(vc) – OCPD) are presented in Table I. The slopes of the lines represent the polarization of the PD, as an increase in the open-circuit potential per 100 mV increase in PD, by voltage clamping. The polarization was 16–31 mV percent in the absence of inhibitors. The voltage-clamp polarization was significantly greater in Na^+-free/Cl^--free/4 mM K^+ than in regular or Na^+-free/Cl^--free/79 mM K^+ solutions ($P < 0.01$). With 10^{-5} M SCH 28080 or 10^{-3} M cimetidine, the polarization was 4–8 mV percent, independent of the ionic composition of the media. With 10^{-2} M SCN^-, the polarization was significantly greater than in the presence of cimetidine or SCH 28080 ($P < 0.01$). The voltage-clamp polarization was significantly lower with SCN^- than in its absence independent of the ionic

TABLE I

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

n, number of experiments. Significant differences refer to slopes.

	n	Slope	Intercept	*r*
Reg. solns.	12	26.0	1.12	0.84
+ cimetidine 10^{-3} M	5	7.6 [a]	−0.28	0.63
+ SCH 28080 $5 \cdot 10^{-5}$ M	6	5.7 [a]	1.53	0.53
+ SCN^- 10^{-2} M	6	17.6 [a,b]	2.08	0.84
Na^+-free/Cl^--free 4 mM K^+	16	35.4 [a]	2.61	0.92
+ cimetidine 10^{-3} M	5	3.4 [c]	1.20	0.36
+ SCH 28080 $5 \cdot 10^{-5}$ M	6	8.2 [c]	2.43	0.44
+ SCN^- 10^{-2} M	5	21.4 [c,d]	1.72	0.73
Na^+-free/Cl^--free 79 mM K^+	21	18.7 [a,c]	2.71	0.86
+ cimetidine 10^{-3} M	5	3.6 [e]	0.94	0.64
+ SCH 28080 $5 \cdot 10^{-5}$ M	6	7.5 [e]	2.07	0.57
+ SCN^- 10^{-2} M	5	11.8 [e,f,g]	4.78	0.71

[a] Significantly different from regular solutions (without inhibitors; ($P < 0.01$).

[b] Significantly different from regular solutions (with other inhibitor; ($P < 0.01$).

[c] Significantly different from Na^+-free/Cl^--free/4 mM K^+ (without inhibitors; $P < 0.01$).

[d] Significantly different from Na^+-free/Cl^--free/4 mM K^+ (with other inhibitors; $P < 0.01$).

[e] Significantly different from Na^+-free/Cl^--free/79 mM K^+ solutions (without inhibitors; $P < 0.01$).

[f] Significantly different from Na^+-free/Cl^--free/79 mM K^+ solutions (with either inhibitor; $P < 0.01$).

[g] Significantly different from Na^+-free/Cl^--free/4 mM K^+ plus SCN^- ($P < 0.05$).

composition of the media ($P < 0.05$). The presence of SCN^- made insignificant the difference of the voltage-clamp polarization between regular and other solutions. As without SCN^-, the voltage-clamp polarization was greater in Na^+-free/Cl^--free/4 mM K^+ than in Na^+-free/Cl^--free/79 mM K^+ solutions ($P < 0.05$).

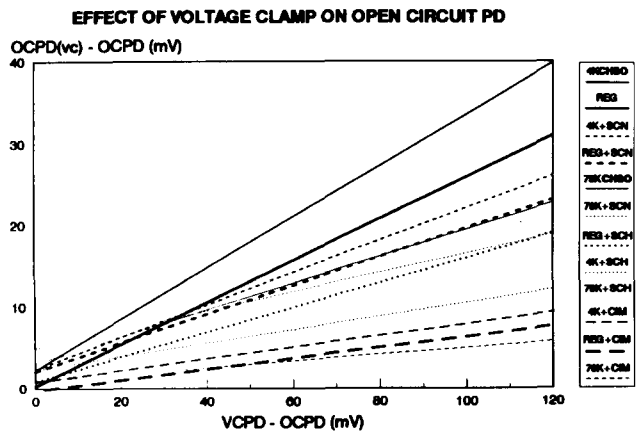

Fig. 4. Effect of voltage clamp on open circuit PD. Increase in OCPD during voltage clamp ($OCPD_{VC} - OCPD$) plotted vs. increase in voltage by voltage clamp over the pre-clamp PD ($VCPD - OCPD$). The slope represents the increment in OCPD (polarization) induced by voltage clamp. Values are presented in Table I.

Discussion

From the work on vesicles obtained from the secretory membrane, there is incontrovertible evidence for the existence of a neutral H^+/K^+-ATPase pump [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 [11,12], we have provided further evidence of the electrogenicity of the proton pump in the intact tissue and presented a phenomenological construct [12,22], in which the two mechanisms were put together. In the most recent publication [12], we showed that the polarization induced by voltage clamping across the gastric mucosa was markedly reduced by two inhibitors of gastric secretion, famotidine, a strong H_2-blocker [14] and omeprazole, which in the active form is a sulfonamide that inhibits the H^+/K^+-ATPase by reacting with its sulfhydryl groups [23,24]. Changing the ionic composition of the media (e.g., NaCl-free and/or 79 mM K^+ solutions) did not induce major changes in the voltage-clamp polarization, except with K^+-free solutions in which the voltage-clamp polarization was zero [12]. Redistribution of ions across the plasma membrane was not a major factor, while the presence of a viable proton pump, in which K^+ is intimately involved, was necessary to induce polarization by voltage clamping.

Was the viability of the pump necessary or was the flow of H^+ necessary to obtain the polarization effects of voltage clamping? The answer is given by the present experiments, in which voltage clamp was done in the presence of three inhibitors of H^+ secretion, including SCN^-, an inhibitor of H^+ secretion [25] that does not affect the pump [16–18,26,27] and the results with SCN^- are compared with the effects of the other two inhibitors, cimetidine, an H_2-blocker [13,14], and SCH 248080, apparently, a reversible competitive inhibitor of the K^+-induced hydrolysis of the H^+/K^+-ATPase [15]. The inhibitory effect of SCN^- has been known for a long time [22,25]. Sanders et al. [16] showed that weak bases could restore acid secretion, after inhibition by SCN^-, which led to the hypothesis of back diffusion of HSCN as the cause of SCN^- inhibition. SCN^- did not alter the active transport mechanism. Gutknecht and Walter [28] supported the hypothesis by showing the high permeability of lipid bilayers to HSCN. Further support for the back diffusion hypothesis was presented with the experiments of Reenstra and Forte [27] and Wolosin and Forte [17] in which they showed an increase of intravesicle loss of H^+ by intravesicular SCN^- [27] and the inhibition of formation of pH gradients in vesicles by SCN^- [17]. The inhibition of H^+ secretion is accompanied by an

increase in the transepithelial resistance [19,22,29]. The major site of the increase in the resistance appears to be in the secretory membrane of the oxyntic cells, not by a reduction in the surface area of the secretory membrane [30] but by a decrease of the conductance of a high-conductance mechanism located in the secretory membrane [20,31]. The fact that the voltage-clamp polarization was not markedly reduced by SCN^-, contrary to what happens with inhibitors of the transport mechanism (omeprazole, SCH 28080, famotidine and cimetidine) supports the concept that it is the transport mechanism (proton pump) that contributes to the polarization and not the flow of H^+ ions per se.

As found previously [12] and in present experiments, polarization was higher in Na^+-free/Cl^--free/4 mM K^+ than in regular solutions and in both of them the polarization was higher than in Na^+-free/Cl^--free/79 mM K^+. The circuit shown in Fig. 5 presents an equivalent circuit for the secretory membrane, in which the emf is mostly the proton pump emf and the parallel resistance, R_x, represents the K^+ and Cl^- conductive pathways. The PD across this circuit is given by

$$V_{CS} = E_P R_x/(R_x + R_P) \quad (1)$$

where V_{CS} is the PD across the secretory membrane, E_P is the pump emf, R_x is the resistance of the parallel (K^+ and Cl^- pathways) and R_P the pump resistance.

If the effects on the transepithelial resistance and PD are mostly due to effects on the secretory membrane, changes in the transepithelial PD should be a reflection of the changes in V_{CS}. Polarization of the transepithelial PD as a result of the polarization of the pump emf (E_P) will depend also on the relative values of the resistances at the time of the polarization. For example, if in going from control to Na^+-free/Cl^--free/4 mM K^+ the parallel resistance, R_x (K^+ and Cl^- pathways), increases due to the removal of Cl^-, the factor $R_x/(R_x + R_P)$ will increase and an equal polarization of E_P should result in a larger polarization of V_{CS} and, therefore, a larger polarization of the transepithelial PD. This was the actual result, that is, the polarization was higher in Na^+-free/Cl^--free/4 mM K^+ than in regular solutions (35 vs. 25 mV%)

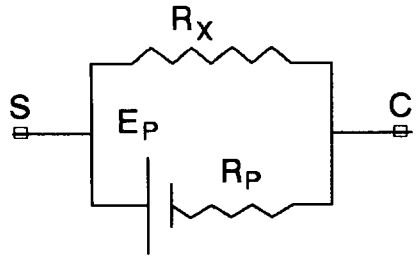

Fig. 5. Equivalent circuit across the secretory membrane of gastric mucosa. E_P is the pump emf and R_P the pump resistance. R_x is the resistance of the parallel (K^+ and Cl^- pathway (see text and Fig. 6).

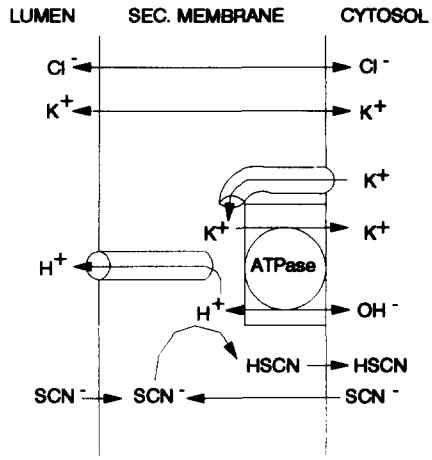

Fig. 6. Model of proton pump which combines a neutral H^+/K^+-ATPase pump with attached K^+ and H^+ conductances to make it electrogenic. Separate K^+ and Cl^- conductances across the secretory membrane are also present. SCN^- reacts with H^+ within the membrane with diffusion of undissociated HSCN into the cytoplasm.

($P < 0.01$). On the other hand, when changing to the Na^+-free/Cl^--free/79 mM K^+ the parallel resistance, R_x (K^+ and Cl^- pathways), will decrease and an equal polarization of E_P should result in a smaller polarization of V_{CS} and, therefore, a smaller polarization of the transepithelial PD. This, again, was the actual result, that is, the polarization was smaller in Na^+-free/Cl^--free/79 mM K^+ (19 mV%) than in regular (25 mV%) or in Na^+-free/Cl^--free/4 mM K^+ solutions (35 mV%) ($P < 0.01$).

The polarization in the presence of SCN^- was greater than the polarization in the presence of inhibitors of the pump, as mentioned above ($P < 0.01$). These data support the concept [16–18,26,27] that SCN^- does not affect the active transport mechanism, the H^+/K^+-ATPase pump, as the other inhibitors do. On the other hand, the polarization was smaller in the presence of SCN^- than in its absence in the three types of solutions tested ($P < 0.05$). This brings us to the model presented in Fig. 6. The model is slightly modified from that presented previously [12,31]. A neutral H^+/K^+-ATPase occupies the hemimembrane (secretory) adjacent to the cytoplasm. Parallel to the exchanger is a K^+ conductance and both are in series with a H^+ conductance. The three elements constitute the electrogenic proton pump. SCN^- enters the membrane and reacts with H^+ to form undissociated HSCN which diffuses into the cytoplasm. The result of the addition of SCN^- should be an increase in the resistance of the 'pump', R_P, by reducing the H^+ entering its pathway. With an increase in R_P, the coefficient of E_P, $R_x/(R_x + R_P)$, will decrease; therefore, any polarization of E_P will result in a smaller change in V_{CS} and, therefore, in a smaller change in the transepithelial PD. As stated above, the experimental results agree with those predicted from the model. As a

matter of fact, in regular solutions there was a significant increase in resistance after addition of SCN^- (see Results). The data on SCN^- could also be explained on the basis of a decrease in the parallel resistance, R_x. In Cl^--free solutions, the resistance did not change after addition of SCN^-. Perhaps the increase in R_P was accompanied by a simultaneous decrease in R_x when SCN^- was added to the solution. As suggested by Reenstra and Forte [18], SCN^- may use the Cl^- channels, which would support the concept that, in Cl^--free solutions, SCN^- may decrease R_x. Both changes, increase in R_P and decrease in R_x, would contribute to a decrease in the value of the coefficient of E_P, $R_x/(R_x + R_P)$. This may explain why the polarization was so low with SCN^-, especially in Na^+-free/Cl^--free/79 mM K^+ solutions (10 mV%). The low polarization found in the presence of inhibitors, other than SCN^-, cannot be attributed to a decrease in R_x, since all inhibitors induced an increase in resistance, especially in regular solutions (see Results).

In conclusion, voltage-clamp polarization of the open circuit potential is markedly reduced by inhibitors of the pump, either by blocking the H_2-receptors or by direct reaction with the enzyme H^+/K^+-ATPase. The polarization is only moderately reduced by SCN^-. This latter result further supports the concept that the pump is electrogenic and SCN^- does not affect the enzyme.

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References

1 Ganser, A.L. and Forte, J.G. (1972) Biochim. Biophys. Acta 307, 169–172.
2 Sachs, G., Chang, H.H., Rabon, E., Schackman, R., Lewin, M. and Saccomani, G. (1976) J. Biol. Chem. 251, 7690–7698.
3 Sachs, G., Berglindh, T., Rabon, E., Stewart, H.B., Barcellona, M.L., Wallmark, B. and Saccomani, G. (1980) Ann. N.Y. Acad. Sci. 341, 312–334.
4 Lee, H.C., Breitbart, H. and Forte, J.G. (1980) Ann. N.Y. Acad. Sci. 341, 297–310.
5 Rehm, W.S. (1950) Gastroenterology 14, 401–417.
6 Heinz, E. and Durbin, R. (1959) Biochim. Biophys. Acta 31, 246–247.
7 Rehm, W.S. and LeFevre, M.E. (1965) Am. J. Physiol. 208, 922–930.
8 Schwartz, M. and MacKrell, T.W. (1969) Proc. Soc. Exp. Biol. Med. 130, 1048–1051.
9 Kidder, G.W., III and Rehm, W.S. (1970) Biophys. J. 10, 215–236.
10 Noyes, D.H. and Rehm, W.S. (1970) Am. J. Physiol. 219, 184–192.
11 Rehm, W.S., Carrasquer, G. and Schwartz, M. (1990) J. Intern. Med. 228 (Suppl. 1), 31–39.
12 Carrasquer, G., Zhang, J., Wu, X., Rehm W.S. and Schwartz, M. (1993) Am. J. Physiol. 27, G630–G636.
13 Blimblecombe, R.W., Duncan, W.A.W., Durant, G.J., Emmett, J.C., Ganellin, C.R. and Parsons, M.E. (1975) J. Intern. Med. Res. 3, 86–92.
14 Lin, J.H. (1991) Clin. Pharmacokinet. 20, 218–236.
15 Scott, C.K., Sundell, E. and Castrovilly, L. (1987) Biochem. Pharmacol. 36, 97–104.
16 Sanders, S.S., Pirkle, J.A., Shoemaker, R.L. and Rehm, W.S. (1978) Am. J. Physiol. 234, E120–E128.
17 Wolosin, J.M. and Forte, J.G. (1983) J. Membr. Biol. 76, 261–268.
18 Forte, J.G., Reenstra, W.W. and Wolosin, J.M. (1983) in Membrane Biophysics II: Physical Methods in the Study of Epithelia, (Dinno, M.A., Callahan, A. and Rozzell, T.C., eds.), pp. 141–158, Alan Liss, New York.
19 Rehm, W.S. (1962) Am. J. Physiol. 203, 63–72.
20 Rehm, W.S., Carrasquer, G. and Schwartz, M. (1986) Am. J. Physiol. 250, G511–G517.
21 Neter, J., Wasserman, W. and Kutner, M.H. (1983) in Applied Linear Regression Models, Richard D. Irwin, Homewood.
22 Schlesinger, H.S., Dennis, W. and Rehm, W.S. (1955) Am. J. Physiol. 183, 75–78.
23 Fellenius, E., Berglindh, T., Sachs, T., Olbe, L., Elander, B., Sjostrand, S.E. and Walmark, B. (1981) Nature. 290, 159–161.
24 Lindberg, P., Nordberg, P., Alminger, P. Branstrom, A. and Wallmark, B. (1986) J. Med. Chem. 429, 1327–1329.
25 Davenport, H.W. (1940) Am. J. Physiol. 129, 505–514.
26 Hersey, S.J., Chew, S.C., Campbell, L. and Hopkins, E. (1981) Am. J. Physiol. 240, G232–G238.
27 Reenstra, W.W. and Forte, J.G. (1986) Am. J. Physiol. 250, G76–G84.
28 Gutknecht, J. and Walter, A. (1982) Biochim. Biophys. Acta 685, 233–240.
29 Forte, J.G. and Machen, T.E. (1975) J. Physiol. 244, 33–51.
30 Forte, J.G., Machen, T.E. and Forte, J.G. (1977) Gastroenterology 69, 1208–1222.
31 Rehm, W.S., Chu, T.C., Schwartz, M. and Carrasquer, G. (1983) Am. J. Physiol. 245, G143–G156.