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Evidence for HCO_3^- conductance pathways in nutrient membrane of resting frog fundus

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The effect on potential difference (PD) and resistance in Cl^- media bathing the resting fundus of *Rana pipiens* was determined for nutrient HCO_3^- changes from 25 mM to several lower concentrations and back to 25 mM. The graph of $|\Delta PD|$ versus $\log[HCO_3^-]$ was linear for changes from 25 down to 3.1 mM and also back to 25 mM, but deviated considerably for changes to 1.6 mM. The fact that changes from higher to lower HCO_3^- gave a less rapid initial PD response than the reverse direction eliminated H^+ conductance pathways as being predominant. Experiments were done in which in the first part changes were made in the nutrient solution from 5% CO_2 and 25 mM HCO_3^- to 0.6% CO_2 and 3 mM HCO_3^- and in the second part, the same changes with the simultaneous change of secretory solution from 5% to 10% CO_2. The magnitude of the PD decrease was greater by 4.0 mV in the second part. This result indicated that HCO_3^- rather than OH^- conductance pathways predominated. On the secretory side, the change from 25 to 3.1 mM HCO_3^- gave a small but significant change in PD. The latter effect was too small to determine whether HCO_3^- pathways existed in the secretory membrane.

Introduction

Recently, using the method of ion substitution, we found that, in addition to conductance pathways for K^+, Cl^-, and Na^+ [1], there exist conductance pathways for HCO_3^- in the nutrient membrane of the bullfrog antrum [2]. It should be noted that in this regard Flemstrom and Sachs [3] reported a change in potential difference (PD) resulting from a change in HCO_3^- not only on the nutrient side but also on the secretory side of the antrum. They did not, however, determine whether a linear relationship exists between $|\Delta PD|$ and the log of the HCO_3^- concentration. In contrast, for the secreting fundus of *Rana pipiens*, Sanders et al. [4] found no significant change in PD with a change in nutrient HCO_3^- concentration and had no evidence for HCO_3^- conductance pathways in the nutrient membrane. In their studies, they determined the change in PD 1 min after the change in nutrient HCO_3^- concentration and the maximum PD which generally occurred within 4 min after the change in HCO_3^- concentration. The reason for their choice of short periods of time is that, in studies involving changes in nutrient K^+ concentration, an increase in the nutrient K^+ concentration from 4 to 20 mM (a 5-fold increase) resulted in a marked decrease in PD of about 25 mV to a constant level within about 4 min after the K^+ increase [4].

If instead the PD response is examined 10 min after the change in nutrient HCO_3^-, it will be seen (vide infra) that there is a small but significant change in PD for moderate secretory rates (about

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2 μequiv.$\cdot$ $h^{-1} \cdot cm^{-2}$) which decreases to about 1 mV or less for relatively high secretory rates (about 4 μequiv.$\cdot$ $h^{-1} \cdot cm^{-2}$). The latter result is in accord with the finding of Sanders et al. [4]. In this paper, some consideration is given to the connection between the rate of H^+ secretion and the magnitude of the PD response and also to the existence of a HCO_3^- conductance in the secretory membrane under resting (inhibited) conditions. The main thrust of the present work, however, it to examine the existence of HCO_3^- conductance pathways in the nutrient membrane of the resting (inhibited) fundus of *Rana pipiens.*

In order to determine HCO_3^- conductance pathways particularly in the nutrient membrane of the resting fundus, three factors considered in some detail in studies of HCO_3^- pathways in the nutrient membrane of the antrum [2] will be reviewed here. The first factor concerns the initial rate of the PD response following a change in HCO_3^- concentration when a diffusion barrier exists between the nutrient membrane and the bathing medium. Such a barrier exists in the nutrient membrane of the frog gastric mucosa and consists of the lamina propria, the muscularis mucosa and the residual submucosa after removal of the external muscle layers. As a consequence of the diffusion barrier, Spangler and Rehm [5] found that, for both cation and anion conductances, an increase in concentration gives a faster initial PD response than a decrease in concentration. While the curve of concentration at the cell border versus time is the same whether the nutrient concentration of the ion involved increases or decreases, the initial PD response is different in these two cases since the PD depends on the logarithm of the concentration ratio across the nutrient membrane (See Ref. 5 for details of this process.). The following point is of importance in the present considerations. When in an increase in concentration of HCO_3^- the initial PD change is sufficiently rapid, then this result would constitute evidence for a conductance pathway for HCO_3^-. It would, indeed, be difficult to explain the rapidity of the response as an indirect effect of HCO_3^- on other ions. The latter possibility arises since, in studies of the nutrient side of the antrum in the absence of HCO_3^-, the PD responses associated with simple conductance pathways and resulting from changes in concentration of K^+, Cl^-, and Na^+ were either reduced or abolished [1,6].

The second factor to determine the existence of a HCO_3^- conductance pathway is that, upon performance of a series of changes of HCO_3^- concentration, an approximate linear relationship between $|\Delta PD|$ and log $[HCO_3^-]$ may occur. In nutrient HCO_3^- changes in studies of the antrum, such a linear relationship was obtained for a range of HCO_3^- concentrations between 3 and 25 mM [2]. However, a curvilinear relationship does not necessarily exclude a HCO_3^- conductance pathway. It would appear that the linear relationship in particular could not be easily explained when the only effect of HCO_3^- was to influence the conductances of other ions. (See Discussion for details of these considerations.)

The third factor requires that the HCO_3^- conductance if it exists needs to be separated from possible OH^- and H^+ conductances. This consideration arises since with constant p_{CO_2} removal of HCO_3^- decreases the pH and adding HCO_3^- back increases the pH. Consequently the decrease in HCO_3^- is accompanied by a decrease in OH^- and an increase in H^+ and vice versa. Moreover, all the changes in e.m.f. values are in the same direction. Therefore, when no changes in PD occur, it follows that the sum of the HCO_3^-, OH^- and H^+ conductances is zero [4]. However, as it will be seen, PD changes do occur and, since the increase in HCO_3^- gives a greater initial PD response than the decrease in HCO_3^-, it would appear that the conductance pathways, if they exist, can be either HCO_3^- and/or OH^- but not H^+ conductance pathways.

Hence the establishment of HCO_3^- conductance pathways, particularly in the nutrient membrane, will require an examination of the three factors considered above. In addition, a separation of the HCO_3^- conductance from a possible OH^- conductance will be considered.

Methods

Experiments were performed on fundi of stomach of *Rana pipiens* by an in vitro method in which the stomachs were mounted between a pair of cylindrical chambers [7]. 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 new Cl^- standard secretory (mucosal) solution which is hypertonic [8] contained: Na^+, 156; K^+, 4; Cl^-, 160. In studies in which the nutrient HCO_3^- concentration was changed from 25 mM HCO_3^- to lower concentrations or from lower concentrations back to 25 mM HCO_3^-, modified, phosphate-free nutrient solutions were used. In all these solutions, the Ca^{2+}, Mg^{2+} and K^+ concentrations were unaltered. One solution with 25 mM $NaHCO_3$ contained 50 mM NaCl, 12.5 mM Na_2SO_4 and 12.5 mM sucrose and a second solution with zero HCO_3^- contained 50 mM NaCl, 25 mM Na_2SO_4 and 25 mM sucrose. Mixtures of these two solutions provided solutions of constant Cl^- concentration and with varying concentrations of HCO_3^-. Other modifications in which the HCO_3^- concentrations were reduced from 25 mM HCO_3^- also contained a fixed concentration of Cl^-. In the studies of the existence of a linear relationship between $|\Delta PD|$ and log $[HCO_3^-]$, both sides of the fundus were gassed with 95% O_2/5% CO_2. In other experiments, the O_2 and CO_2 percentages were varied.

In these experiments, the transmembrane resistance and the transmembrane 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. The H^+ secretory rate was determined by the pH stat method of Durbin and Heinz [9]. The pH of the secretory solution during secretion was maintained generally between 4.7 and 5.0. A concentration of 10 to 20 mM SCN in the secretory solution was used to decrease the H^+ secretory rate to zero. On occasion 1 mM cimetidine was used to inhibit the fundus. Inspection of the data indicated no difference between SCN-inhibited and cimetidine-inhibited fundi. Hence for presentation herein, the data are lumped.

In the change of the concentration of HCO_3^- in the nutrient solution, due to the existence of a diffusion barrier between the nutrient solution and the nutrient membrane, it takes about 10 min (approx. five time constants) for the concentration of the ion at the cell membrane to attain the new concentration in the nutrient solution [4]. In the present experiments, the PD change at the 10 min mark was recorded.

Results

Effect of varying H^+ secretory rates on the PD response to changes in HCO_3^- concentration in the nutrient solution

Prior to a detailed examination of HCO_3^- conductance pathways in the nutrient membrane of the resting fundus, we consider some observations of HCO_3^- conductances in three states of H^+ secretion. The latter, as shown in Table I, refer to a relatively high H^+ secretory rate (3.81 μequiv.$\cdot h^{-1} \cdot cm^2$), a more moderate secretory rate (1.76 μequiv.$\cdot h^{-1} \cdot cm^{-2}$) and a zero rate. At the relatively high secretory rate, the change in HCO_3^- concentration in the nutrient solution from 25 to 3 mM gave an insignificant change in PD; at the more moderate rate, a decrease in PD of about 5 mV and at the zero rate (due to the absence of histamine), a decrease in PD of about 13 mV. The first case is in agreement with the finding of Sanders et al. [4]. Moreover, as Table I shows, the resistance is greatest in the absence of H^+ secretion and the change in resistance with the decrease in nutrient HCO_3^- concentration is also greatest in this case. (See Discussion for a possible explanation.)

Changes in PD and resistance due to changes in HCO_3^- concentration in the nutrient solution of the resting fundus

Experiments were performed on the resting fundus in which the nutrient HCO_3^- concentration was changed from 25 to 12.5 mM and back to 25 mM, then from 25 to 6.25 mM and back to 25 mM and so forth to 3.1 and 1.6 mM. Fig. 1 is a plot of resistance and PD versus time of part of a representative experiment for changes of nutrient HCO_3^- concentration from 25 to 3.1 mM and back to 25 mM. The decrease from 25 to 3.1 mM HCO_3^- gave a decrease in PD and an increase in resistance.

TABLE I

EFFECT ON PD AND RESISTANCE DUE TO A DECREASE IN NUTRIENT HCO_3^- FROM 25 TO 3.1 mM WITH CHANGES IN H^+ SECRETORY RATE

Values are means ± S.D. Student's *t*-test using paired observations was used to determine the level of significance. Columns labeled PD and *R* refer to the control values of transmembrane potential difference and resistance, respectively, and columns labeled ΔPD and ΔR refer to changes in the two parameters following the change to 3.1 mM HCO_3^-.

No. of expts.	PD (mV)	ΔPD (mV)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
	Relatively high secretory rates (3.81 ± 0.65 μequiv.$\cdot h^{-1} \cdot cm^{-2}$)			
6	15.4 ± 5.5	−0.5 ± 0.5	122 ± 46	−6 ± 24
	Moderate secretory rates (1.76 ± 0.65 μequiv.$\cdot h^{-1} \cdot cm^{-2}$)			
4	18.4 ± 6.4	−4.9 ± 0.9 [a]	202 ± 43	40 ± 25 [b]
	Zero secretory rate			
4	21.7 ± 5.9	−12.9 ± 3.1 [a]	324 ± 83	190 ± 48 [a]

[a] $P < 0.01$.
[b] $P < 0.05$.

The return to 25 mM HCO_3^- brought the PD and resistance back towards control values. As Fig. 1 shows, the PD changes less rapidly in the initial phase in going from 25 to 3.1 mM HCO_3^- than in the reverse direction. As described in the Introduction, the rapidity of the initial increase in PD in going from low to high HCO_3^- is evidence for a HCO_3^- conductance pathway since it would be difficult to account for the difference in the time-course of the PD responses to a decrease and an increase in HCO_3^- as an indirect effect of HCO_3^- on other ions. As indicated above, the concentration at the cell border versus time is the same for both decreases and increases of HCO_3^-.

In Table II, the effect on PD and resistance due to changes in HCO_3^- concentration on the nutrient side in Cl^- solutions is shown. Decreases in HCO_3^- concentration gave decreases in PD and increases in resistance while increases in HCO_3^- concentration returned the PD and resistance towards control values.

In Fig. 2 (based on values in Table II), the absolute value of the change in PD, $|\Delta PD|$, is plotted versus the logarithm of the initial or final

TABLE II

EFFECT ON PD AND RESISTANCE OF CHANGES IN HCO_3^- CONCENTRATIONS ON THE NUTRIENT SIDE IN Cl^- SOLUTIONS

Values are means ± S.D. of nine experiments. Student's *t*-test using paired observations was used to determine the level of significance. Columns labeled PD and *R* refer to the control values of transmembrane potential difference and resistance, respectively, and columns labeled ΔPD and ΔR refer to changes in the two parameters following the change to the final solution.

Original soln. [HCO_3^-] (mM)	Final soln. [HCO_3^-] (mM)	PD (mV)	ΔPD (mV)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
25.0	12.5	25.3 ± 5.1	−4.6 ± 1.2 [a]	248 ± 71	53 ± 28 [a]
25.0	6.2	24.1 ± 5.5	−9.3 ± 2.5 [a]	252 ± 90	96 ± 32 [a]
25.0	3.1	22.5 ± 4.9	−13.2 ± 2.0 [a]	260 ± 90	169 ± 42 [a]
25.0	1.6	21.4 ± 5.5	−14.6 ± 1.6 [a]	260 ± 84	148 ± 57 [a]
12.5	25.0	20.1 ± 5.1	4.3 ± 1.0 [a]	303 ± 87	−54 ± 28 [a]
6.2	25.0	14.1 ± 5.4	8.5 ± 2.5 [a]	356 ± 92	−100 ± 27 [a]
3.1	25.0	8.8 ± 5.0	12.8 ± 1.8 [a]	429 ± 109	−186 ± 93 [a]
1.6	25.0	4.9 ± 6.2	14.9 ± 2.3 [a]	427 ± 120	−161 ± 93 [a]

[a] $P < 0.01$.

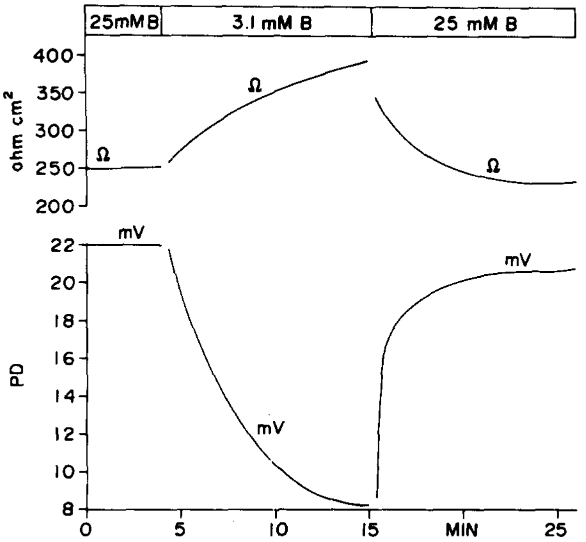

Fig. 1. Effect of changes in HCO_3^- concentration on the nutrient side from 25 to 3.1 mM HCO_3^- and back to 25 mM HCO_3^-. Resistance and PD are plotted vs. time. B refers to HCO_3^-.

concentration of nutrient HCO_3^- for a range of concentrations between 3.1 and 25 mM (an 8-fold change). The closed circles are for an initial nutrient concentration of 25 mM HCO_3^-. The open circles are for a final nutrient concentration of 25 mM HCO_3^-. Within the limits of experimental error (note the half lines for S.E.), there is a linear relationship for concentrations from 25 to 3.1 mM nutrient HCO_3^-. Not shown in the graph is the change from 25 to 1.6 mM HCO_3^- and vice versa. For low nutrient HCO_3^- concentrations such as 1.6 mM, there is a marked deviation from the straight line (see values in Table II), much more so than for the same concentration change in the bullfrog antrum [2]. For the straight lines in Fig. 2, the slopes are about 14 and 15 mV per 10-fold change in HCO_3^- compared to about 9 mV per 10-fold change in the bullfrog antrum [2].

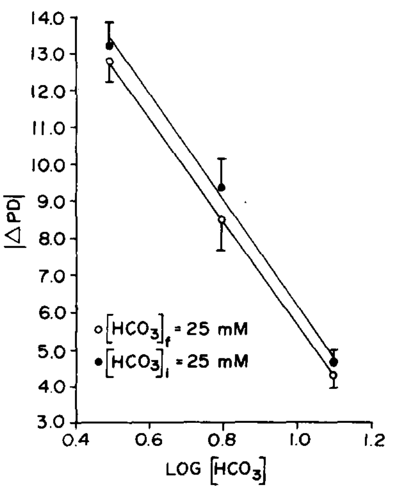

Fig. 2. Absolute value of the change in PD, $|\Delta PD|$, is plotted vs. log $[HCO_3^-]$ in nutrient solution for an initial $[HCO_3^-]_i = 25$ mM (closed circles) or for a final $[HCO_3^-]_f = 25$ mM (open circles). Half lines represent the standard errors of the mean.

Experiments determining HCO_3^- conductance pathways as more likely than OH^- pathways in the nutrient membrane

A series of eight experiments were performed, each consisting of two parts. In the first part, the nutrient HCO_3^- was decreased from 25 to 3 mM and simultaneously the nutrient gas mixture was changed from 95% O_2/5% CO_2 to 99.4% O_2/0.6% CO_2. In this way, the ratio of $[HCO_3^-]$ to $[CO_2]$ in the nutrient solution remained constant and the pH was essentially unchanged. However, the decrease in nutrient CO_2 very likely caused CO_2 to diffuse out of the cells with the consequent rise of cellular pH and cellular OH^- concentration. Thus, the decrease in nutrient HCO_3^- and the increase in cellular OH^- could each decrease the PD. Thus this part of the experiment cannot by itself differentiate which conductive pathways (HCO_3^- or OH^-) are dominant. Fig. 3 shows a typical experiment in which the PD decreased markedly 10 min after control. Table III shows that the average decrease in PD for eight experiments was −10.6 mV in the fundus. The comparative value in the antrum was −5.6 mV [2].

In the second part of each experiment, in addition to the same changes in HCO_3^- and CO_2 and in the gas mixture in the nutrient solution, the gas mixture on the secretory side was simultaneously changed from 95% O_2/5% CO_2 to 90% O_2/10% CO_2. The last maneuver should cause CO_2 to diffuse into the cells, thereby decreasing cellular pH relative to the first part. Consequently, the OH^- concentration is less in the second part compared to the first part. If then the conductive pathways were primarily OH^-, we would expect the magnitude of the PD decrease to be less in the second part than in the first part. As Fig. 3 shows, the exact opposite is true when we compare the decrease in PD in the second half of the experi-

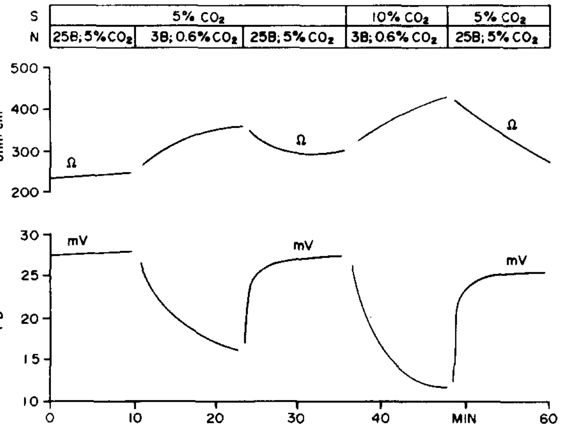

Fig. 3. First part: Effect of changes in HCO_3^- concentration on the nutrient side from 25 to 3 mM HCO_3^- and at same time change of nutrient gases from 95% O_2/5% CO_2 to 99.4% O_2/0.6% CO_2. Second part: Same changes from control with simultaneous change of secretory gases from 95% O_2/5% CO_2 to 90% O_2/10% CO_2 and finally return to standard solutions and gases. Resistance and PD vs. time. S refers to secretory side and N refers to nutrient side.

ment with the first half. Table III shows that the decrease in the second part 10 min after the change from control is −14.6 mV, i.e. about a 43% increase in magnitude. In the antrum the change was from −5.6 mV, i.e. almost an 80% increase in magnitude [2]. In both the fundus and the antrum the average difference in PD between part one and part two is statistically significant ($P < 0.01$).

To account for the greater magnitude of the PD decrease in the second part, we consider the explanation given for the antrum [2]. The increase in cellular CO_2 due to the increase in secretory CO_2 (from 5% to 10%) causes some CO_2 in the cell to combine with H_2O to form H_2CO_3 which, in turn, would lead to an increase in H^+ and HCO_3^-. If the protein buffering capacity of the cells is comparable to that of other cells, e.g. the red cell [10], the HCO_3^- concentration would increase significantly in the cells of the fundus. Consequently, the ratio of HCO_3^- in the cell to that in the nutrient fluid would increase with the increase in CO_2 in the secretory gas. Thus, the difference between −10.6 and −14.6 mV can be explained as the result of HCO_3^- conductive pathways in the nutrient membrane.

As in the case of the antrum, a series of experiments were performed in which the nutrient HCO_3^- was maintained at 3 mM and the nutrient gas mixture was 99.4% O_2/0.6% CO_2 and the only change was the periodic variation of the gas mixture on the secretory side from 0.6% to 10% CO_2 and back to 0.6% CO_2. In these experiments, it was found that a decrease in secretory p_{CO_2} increased the PD and that an increase in secretory p_{CO_2} decreased the PD. In nine experiments, the change from 10% to 0.6% CO_2 increased the PD on the average by 3.1 ± 1.8 (S.D.) mV and, in seven experiments, the change from 0.6% to 10% decreased the PD on the average by 3.5 ± 1.4 (S.D.) mV. These changes in PD due to the change in secretory CO_2 agree with our interpretation of the PD decrease as resulting from an increase of

TABLE III

EFFECT ON PD AND RESISTANCE OF CHANGES IN HCO_3^- CONCENTRATIONS ON THE NUTRIENT SIDE AND CO_2 CONCENTRATIONS ON BOTH SIDES

Values are means ± S.D. of six experiments. Student's *t*-test using paired observations was used to determine the level of significance. Columns labeled PD and *R* refer to the control values of transmembrane potential difference and resistance, respectively, and columns labeled ΔPD and Δ*R* refer to changes in the two parameters following the change to the final solution. N refers to nutrient and S to secretory. The secretory CO_2 is 5% except for the period when it is raised to 10%.

PD (mV)	ΔPD (mV)	*R* ($\Omega \cdot cm^2$)	Δ*R* ($\Omega \cdot cm^2$)
Part I: N ΔHCO_3 from 25 to 3 mM and N ΔCO_2 from 5% to 0.6%			
25.3 ± 3.5	−10.6 ± 1.5 [a]	282 ± 34	98 ± 40 [a]
Part 2: Same as Part 1 and simultaneously S ΔCO_2 from 5% to 10%			
24.8 ± 3.8	−14.6 ± 2.9 [a]	299 ± 94	166 ± 94 [a]

[a] $P < 0.01$.

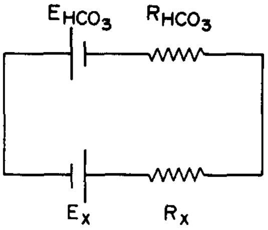

Fig. 4. Equivalent circuit for nutrient membrane comprising a HCO_3^- limb and an X limb representing all other ionic pathways. Right-hand side is nutrient and left-hand side is cell.

cellular HCO_3^- and the PD increase, from a decrease in cellular HCO_3^-.

Effect of changes in HCO_3^- in the secretory solution on PD and resistance of the resting fundus

In six experiments the regular secretory solution on the secretory side was replaced with a phosphate-free regular nutrient solution containing 10 to 15 mM SCN. This solution was then replaced with a phosphate-free nutrient solution containing 3.1 mM HCO_3^- and also containing 10 to 15 mM SCN. The change from 25 to 3.1 mM in the secretory solution resulted in an increase in PD of 1.6 ± 1.1 (S.D.) mV and no significant change in resistance. The return to 25 mM HCO_3^- gave a decrease in PD of -0.5 ± 1.3 (S.D.) mV, an insignificant decrease, and a small decrease in resistance of 22 ± 18 (S.D.) $\Omega \cdot cm^2$.

The significant change in PD resulting from the change in HCO_3^- from 25 to 3.1 mM is too small to permit further analysis. In other words, it would not be possible to determine whether there is a high resistance HCO_3^- conductance pathway or whether the effect is associated with H^+ and/or OH^- conductance pathways.

Discussion

The principal effort in this paper was to determine whether HCO_3^- conductance pathways exist in the nutrient membrane of the resting (inhibited) fundus. Preliminary studies showed that, for the same change in HCO_3^- concentration (25 to 3.1 mM) in the nutrient solution, the PD change was greater at low H^+ rates than at higher rates. This result could be due to one or both of the following factors. The first is that the characteristics of the HCO_3^- conductance pathway change with the degree of secretion. The second is that the characteristics of this pathway do not change with varying H^+ secretory rates but becomes more manifest because the net conductance of the parallel pathways decrease.

From the Introduction, it is evident that the change in PD could be due not only to HCO_3^- but also to OH^- and H^+. However, the rapidity of the initial PD response due to H^+ should be opposite to that shown in Fig. 1 and hence the H^+ conductance could not be the major determinant of the response. Thus HCO_3^- and OH^- which increase or decrease together could each account for the initial PD response. Furthermore, the rapidity of the initial PD response in going from a low to a high HCO_3^- concentration makes it unlikely that a HCO_3^- effect on other ions could account for the PD change. In addition, the linear relationship between $|\Delta PD|$ and log $[HCO_3^-]$ argues against the PD response resulting solely from an effect of HCO_3^- on the conductance of ionic pathways other than HCO_3^-.

To see how linearity can arise, we review briefly the considerations used before for the antrum [2]. For this purpose, we refer to the equivalent circuit of Fig. 4 for the nutrient membrane comprising two conductive pathways, one a HCO_3^- limb and the other an X limb for all other ionic pathways. Each limb consists of a resistance R and an e.m.f. E. For a change in HCO_3^- concentration, it follows from the circuit (since $\Delta E_X = 0$) that

$$\Delta PD = -R_X(R_{HCO_3} + R_X)^{-1} \Delta E_{HCO_3}$$

provided that R_X and R_{HCO_3} are constant. However, since the changes in HCO_3^- concentration show changes in resistance for the linear part, some hypothesis other than constancy of R_X and R_{HCO_3} needs to be considered. As a possibility, if R_X and R_{HCO_3} both varied inversely with the HCO_3^- concentration, then $R_X/(R_{HCO_3} + R_X)$ would remain constant. It would be, indeed, very difficult to explain the linear relationship based on a model in which either resistance alone of the two parallel pathways would change with variation in HCO_3^- concentration.

From experiments like the one in Fig. 3, it was evident that HCO_3^- and not OH^- was involved in the linear relationship. The argument based on the buffering capacity of fundic cells (see Results) indicates that a HCO_3^- conductance pathway is principally responsible for the PD changes among the three ions HCO_3^-, OH^-, and H^+.

As Table II shows, the change in PD was -14.6 mV when the secretory gas was changed from 5% to 10% CO_2 with simultaneous decrease of HCO_3^- and -10.6 mV when the secretory gas remained at 5%. One might further expect that changing the secretory gas by itself from 5% to 10% CO_2 might result in ΔPD equal to the difference between -10.6 and -14.6 mV, i.e. -4.0 mV. In actuality, the magnitude of the PD decrease was 3.5 mV (and thus close to 4.0 mV) for a change not from 5% to 10% CO_2 but for a change from 0.6 to 10% CO_2. We are unable at this time to account for the difference.

In the present studies of the resting fundus unlike those of the antrum [2], there was a significant (although small) increase in PD upon changing HCO_3^- on the secretory side from 25 to 3.1 mM. However, the change in PD was too small to attempt to determine whether the HCO_3^- pathway predominated. Perhaps as mentioned in the paper on the antrum, the use of prostaglandins might enhance the PD response with a change in HCO_3^- concentration on the secretory side.

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