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Evidence for HCO_3^- conductance pathways in nutrient membrane of bullfrog antrum

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The effect of changing the nutrient-side HCO_3^- concentration on potential difference (PD) and resistance in bullfrog antrum bathing in Cl^- media was determined. Changes in HCO_3^- concentration were from 25 mM to several lower concentrations and back to 25 mM. A plot of $|\Delta PD|$ versus log $[HCO_3^-]$ gave a linear relation for changes of HCO_3^- concentration from 25 down to 3.1 mM and back to 25 mM but deviated to some extent for changes to 1.6 mM. In these experiments, changes from higher to lower HCO_3^- concentrations gave a less rapid initial PD response than those in the reverse direction. This result eliminated H^+ conductance pathways as being predominant. Experiments were done in which in the first part changes were made in 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 a simultaneous change of secretory solution from 5% to 10% CO_2. The magnitude of PD decrease was greater by 4.5 mV in the second part. This result indicated that HCO_3^- conductance pathways rather than OH^- conductance pathways predominated. There was no evidence of HCO_3^-, OH^- and H^+ conductance pathways in secretory membrane.

Introduction

It was previously found that, with Cl^- solutions bathing both sides of the bullfrog antrum, a decrease in HCO_3^- from 25 mM to zero in the nutrient solution increased the negativity of the nutrient (i.e., the PD decreased) and the return to 25 mM HCO_3^- decreased the negativity of the nutrient [1,2]. Moreover, in a plot of PD versus time, the PD changed less rapidly in the initial phase in going from 25 mM to zero HCO_3^- than in the reverse direction. The latter result is compatible with HCO_3^- conductance pathways. In general, Spangler and Rehm [3] found that, for both cation and anion conductances, an increase in concentration gives a faster initial PD response than does a decrease in concentration. This effect, as explained elsewhere [3,4], is a consequence of the existence of a diffusion barrier; it takes time for the concentration of an ion at the border between the nutrient membrane and the diffusion barrier to reach the new concentration in the nutrient solution. The curve showing the concentration at the cell border versus time is the same whether the nutrient concentration of the ion involved increases or decreases. The graph of concentration versus time is curvilinear and is given by a sum of exponentials (see ref. 3 for details). However, since the PD depends on the logarithm of the concentration ratio across the nutrient membrane, the PD at the nutrient membrane changes less in going, for example, from 25 to 22 mM HCO_3^- on the way to a lower concentration, say 3 mM HCO_3^-, than in going from 3 to 6 mM on the way to 25 mM HCO_3^-. The first change gives a ratio of 25 : 22, whereas the second change gives a ratio of 6 : 3.

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The greater the ratio, the greater is the initial PD change.

A further factor which has to be considered in determining whether there are HCO_3^- conductance pathways in the nutrient membrane is the effect on pH of a change in HCO_3^- concentration without changing p_{CO_2}. Under the latter circumstance, removal of HCO_3^- decreases the pH and return of HCO_3^- increases the pH. Thus the decrease in HCO_3^- is accompanied by a decrease in OH^- and an increase in H^+ and vice versa. If no changes in PD occur, then we can conclude that the sum of the HCO_3^-, OH^- and H^+ conductances is zero [5]. However, since 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 HCO_3^- and/or OH^- but not H^+ conductance pathways.

A question which has to be answered is whether the change in PD accompanying a change in HCO_3^- concentration is not simply the consequence of changes in resistance of other ionic pathways such as K^+ and Cl^-. The latter changes have been found [1,2]. In those studies, the HCO_3^- was changed from 25 mM to zero (SO_4^{2-} + sucrose for HCO_3^-) and the accompanying changes in K^+ and Cl^- concentration resulted in reduced PD responses. Under such circumstances, it cannot be definitely decided whether a HCO_3^- and/or OH^- conductance pathway exists. If, however, we perform a series of changes in HCO_3^- concentrations and obtain an approximate linear relationship between $|\Delta PD|$ and $\log[HCO_3^-]$, then it would be difficult to explain the PD response as an effect of HCO_3^- on other ionic pathways alone. This problem will be dealt with in detail in the Discussion.

In previous studies of *Necturus* and bullfrog antra, Flemstrom and Sachs [6] found evidence for HCO_3^- pathways in both membranes. However, they did not determine whether a linear relationship between $|\Delta PD|$ and $\log[HCO_3^-]$ exists and they did not determine whether the conductance pathways were HCO_3^- and/or OH^-. Both of these considerations are examined in the present paper on the bullfrog antrum.

Methods

Experiments were performed on antra of stomachs of the bullfrog, *Rana catesbeiana*, 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 somewhat 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 antrum 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. To assure that the antrum was used, histamine was added to the nutrient solution to a concentration of 0.1 mM to see if H^+ secretion occurred. If no H^+ secretion took place, we concluded that we were dealing with the antrum. Two pairs of electrodes were used, one for sending a 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 lack of a H^+ secretory rate was determined by the pH stat method of Durbin and Heinz [9].

When we change 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. 5 time constants) for the concentration of the ion at the cell membrane to attain the new concentration in the nutrient solution [3]. In the present experiments, the PD change at the 10 min mark was recorded.

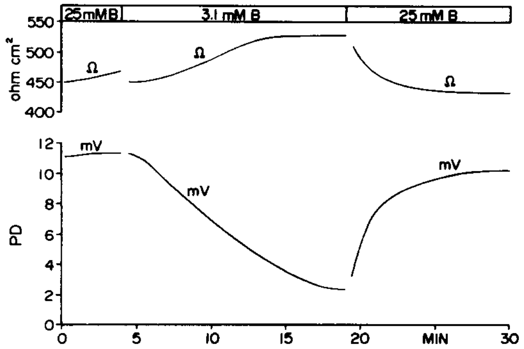

Fig. 1. Effect of changes in HCO_3^- concentration on the nutrient side from 25 to 3.1 HCO_3^- and back to 25 mM HCO_3^-. Resistance and PD are plotted vs. time. B refers to HCO_3^-.

Results

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

Experiments were performed 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 3.12 and 1.56 mM. Fig. 1 is a plot of resistance and PD versus time of part of a representative experiment for changes of 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. Upon return to 25 mM HCO_3^- the PD and resistance returned 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. The reason for the difference in the initial rapidity of the PD response was described in the Introduction.

In Table I, 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 whereas increases in HCO_3^- con-

TABLE I

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 performed to determine the level of signficance. Columns labeled PD and *R* refer to the control values of transmembrane potential difference and resistance, respectively. Columns labeled ΔPD and ΔR refer to the changes in the two parameters following the change to the final solution.

Original soln. $[HCO_3^-]$ (mM)	Final soln. $[HCO_3^-]$ (mM)	PD (mV)	Δ PD (mV)	*R* ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
25.0	12.5	19.6 ± 4.0	-1.9 ± 0.7 [a]	400 ± 98	4 ± 29
25.0	6.2	18.2 ± 5.2	-4.5 ± 1.3 [a]	479 ± 195	50 ± 43 [a]
25.0	3.1	18.0 ± 5.3	-7.1 ± 2.0 [a]	473 ± 177	69 ± 64 [b]
25.0	1.6	17.4 ± 5.8	-9.2 ± 2.0 [a]	460 ± 161	100 ± 70 [a]
12.5	25.0	17.0 ± 4.2	2.0 ± 0.9 [a]	421 ± 115	-37 ± 33 [a]
6.2	25.0	13.2 ± 5.8	4.6 ± 1.8 [a]	527 ± 188	-64 ± 48 [a]
3.1	25.0	9.6 ± 6.1	7.2 ± 2.6 [a]	569 ± 195	-98 ± 60 [a]
1.6	25.0	6.1 ± 6.0	10.1 ± 3.0 [a]	605 ± 196	-153 ± 74 [a]

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

centration returned the PD and resistance essentially to control values.

In Fig. 2 (based on values of Table I), the absolute value of the change in PD, $|\Delta \mathrm{PD}|$, is plotted versus the logarithm of the initial or final concentration of HCO_3^-. 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 concentration from 25 mM HCO_3^- to lower concentration values and also for concentrations from lower concentration values to 25 mM. With the exception of the change from 25 to 1.56 mM HCO_3^- and back to 25 mM, the plotted points can be represented essentially by a single straight line of slope equal to about 9 mV per 10-fold change in HCO_3^- concentration. For the change from 25 to 1.56 mM HCO_3^- or for the reverse change, $|\Delta \mathrm{PD}|$ deviates from the straight line to a small extent.

From these results, it would appear as discussed in the Introduction that either HCO_3^- conductance pathways and/or OH^- conductance pathways exist in the nutrient membrane. For more detailed considerations, see the Discussion.

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

A series of six experiments were performed, each consisting of two parts. In the first part, the nutrient HCO_3^- was changed from 25 to 3 mM and, at the same time, 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 $[CO_2]$ was maintained constant in the nutrient solution and, therefore, the nutrient pH remained constant. However, the reduction of CO_2 in the nutrient solution undoubtedly results in CO_2 diffusing out of the cells and, consequently the cellular pH rises and the cellular OH^- concentration rises (see below). Thus the decrease in nutrient HCO_3^- and the increase in cellular OH^- could each contribute to the decrease in PD. We could not decide by this part of the experiment alone which conductive pathways (HCO_3^-) and/or OH^-) predominate. As Fig. 3 illustrates for one such experiment the PD decrease in the first 10 min after the change from control was about 5 mV. Table II shows that the average decrease for six experiments was 5.6 mV.

In the second part of each experiment, the same changes in the nutrient solution were made as in the first part but simultaneously the gas mixture of the secretory solution was changed from 95% O_2/5% CO_2 to 90% O_2/10% CO_2. The increase in secretory CO_2 would cause CO_2 to diffuse into the cells, thereby decreasing the cellular pH relative to the first part. As a consequence, the OH^- concentration in the cell in the second part should be

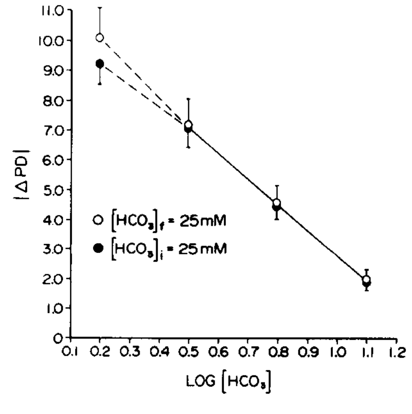

Fig. 2. Absolute value of the change in PD, $|\Delta PD|$, plotted vs. $\log[HCO_3^-]$ in nutrient solution for $[HCO_3^-]_i = 25$ mM (closed circles) and for $[HCO_3^-]_f = 25$ mM (open circles). Half lines represent the standard errors of the mean.

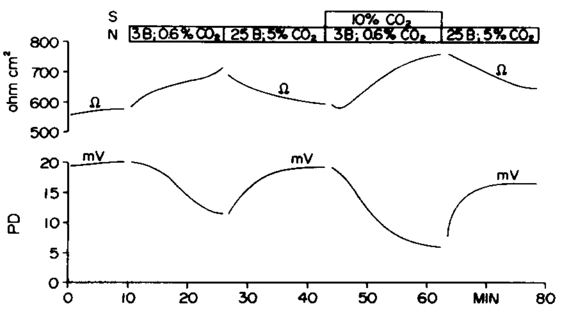

Fig. 3. First part: Effect of changes in HCO_3^- concentration on the nutrient side from 25 to 3 mM HCO_3^- and at same time change of nutrient gases from 95% O_2/5% CO_2 to 99.4% O_2/0.6% CO_2 and finally return to standard solutions and gases. Second part: Same nutrient changes with simultaneous change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

TABLE II

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 obsrvations was performed to determine the level of significance. Columns labeled PD and *R* refer to the control values of transmembrane potential difference and resistance, respectively. Columns labeled ΔPD and ΔR refer to the 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 ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
Part I: NΔHCO_3^- from 25 to 3 mM and NΔCO_2 from 5% to 0.6%			
17.2 ± 3.1	-5.6 ± 1.0 [a]	604 ± 144	71 ± 40 [a]
Part 2: Same as Part 1 and simultaneously SΔCO_2 from 5% to 10%			
17.0 ± 3.9	-10.1 ± 2.4 [a]	607 ± 132	121 ± 67 [a]

[a] $P < 0.01$.

lower than in the first part. If then the conductive pathways responsible for the PD changes were primarily OH^- pathways, the magnitude of the PD decrease in the second part should be less than the magnitude of the PD decrease in the first part. As Fig. 3 shows, the PD decrease in the second part 10 min after the change from control is about 10 mV, i.e. about 100% increase in magnitude compared to the first part. Table II shows that the average decrease in PD for six experiments was 10.1 mV. Comparison of the PD results from the two parts (−5.6 mV versus −10.1 mV) of the six experiment indicates that the average difference of 4.5 mV is statistically significant ($p < 0.01$).

How then do we account for the greater magnitude of the PD decrease in the second part? The increase in CO_2 in the cell due to the 10% CO_2 in the secretory solution would cause 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 we assume that the protein buffering capacity of the antral cells is comparable to that of other cells, e.g., the red cell [10], the HCO_3^- concentration would increase signficantly in the antral cells and hence the ratio of HCO_3^- in the cells to that in the nutrient fluid would be greater when the secretory gas is 90% O_2/10% CO_2 than when it is 95% O_2/5% CO_2. Therefore, the difference between −5.6 mV and −10.1 mV could be explained as the consequence of HCO_3^- conductive pathways in the nutrient membrane.

We might also ask whether the change in PD might be attributed to an effect on the secretory membrane resulting from the change in CO_2 concentration across the secretory membrane. Experiments in which the HCO_3^- concentration on the secretory side was changed from 25 to 3 mM and back to 25 mM showed no significant changes in PD. As mentioned in the Introduction, if no changes in PD occur, then the sum of the HCO_3^-, OH^- and H^+ conductances is zero. Thus it would appear that the PD changes are independent of the secretory membrane.

As Fig. 3 and Table II show, in both parts of the experiment, the resistance increases. In the first part, the resistance increase averaged 71 ohm $\cdot$ cm^2 and, in the second part, the resistance increase averaged 121 ohm $\cdot$ cm^2. The difference, however, is not statistically significant.

In another series of experiments, the nutrient HCO_3^- was maintained at 3 mM and the nutrient gas mixture was 99.4% O_2/0.6% CO_2. The gas mixture on the secretory side was periodically changed with extreme changes from 0.6% to 10% CO_2 and back to 0.6% CO_2. In seven out of eight experiments, it was found that an increase in secretory p_{CO_2} decreased the PD and that a decrease in secretory p_{CO_2} increased the PD. In the change from 0.6% to 10% CO_2, the PD decreased on average by -2.9 ± 1.6 (S.D.) mV and, in the return to 0.6% CO_2, the PD increased on the average by 2.4 ± 1.1 (S.D.) mV. These changes in PD due to the change in the secretory gas alone are in accord with our interpretation of the previous data in that the PD decrease is due to an increase of cellular HCO_3^- and the PD increase is due to a decrease of cellular HCO_3^-. In one experiment, the PD results were exactly opposite to those in the other seven experiments.

Discussion

As mentioned in the Introduction, one of the main purposes of this paper was to determine whether a linear relationship existed between $|\Delta PD|$ and log $[HCO_3^-]$. In order to see why a part of the curve is linear or approximately linear, let us refer to the equivalent circuit of Fig. 4 for the nutrient membrane comprising a HCO_3^- conductive limb and an X-conductive limb representing all other ionic pathways. Then, for these two limbs, the PD is given by

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

If HCO_3^- is changed in the nutrient solution, Eqn. 1 becomes

$$\Delta PD = -R_X(R_{HCO_3} + R_X)^{-1}\Delta E_{HCO_3} \quad (2)$$

provided R_X and R_{HCO_3} remain essentially constant. In previous work with K^+ [3,11], a linear relationship was obtained for changes of K^+ in the nutrient solution for the fundus and the antrum beyond a certain K^+ concentration (10 mM in the fundus and 20 mM in the antrum). For higher concentrations, further increases in concentration did not alter the resistance. Unlike the changes in K^+ concentration, the changes in HCO_3^- concentration show resistance changes throughout the range of concentrations under consideration (see Table I). Since it is known that, for HCO_3^- concentration changes, the resistances of other ionic pathways change [1,2], another possibility is that both R_X and R_{HCO_3} increase or decrease essentially proportionally to each other. For example, if as the concentration of HCO_3^- decreases, R_X and R_{HCO_3} increase inversely proportional to the concentration, then $R_X/(R_{HCO3} + R_X)$ is a constant independent of concentration. On the other hand, if only R_X or R_{HCO_3} varies, then there can be no linear relationship between $|\Delta PD|$ and log $[HCO_3^-]$. At the very best, we would be hard pressed to develop a model giving a linear relationship based on the variation of R_X alone with a change in HCO_3^- concentration.

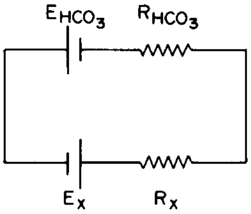

Fig. 4. Equivalent circuit for nutrient membrane comprising a HCO_3^- limb and an X limb representing all other ionic pathways. Right-hand side is nutrient and left-hand side is cell.

We next look at the relationship existing among HCO_3^-, OH^- and H^+. From the discussion in the Introduction, the rapidity of the initial PD response due to H^+ should be opposite to that found as shown in Fig. 1, i.e. the H^+ conductance channel could not be the major determinant of the PD change. It followed that HCO_3^- and OH^- which increased or decreased in concentration together could each acccount for the initial PD response.

From experiments like the one illustrated by Fig. 3, it was evident that HCO_3^- and not OH^- was involved in the linear relationship. From the argument based on the possible buffering capacity of the antral cells (see Results), the existence of a HCO_3^- conductance pathway was indicated as the predominant pathway among the three ions, namely, HCO_3^-, OH^- and H^-.

It will be recalled from Table II that the change in PD was -10.1 mV when the secretory gas was changed from 5% to 10% CO_2 with simultaneous decrease of HCO_3^- and -5.6 mV when the secretory gas was maintained at 5%. One might predict that changing the secretory gas by itself would result in a ΔPD which could be equal to the difference between -5.6 and -10.1 mV, i.e., -4.5 mV. However, from the results it should be noted that when the gas in the secretory solution was changed without HCO_3^- change, the magnitude of the decrease in PD was 2.9 mV. At present we are unable to account for the difference between these results, namely 4.5 and 2.9 mV. Our attempts to explain this discrepancy involve too many unproved postulates and hence we do not feel justified in presenting an explanation.

In the experiments of Flemstrom and Sachs [6], they inferred the existence of a HCO_3^- conductance in the nutrient membrane based on the change in transmucosal PD with variation in the HCO_3^- concentration. They did not attempt to determine whether there was a linear relationship between $|\Delta PD|$ and $\log [HCO_3^-]$ and they did not attempt to differentiate among the ions HCO_3^-, OH^- and H^+. On the secretory membrane, we were unable to confirm their inference of a HCO_3^- pathway since we found no significant variation in PD with a change in HCO_3^- concentration. In our experiments, we used a hypertonic solution [8] on the secretory side and, under these circumstances, there was no evidence of a HCO_3^- conductance pathway in the secretory membrane. However, Johansson et al. [12] reported that E_2 prostaglandins stimulate gastric HCO_3^- secretion. In future experiments, it would be of interest to determine whether prostaglandins will evoke a PD response as suggested by G. Sachs (personal communication).

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