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EFFECT OF REDUCED pH IN ABSENCE OF HCO_3^- ON ANOMALOUS AND NORMAL POTENTIAL RESPONSES IN BULLFROG ANTRUM

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Effect of changing [K^+], [Na^+] and [Cl^-] in nutrient solution on potential difference (PD) and resistance was studied in bullfrog antrum with and without nutrient HCO_3^- but with 95% O_2/5% CO_2 in both cases. In both cases, changing from 4 to 40 mM K^+ gave about the same initial PD maximum (anomalous response) which was followed by a decrease below control level. Latter effect was much less with zero than with 25 mM HCO_3^-. Changing from 102 to 8 mM Na^+ gave initial normal PD response about the same in both cases. However, 10 min later the change in PD with zero HCO_3^- was insignificant but with 25 mM HCO_3^- the PD decreased (anomalous response of electrogenic NaCl symport). PD maxima due to K^+ and Na^+ were largely related to ($Na^+ + K^+$)-ATPase pump. Changes in nutrient Cl^- from 81 to 8.1 mM gave only a decrease in PD (normal response). Initial PD increases are explained by relative increases in resistance of simple conductance pathways and of parallel pathways of ($Na^+ + K^+$)-ATPase pump and Na^+/Cl^- symport. Removal of HCO_3^- and concurrent reduction of pH modify resistance of these pathways.

Introduction

In studies of the antrum of the bullfrog, *Rana catesbeiana*, changes in K^+ concentration in the nutrient solution in Cl^- media result in two types of PD response [1,2]. First an increase of the nutrient K^+ concentration from 0 or 4 mM to higher concentrations decreases the potential difference of the nutrient relative to the secretory side of the antrum and a decrease of the nutrient K^+ concentration increases the PD. This response is defined as a normal PD response and is attributed to passive, conductance pathways for K^+ in the nutrient membrane. Second, after the antrum is bathed in Cl^- solutions without K^+ for 20 min or more, an increase in nutrient K^+ to 4 mM increases the positivity of the nutrient and a return within a few minutes to zero K^+ decreases the positivity of the nutrient. This response is defined as an anomalous PD response and is attributed because of its abolition by ouabain to a ($Na^+ + K^+$)-ATPase pump on the nutrient-facing membrane in which more Na^+ than K^+ is transported per cycle across the nutrient membrane.

Recently, the anomalous PD response was obtained for a change from a non-zero concentration of K^+ (4 mM) in the nutrient solution to a higher concentration (40 mM) [3]. This was found under the condition of zero HCO_3^- in the nutrient solution with phosphate buffer in that solution to maintain pH at 7.3 while both sides of the antrum were gassed with 100% O_2. In this case, the initial response of about 8 mV above the control level (anomalous response) was followed by a decrease in PD below the control level (normal response). Under similar conditions, a decrease in Na^+ concentration from 102 to 8 mM gave an initial increase in PD of about 6 mV above the control

Abbreviation: PD, potential difference.

level (normal response) followed by a decrease in PD below the control level (anomalous response). The initial anomalous response of K^+ and a major part of the initial normal response of Na^+ are associated with the $(Na^+ + K^+)$-ATPase pump since ouabain (1 mM) eliminates the complete response due to K^+ and the major part due to Na^+. The effects following the initial responses, the normal response due to K^+ and the anomalous response due to Na^+, are associated respectively with K^+ conductive pathways [3] and with an electrogenic Na^+/Cl^- symport [3,4], both in the nutrient membrane. With 95% O_2/5% CO_2 gassing both sides of the antrum and with HCO_3^- in the nutrient solution, the initial PD response of K^+ was either small (about 2.5 mV) or absent and the initial PD response of Na^+ was generally small (also about 2.5 mV). For this reason they were not seriously considered until their accentuation under the conditions described above for 100% O_2 and zero HCO_3^-. At the same time the latter conditions depressed the PD effects following the initial response.

In the present study, both sides of the antrum are gassed with 95% O_2/5% CO_2 throughout each experiment. The effect of the removal of nutrient HCO_3^- with the concurrent decrease in nutrient pH to about 5 on normal and anomalous PD responses is examined for changes in concentration of K^+, Na^+ and Cl^- in the nutrient solution.

Methods

Experiments were performed an 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 [5]. 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: Na^+, 102; K^+, 4; and Cl^-, 106. In all experiments both sides of the antrum were gassed with 95% O_2/5% CO_2. For the HCO_3^--free nutrient solution, the 25 mM HCO_3^- was replaced with 25 mM Cl^- or, in Cl^- changes, with 12.5 mM SO_4^{2-} plus sucrose.

In these studies, the transmembrane resistance and the transmembrane potential difference were measured. To assure that the antrum was being 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 we were dealing with the antrum. Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The PD was considered positive when the nutrient side was positive relative to the secretory side of the frog 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 [6].

For the PD response on the nutrient side of the gastric mucosa, an ion substitution method was used. In K^+ concentration changes, K^+ was replaced with Na^+; in Na^+ concentration changes, Na^+ was replaced with choline; and in Cl^- concentration changes, Cl^- was replaced with SO_4^{2-}. In the last case, sucrose was added to make up any osmotic deficit. In the technique of ion substitution [7], the concentration of a given ion is rapidly changed and the time-course of the change in PD is recorded. 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

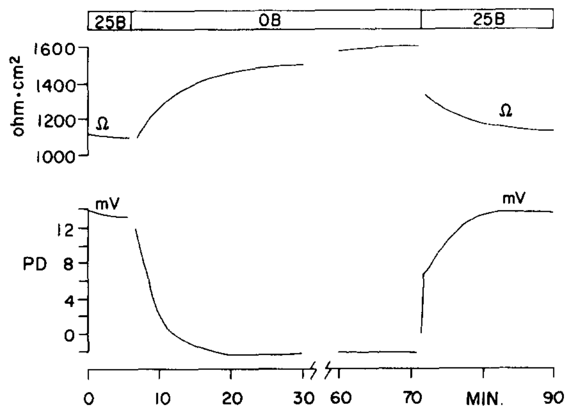

Fig. 1. Effect of changes in HCO_3^- concentration on the nutrient side from 25 mM to zero HCO_3^- and back to 25 mM HCO_3^-. Resistance and PD are plotted vs. time. B refers to HCO_3^-.

at the cell membrane to attain the new concentration in the nutrient solution. Consequently, most of the PD change of interest occurs during the first 10 min.

In the present studies for K^+ and Na^+, PD readings were used as follows. For an increase in K^+ from 4 to 40 mM in the nutrient solution or a decrease in Na^+ from 102 to 8 mM in the nutrient solution, there was an initial maximum in PD within the first minute or so. This maximum was determined. The other reading used was the 10-min value of the PD. For reverse changes, the initial minimum if present was determined and also the 10-min value of the PD.

Results

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

Fig. 1 is a plot of resistance and PD versus time of a representative experiment for changes of HCO_3^- concentration from 25 mM to zero in the nutrient solution and the return to 25 mM. The decrease from 25 mM HCO_3^- to zero gave a marked decrease in PD and a marked increase in resistance. Upon return to 25 mM HCO_3 one hour later, the PD and resistance returned to control values. As Fig. 1 shows, the PD changes less rapidly in the initial phase in going from 25 mM to zero HCO_3^- than in the reverse direction. In general, for both cation and anion conductances, an increase in concentration gives a faster initial PD response than a decrease in concentration.

The latter effect, as explained elsewhere [3,7,8], is a consequence of the existence of a diffusion barrier so that 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 of concentration at the cell border versus time is the same whether the concentration of the nutrient solution is increased or decreased. However, since the PD depends on the logarithm of the concentration ratio across the nutrient membrane, the PD at the nutrient membrane changes less rapidly in going, e.g., from 25 to 22 mM HCO_3^- on the way to 1 mM HCO_3^- than in going from 1 to 4 mM HCO_3^- on the way to 25 mM HCO_3^-. The change from 25 to 22 mM HCO_3^- gives a ratio of 25 : 22 whereas the change from 1 to 4 gives a ratio of 4 : 1. The greater the ratio, the greater is the initial PD change. These considerations are compatible with the existence of HCO_3^- conductance channels. However, since removal of HCO_3^- decreases the pH and return of HCO_3^- increases the pH, the decrease in HCO_3^- is accompanied by a decrease in OH^- and an increase in H^+ and vice versa. This further consideration implies the possible existence of OH^- but not H^+ conductance pathways in the nutrient membrane. Without further studies using other HCO_3^- concentrations and other pH changes, the implication remains tentative. Moreover, changes in HCO_3^- and pH may also influence the conductive pathways of other ions.

In five experiments after about one hour with zero HCO_3^- nutrient solutions, the PD decreased by 18.3 ± 5.1 (S.D.) mV from a control of 16.7 ± 5.4 (S.D.) mV and the resistance increased by 403 ± 108 (S.D.) $\Omega \cdot cm^2$ from a control of 802 ± 247 (S.D.) $\Omega \cdot cm^2$. Upon return to 25 mM HCO_3^-, the PD and resistance returned to control values.

In a previous paper [3], a similar increase in the magnitude of the resistance under the condition of zero HCO_3^-, pH 7.3 and 100% O_2 was found. There was a marked increase in the anomalous PD response due to K^+ which was attributed to the high resistance (for details, see Ref. 3 and Discussion). The question naturally arises whether there is a similar anomalous PD response due to K^+ under present conditions (zero HCO_3^-, low pH).

Normal and anomalous PD responses due to changes in K^+ concentration in zero HCO_3^- nutrient solutions

Fig. 2 shows the changes in PD and resistance with 25 mM and zero HCO_3^- nutrient solutions in changing in each case the nutrient K^+ concentration from 4 to 40 mM and back to 4 mM. With 25 mM HCO_3^- nutrient solutions, the increase in nutrient K^+ results in an initial PD maximum of 0.6 mV above a control of 22 mV and 10 min later in a decrease of the PD by 12 mV below the control level. Upon return to 4 mM K^+, no PD minimum occurs (see Discussion) and the PD returns to a near control level. During the K^+ increase, the resistance decreases markedly and, during the K^+ decrease, the resistance increases to

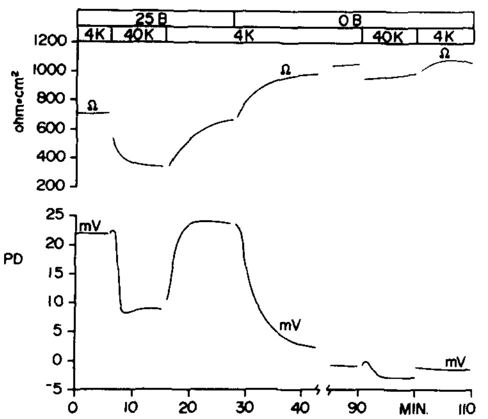

Fig. 2. Effect of changes in K^+ concentration on the nutrient side from 4 to 40 mM K^+ and back to 4 mM K^+ with 25 mM and zero HCO_3^- nutrient solutions. Resistance and PD vs. time.

the control level. After about 1 h in zero HCO_3^-, the PD falls to about -1 mV and the resistance rises to about 1050 $\Omega \cdot cm^2$. Increasing the nutrient K^+ concentration to 40 mM results in an initial PD maximum of about 1 mV above the control level and 10 min later in a small decrease in PD of about 2 mV below the control level. The return to 4 mM K^+ gave no PD minimum and in 10 min a return to the control level. During the K^+ increase, the resistance decreased in 10 min by about 60 $\Omega \cdot cm^2$ (an atypical result; see Table I) and, during the K^+ decrease, the resistance increased in 10 min by about 10 $\Omega \cdot cm^2$.

In Table I, the initial PD maximum and the PD effect 10 min after the change in nutrient K^+ concentration are shown for zero HCO_3^- nutrient solutions. For an increase from 4 to 40 mM K^+, the initial PD increase (anomalous response) was followed by a PD decrease below the control level (normal response). The initial increase of 1.2 mV compares to 1.7 mV with 25 mM HCO_3^- nutrient solutions [3] and the decrease after 10 min of 2.6 mV below the control level is much less than the decrease of 13 mV below the control level with 25 mM HCO_3^- [3].

With zero HCO_3^- nutrient solutions, the resistance changes after 10 min were insignificant whereas with 25 mM HCO_3^- nutrient solutions, the resistance decrease with an increase in K^+ and the resistance increase with a decrease in K^+ were marked and significant in both cases [3]. For both 25 mM and zero HCO_3^-, the resistance showed a decrease at the PD maximum (see Table I and Ref. 3).

It is clear that under present conditions with zero HCO_3^- and low nutrient pH, the anomalous PD response to K^+ is much less than under conditions with zero HCO_3^- and nutrient pH = 7.3.

Normal and anomalous PD responses due to changes in Na^+ concentration in zero HCO_3^- nutrient solutions

Table II summarizes the principal results in zero HCO_3^- nutrient solutions. The change in Na^+

TABLE I

EFFECT ON PD AND RESISTANCE OF CHANGES IN K^+ CONCENTRATIONS ON THE NUTRIENT SIDE WITHOUT HCO_3^-

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 the transmembrane potential difference and corresponding resistance and columns labeled ΔPD and ΔR refer to changes in the two parameters following the change to the final concentration of K^+.

Original solution $[K^+]$ (mM)	Final solution $[K^+]$ (mM)	PD (mV)	ΔPD (mV)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
Maximum PD					
4	40	2.4 ± 3.2	1.2 ± 1.2 [b]	1252 ± 599	−79 ± 53 [a]
PD 10 min after control					
4	40	2.4 ± 3.2	−2.6 ± 1.3 [a]	1252 ± 599	27 ± 55
40	4	−0.5 ± 2.0	1.5 ± 0.7 [a]	1221 ± 487	83 ± 101

[a] $P < 0.01$ for seven experiments.
[b] $P < 0.05$ for seven experiments.

TABLE II

EFFECT ON PD AND RESISTANCE OF CHANGES IN Na^+ CONCENTRATIONS ON THE NUTRIENT SIDE WITHOUT HCO_3^-

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 the transmembrane potential difference and corresponding resistance and columns labeled ΔPD and ΔR refer to changes in the two parameters following the change to the final concentration of Na^+.

Original solution $[Na^+]$ (mM)	Final solution $[Na^+]$ (mM)	PD (mV)	ΔPD (mV)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
Maximum PD					
102	8	2.6 ± 2.7	3.4 ± 2.2 [a]	1230 ± 320	18 ± 102
Minimum PD					
8	102	2.3 ± 1.6	−2.5 ± 2.2 [a]	1259 ± 360	−86 ± 101 [b]
PD 10 min after control					
102	8	2.6 ± 2.7	−0.1 ± 1.9	1230 ± 320	49 ± 140
8	102	2.3 ± 1.6	−1.4 ± 2.3	1259 ± 360	−11 ± 115

[a] $P < 0.01$ for ten experiments.
[b] $P < 0.05$ for ten experiments.

concentration from 102 to 8 mM in the nutrient solution gave an initial PD maximum of 3.4 mV above the control level (normal response) and no significant PD change 10 min later. The return to 102 mM Na^+ gave an initial minimum of 2.5 mV below the control level and again no significant PD change 10 min later. With 25 mM HCO_3^- nutrient solutions, the PD maximum was 2.5 mV above the control level and the PD minimum was about 1.0 mV below the control level [3]. Moreover with 25 mM HCO_3^-, the decrease in Na^+ concentration from 102 to 8 mM Na^+ resulted 10 min later in a PD decrease of 6.8 mV and the return to 102 mM Na^+ resulted in a PD increase of 7.5 mV. Both of the latter PD changes are anomalous PD responses associated with a Na^+/Cl^- symport in the antrum [3,4].

With zero HCO_3^- in the nutrient solution, the only significant change in resistance occurred at the PD minimum, a decrease of 86 $\Omega \cdot cm^2$. With 25 mM HCO_3^- in the nutrient solution, there was a smaller decrease at the PD minimum of 45 $\Omega \cdot cm^2$. In addition with 25 mM HCO_3^-, larger significant changes occurred after 10 min, namely an increase in resistance with the decrease in Na^+ concentratoin from 102 to 8 mM and a decrease in resistance with the return to 102 mM Na^+. Both changes were of the order of 100 $\Omega \cdot cm^2$ [3].

Normal PD responses due to changes in Cl^- concentration in zero HCO_3^- nutrient solutions

Table III summarizes the principal results in zero HCO_3^- nutrient solutions. There were no maxima or minima in PD. The decrease in Cl^- concentration from 81 to 8.1 mM in the nutrient solution gave in 10 min a decrease of 8.8 mV and the return to 81 mM, an increase of 7.4 mV. With 25 mM HCO_3^-, the respective changes in PD were −15.3 and 15.3 mV [3]. With zero HCO_3^-, the resistance increased with a decrease in Cl^- concentration and decreased with an increase in Cl^- concentration. With 25 mM HCO_3^-, only the increase in Cl^- from 8.1 to 81 mM gave a significant change, a decrease somewhat smaller than the decrease with zero HCO_3^-.

The Cl^- results are in accord with a model of a Na^+/Cl^- symport (in which more Cl^- than Na^+ is transported per cycle) in parallel with a simple, passive conductance pathway for Cl^-. For both limbs in the nutrient membrane, the PD response to Cl^- changes is normal. Since Table II shows that with zero HCO_3^- the anomalous PD response of Na^+ is abolished, it seems that with zero HCO_3^- the Cl^- is transported only through the simple conductance pathway. The decrease in ΔPD from 15.3 mV with 25 mM HCO_3^- to 8.8 mV with zero HCO_3^- is consistent with the inhibition of the

TABLE III

EFFECT ON PD AND RESISTANCE OF CHANGES IN Cl^- CONCENTRATIONS ON THE NUTRIENT SIDE WITHOUT HCO_3^-

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 the transmembrane potential difference and corresponding resistance and columns labeled ΔPD and ΔR refer to changes in the two parameters following the change to the final concentration of Cl^-.

Original solution $[Cl^-]$ (mM)	Final solution $[Cl^-]$ (mM)	PD (mV)	ΔPD (mV)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
81	8.1	0.6 ± 2.2	-8.8 ± 1.4 [a]	888 ± 249	128 ± 75 [a]
8.1	81	-8.1 ± 1.9	7.4 ± 1.9 [a]	1014 ± 130	-112 ± 116 [b]

[a] $P < 0.01$ for eight experiments.
[b] $P < 0.05$ for eight experiments.

Na^+/Cl^- symport but can arise at least in part from a change in resistance of other pathways in going from 25 mM to zero HCO_3^- nutrient solutions.

Discussion

We shall first consider the anomalous PD response in the antrum obtained previously in two different ways upon increasing the K^+ concentration from zero K^+ or from 4 mM K^+ (see Introduction). In particular, the PD maxima is about 2 mV above the control level in the increase from 4 to 40 mM K^+ in 25 mM HCO_3^- nutrient solutions and is enhanced to 7.8 mV in the same K^+ change

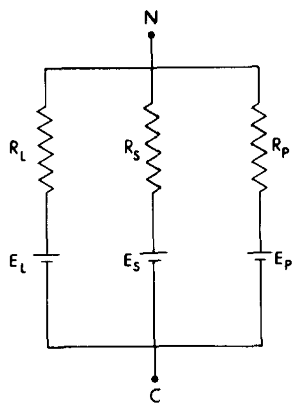

Fig. 3. Equivalent circuit for nutrient membrane comprising L limb representing the leak pathway, S limb representing the NaCl symport pathway and P limb representing the $(Na^+ + K^+)$-ATPase pump pathway. C refers to cell and N to nutrient.

in zero HCO_3^- nutrient solutions containing phosphate buffer to maintain the pH at 7.3. In the first case, both sides of the antrum were gassed with 95% O_2/5% CO_2 and, in the second case, both sides were gassed with 100% O_2 [3]. We shall briefly review our explanation of the enhancement in PD [3]. For this purpose, we consider first the $(Na^+ + K^+)$-ATPase pump and second the electrical circuit (Fig. 3) comprising three limbs: the $(Na^+ + K^+)$-ATPase pump pathway, the Na^+/Cl^- symport pathway and the conductance or leak pathway for K^+, Cl^-, HCO_3^-, etc.

Since ouabain or lack of Na^+ in the nutrient solution abolished the anomalous PD response of K^+, it was reasonable to assume that this response was caused by the $(Na^+ + K^+)$-ATPase pump. Thermodynamic considerations [1,2] gave a relation for the emf E_P of the electrogenic $(Na^+ + K^+)$-ATPase pump under equilibrium conditions with $n \neq m$, namely

$$E_P = \frac{nRT}{(n-m)F} \ln \frac{[Na^+]_C}{[Na^+]_N} + \frac{mRT}{(n-m)F} \ln \frac{[K^+]_N}{[K^+]_C} + E_X \quad (1)$$

$$E_X = \frac{RT}{(n-m)F} \ln k \frac{[ATP]}{[ADP][P_i]} \quad (2)$$

where n is the number of Na^+ ions transported out of the cell (C) and m is the number of K^+ ions transported from the nutrient (N) in to the cell in each cycle; R, T and F have their usual meanings; E_X is the contribution of the active transport energy to the emf [2]. If then, $n > m$, it follows from Eqn. 1 that increasing the nutrient K^+ concentration increases E_P (anomalous response) and increasing the nutrient Na^+ concentration decreases E_P (normal response).

In order to determine the conditions under which the anomalous response will occur we next refer to the electrical circuit (Fig. 3). In this circuit E_P, E_S, and E_L represent the emf's of the pump, symport, and leak pathways and R_P, R_S, and R_L their respective resistances. For changes in nutrient K^+ concentrations, the symport which involves Na^+ and Cl^- concentration changes can be ignored. Then the PD due to the two pathways of pump and leak is given by

$$PD = \frac{R_L E_P + R_P E_L}{R_P + R_L} \tag{3}$$

For a change in K^+ concentration in the nutrient membrane, the change in PD with R_L and R_P assumed constant is

$$\Delta PD = \frac{R_L \Delta E_P + R_P \Delta E_L}{R_P + R_L} \tag{4}$$

Then for increases in K^+ concentration, ΔE_P is positive and ΔE_L is negative. For an anomalous PD response, it follows from Eqn. 4 that the magnitude of $R_L \Delta E_P$ is greater than the magnitude of $R_P \Delta E_L$. If we assume for simplicity $n = 2$, $m = 1$, then for a 10-fold increase in K^+, $\Delta E_P = 60$ mV from Eqn. 1 and $\Delta E_L = -60$ mV from the Nernst equation. The anomalous response occurs for $R_P < R_L$ and the normal response occurs for $R_P > R_L$. Thus with a reasonably high leak resistance, an anomalous response occurs. Under the conditions of zero HCO_3^-, 100% O_2 and pH = 7.3, the maximum PD (anomalous response) was enhanced. The implication of this model is that R_L has increased relative to R_P to account for the increase in the anomalous PD.

In zero HCO_3^- and low pH nutrient solutions, with 95% O_2/5% CO_2, the resistance in seven experiments averages 1252 ± 599 (S.D.) $\Omega \cdot cm^2$ (see Table I) and with 100% O_2 and pH = 7.3, the resistance in eight experiments averages 1007 ± 172 (S.D.) $\Omega \cdot cm^2$ [3]. One might be tempted to attribute the decrease in the maximum PD to the increase of 245 $\Omega \cdot cm^2$ such that the latter increase brings R_P closer to R_L. However, statistical analysis shows that the difference in resistance is not significant. Nevertheless, within the framework of the model, it is not unreasonable to assume that under the conditions of the present experiments the increased resistance compared to control levels does not increase R_L relative to R_P but increases R_P as well as R_L. Consequently, the maximum PD is not enhanced as in the case of 100% O_2 and pH = 7.3 but is of the same order of magnitude as in the case of the control conditions of 95% O_2/5% CO_2 and 25 mM HCO_3^-.

We now consider the initial normal PD response due to Na^+. This response is associated largely with the anomalous response due to K^+. With zero HCO_3^-, 100% O_2 and pH = 7.3, this accentuated effect is largely due to the ($Na^+ + K^+$)-ATPase pump but after ouabain a small part (about 2 mV) remains. The latter is attributed to a high-resistance Na^+ conductance pathway [3]. In order to explain why an initial enhanced normal response occurs, we need to consider the Na^+/Cl^- symport and then again the electrical circuit.

It was shown [4] that the emf due to a passive, electrogenic symport for $p \neq q$ was given by

$$E_S = \frac{q}{q-p}\frac{RT}{F}\ln\frac{[Cl^-]_N}{[Cl^-]_C} + \frac{p}{q-p}\frac{RT}{F}\ln\frac{[Na^+]_N}{[Na^+]_C} \tag{5}$$

where E_S is the electromotive force of the symport and where p is the number of Na^+ and q the number of Cl^- transported in each cycle. If now $q > p$, the PD is anomalous for Na^+ and normal for Cl.

Next we turn to the electrical circuit. Since the Na^+ leak pathway is assumed to have a high resistance, we can again consider two limbs primarily, the Na^+/Cl^- symport pathway and the ($Na^+ + K^+$)-ATPase pump pathway. For the two limbs, the PD is given by

$$PD = \frac{R_S E_P + R_P E_S}{R_P + R_S} \tag{6}$$

If the Na^+ concentration is changed, then for constant resistances R_P and R_S

$$\Delta PD = \frac{R_S \Delta E_P + R_P \Delta E_S}{R_P + R_S} \tag{7}$$

If now, for the sake of discussion, we assume

$m = 1$, $n = 2$, $p = 1$, $q = 2$, we obtain for a 10-fold decrease in Na^+ concentration from Eqns. 1 and 5 $\Delta E_P = 120$ mV and $\Delta E_S = -60$ mV. Under these conditions from Eqn. 7, a normal PD response occurs if $2R_S > R_P$ and an anomalous PD response if $2R_S < R_P$ (see Ref. 3 for details).

As in the case of K^+, Na^+ also shows no significant difference in resistance between the case of 95% O_2/5% CO_2 and the case of 100% O_2, pH = 7.3, for zero HCO_3^- in both cases. Again as for K^+, R_P increases so that for the normal PD response R_P is closer to $2R_S$. Hence the difference between $2R_S$ and R_P is less than in the case of 100% O_2, pH = 7.3. Under these circumstances the PD maximum is reduced compared to that in 100% O_2, pH = 7.3. The elimination of the anomalous PD response for Na^+ can also be explained by an increase in R_P such that $2R_S$ and R_P have very little difference.

In the case of changing from 40 to 4 mM K^+ with zero HCO_3^-, 100% O_2 and pH = 7.3, there was no initial PD minimum [3]. This was attributed to the decrease in resistance so that R_L was not greater than R_P [3]. In the present case, the resistance did not change significantly. Hence one might expect an initial PD minimum. However, the initial maximum PD while significant was small (ΔPD = 1.2 mV) so that any initial PD minimum would likely at best be small.

The change in Cl^- concentration showed no anomalous PD response. The electrogenic Na^+/Cl^- symport and/or the simple conductance pathway for Cl^- can explain the normal PD responses.

The present studies together with previous studies [3] demonstrate that removal of HCO_3^- from the bathing media modifies the characteristics of the mucosa. With zero HCO_3^- and 95% O_2/5% CO_2 (pH = 5), all PD responses to Na^+, K^+ and Cl^- are reduced or abolished. In contrast, with zero HCO_3^- and 100% O_2 (pH = 7.3), the PD responses associated with the ($Na^+ + K^+$)-ATPase pump, the anomalous PD response due to K^+ and the normal PD response due to Na^+, are enhanced while all other PD responses to Na^+, K^+ and Cl^- are reduced. However, there is a need to determine more affirmatively whether HCO_3^- has a conductance pathway in the nutrient membrane and how HCO_3^- and pH might influence the conductive pathways of other ions.

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