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POTENTIAL DIFFERENCE RESPONSES DUE TO K^+, Na^+ AND Cl^- CHANGES IN BULLFROG ANTRUM WITH AND WITHOUT HCO_3^-

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The effect of changing $[K^+]$, $[Na^+]$ and $[Cl^-]$ in nutrient solution was studied in bullfrog antrum with and without HCO_3^- in nutrient. In 25 mM HCO_3^- (95% O_2/5% CO_2) and in zero HCO_3^- (100% O_2), nutrient pH was maintained at 7.3. Changing from 4 to 40 mM K^+ or from 81 to 8.1 mM Cl^- gave a decrease 10 min later in transmucosal PD (nutrient became more negative) – a normal response. These responses were less in zero than in 25 mM HCO_3^-. A decrease from 102 to 8 mM Na^+ decreased PD (anomalous response of electrogenic NaCl symport). This effect was attenuated or eliminated in zero HCO_3^-. In contrast, change from 4 to 40 mM K^+ gave initial anomalous PD response and change from 102 to 8 mM Na^+, initial normal PD response with either zero or 25 mM HCO_3^-. Both responses were associated with $(Na^+ + K^+)$-ATPase pump and were greater in zero than in 25 mM HCO_3^-. Initial PD increases in zero HCO_3^- are explained as due to increase in the resistance of passive conductance and/or NaCl symport pathways. Thus, removal of HCO_3^- modifies conductance pathways of nutrient membrane.

Introduction

In previous studies of the antrum of the bullfrog, *Rana catesbeiana*, it was found that changing the K^+ concentration in the nutrient solution in Cl^- media gave two types of PD response. First, increasing the nutrient K^+ concentration from 4 to 40 mM decreased the potential difference (PD) of the nutrient relative to the secretory side of the antrum about 16 mV and a return to 4 mM increased the PD about 16 mV [1]. This type of response is defined as a normal PD response and is attributed to passive, conductance pathways for K^+ in the nutrient membrane. Second, if the antrum were bathed with Cl^- solutions without K^+ for 20 min or more, then an increase in nutrient K^+ to 4 mM increased the positivity of the nutrient up to a maximum of about 12 mV and a return shortly thereafter to zero K^+ decreased the positivity of the nutrient. This type of 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 [1].

The question arises: Can one obtain the anomalous response for changes from non-zero to higher concentrations of K^+? Two bits of evidence indicated that an anomalous PD response starting with a non-zero concentration of K^+ might be possible. First, the change from 4 to 40 mM K^+ for the antrum showed, in two-thirds of the experiments in the initial minute or so after the change, a small increase of about 2 mV followed by a rapid decrease by some 16 mV below control

Abbreviation: PD, potential difference.

levels. Since the initial response was small and did not occur in all experiments, it was at first ignored. Second, previous considerations [1,2] indicated that, in order to unmask the anomalous response, the resistance of the passive K^+ conductance pathway has to increase relative to the resistance of the $(Na^+ + K^+)$-ATPase pathway. In this way, the anomalous PD response becomes dominant over the normal PD response. As it turns out, the antrum with zero K^+ in the nutrient solution has a high transmembrane resistance, whereas the antrum with 4 mM K^+ in the nutrient solution usually has a lower resistance. How then might the transmembrane resistance of the mucosa be increased without going to zero K^+ and thereby possibly increasing the resistance of the nutrient K^+ conductance pathway relative to the resistance of the (Na^+K^+)-ATPase pump?

Spenney et al. [3] found that, gassing both sides of the antral gastric mucosa with 95% O_2/5% CO_2 and changing the serosal solution from 18 mM HCO_3^- to zero HCO_3^- increased the cellular resistance. More recently, Manning and Machen [4] found that in the non-secreting fundus (metiamide-treated) with either 95% O_2/5% CO_2 or 100% O_2, HCO_3^--free solutions caused large decreases in transmucosal conductance (i.e., large increases in transmembrane resistance). Hence, experiments with zero HCO_3^- in both the luminal and serosal solutions seemed to offer a means to raise the resistance and to determine an anomalous response, starting from solutions containing K^+.

We, therefore, decided to see whether the anomalous response might be elicited by changing from 2 or 4 mM K^+ to higher K^+ concentrations and using HCO_3^--free solutions and gassing with either 95% O_2/5% CO_2 or 100% O_2. Since HCO_3^--free solutions, 100% O_2 on both sides and a PO_4 buffer to maintain the pH of the nutrient solution constant gave the most pronounced anomalous response for K^+, this paper is limited to PD responses under these conditions. The study was broadened to consider the effects of changes in concentrations of Na^+ and Cl^- as well as K^+ in the nutrient solution on the normal and anomalous PD responses. A preliminary report of some aspects of this work is provided elsewhere [5].

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 [6]. 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)/glucose (10) and the Cl^- secretory (mucosal) solution: Na^+ (102)/K^+ (4)/Cl^- (106). In control experiments, the nutrient contained 25 mM HCO_3^- and both sides of the mucosal were gassed with 95% O_2/5% CO_2. On changing to a HCO_3^--free nutrient solution, both sides of the mucosa were always gassed with 100% O_2 and a PO_4 buffer was present in the nutrient solution to maintain the nutrient pH constant at 7.2 or 7.3. In this case, the standard Cl^- nutrient solution contained: Na^+ (100)/K^+ (4)/Ca^{2+} (1)/Mg^{2+} (0.8)/Cl^- (81)/SO_4^{2-} (0.8)/HPO_4^{2-} (11)/$H_2PO_4^-$ (3)/glucose (10)/sucrose (11.5).

In these studies, the transmembrane resistance and the transmembrane potential difference (PD) were measured. As a test that the antrum was being used, histamine was added to the nutrient solution to a concentration of $1 \cdot 10^{-4}$ M to see if H^+ secretion occurred. The tissue area was 1.3 cm^2 and, since no H^+ secretion took place, we were certain 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 taken as positive when the nutrient side was positive relative to the secretory side of the frog gastric mucosa. The resistance was determined as the change in PD/unit of applied current. Current (20 μA/1.3 cm^2) was applied for 1 or 2 s, first in one direction and 2 or 3 s later, in the other direction. No significant rectification was observed. The lack of a H^+ secretory rate was determined by the pH stat method of Durbin and Heinz [7]. To determine if a PD were associated with the $(Na^+ + K^+)$-ATPase pump, ouabain was added to the nutrient solution to a concentration of $1 \cdot 10^{-3}$ M for maximal effect.

For the PD response on the nutrient side of the

gastric mucosa, an ion substitution method was used in which Na^+ is replaced by K^+ or K^+ by Na^+; Na^+ by choline or choline by Na^+; and Cl^- by SO_4^{2-} or SO_4^{2-} by Cl^-. In the last case, sucrose was added to make up any osmotic deficit. In the technique of ion substitution [8], the concentration of a given ion is rapidly changed and the time course of the change in PD is recorded. The fact that the PD does not attain its maximum value at the nutrient membrane immediately is attributed to the existence of a diffusion barrier between the nutrient solution and nutrient membrane. The barrier consists of the lamina propria, the muscularis mucosa and part of the submucosa. For a change from a low to a higher ion concentration such as K^+ or Cl^-, Spangler and Rehm [8] found that the absolute value of the change in PD, $|\Delta PD|$ vs. time usually passed through a maximum. For the reverse change, $|\Delta PD|$ vs. time gave a point of inflection. For cases in which a maximum or point of inflection was not obtained, the PD recorded about 10 min after the change of solution was used. Since the time constant for diffusion across the barrier is about 2 min, a period of 10 min assures that the concentration change in the nutrient solution is attained essentially at the nutrient membrane [8].

In the present studies for K^+ and Na^+, PD readings were taken 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 taken was the 10 min value of the PD to minimize the influence of the initial maximum. For reverse changes, the initial minimum if present was determined and also the 10 min value of PD.

Results

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

The HCO_3^- concentration was changed from 25 mM to zero in the nutrient solution and later returned to 25 mM. When the change was made to zero HCO_3^- as previously stated, both sides of the antrum were gassed with 100% O_2 and the pH of the nutrient side was maintained at 7.3. Upon return to 25 mM HCO_3^- in the nutrient solution, both sides were gassed with 95% O_2/5% CO_2. Fig. 1 shows the results of these changes. In this figure, both the resistance and PD are plotted versus time.

The decrease from 25 mM HCO_3^- to zero on the nutrient side results in a gradual but marked decrease in PD and a gradual but marked increase in resistance. Upon return to 25 mM HCO_3^- about 1 h later, there is excellent reversibility, a rapid increase in PD and a rapid decrease in resistance to control levels. Moreover, it is evident from Fig. 1 that the decrease in PD is less rapid than the increase in PD.

The latter effect can be accounted for, at least in part, as follows. It was previously found [8] that a solution of Fick's second equation applied to a model containing a membrane in series with a diffusion barrier yielded a curve for the concentration of an ion at the cell border vs. time which could represent both increases and decreases of the ion concentration in the bathing medium. 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 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^-

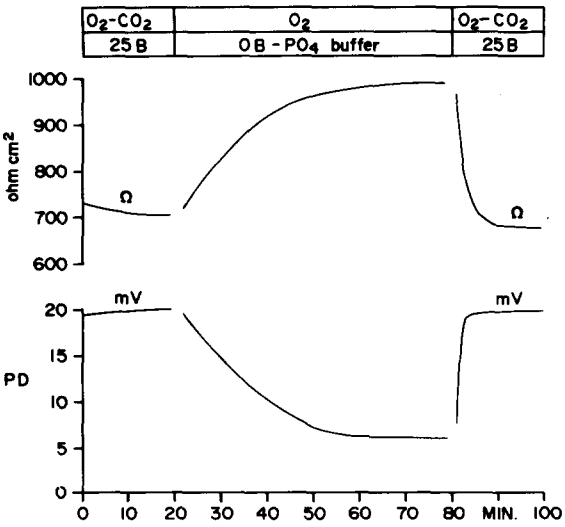

Fig. 1. Effect of changes in HCO_3^- concentration on the nutrient side from 25 mM HCO_3^- to 0 HCO_3^- and back to 25 mM HCO_3^-. Resistance and PD are plotted vs. time. O_2/CO_2 refers to 95% O_2/5% CO_2; O_2, to 100% O_2; and B, to HCO_3^-.

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 mM gives a ratio of 4 : 1. The greater the change, the greater is the initial response. This analysis accounts in part for the decrease in PD since it is somewhat slower than previously observed in other cases of ionic change. (See Refs. 8 and 9 for a detailed analysis of the time constant of these responses.) In the decrease in HCO_3^- to zero, the gassing is changed from 95% O_2/5% CO_2 to 100% O_2 and this change undoubtedly also affects the characteristics of the antrum but in a manner not as yet understood. The PD increase with an increase in HCO_3^-, however, occurs very rapidly irrespective of whether the gases are changed or not (unpublished data) and undoubtedly is explicable mainly in terms of the diffusion barrier.

After about an hour or so in zero HCO_3^-, as already mentioned, the PD decreased markedly and the resistance increased markedly. In 15 experiments, the control PD was 21.9 ± 5.4 (S.D.) mV and the resistance was 748 ± 225 (S.D.) $\Omega \cdot cm^2$. An hour after the change to zero HCO_3^-, 100% O_2 with PO_4 buffer to maintain pH constant, the PD decreased to 8.2 ± 6.9 (S.D.) mV and the resistance increased to 1104 ± 296 (S.D.) $\Omega \cdot cm^2$. As already indicated in the Introduction, the increase in resistance becomes important in obtaining the anomalous PD response with an elevation of the K^+ concentration. The latter will be considered along with normal responses due to changes in K^+ concentration in the next section.

Normal and anomalous PD responses due to changes in K^+ concentration in zero and 25 mM HCO_3^- nutrient solutions

In contrast to the fundus in the acid-secretion state [10], the antrum seemingly exhibits a HCO_3^- conductance, i.e., with a HCO_3^- conductance one would predict, at least in part, the PD changes observed as resulting from changes in HCO_3^- concentration. On the other hand, it is possible that the absence of HCO_3^- indirectly affects the characteristics of other ion pathways. Consequently, with HCO_3^--free solutions, the already high resistance of the antrum is raised still further and thereby creates conditions favorable for an anomalous PD response due to K^+. With this in mind, let us consider what happens when we change from 4 to 40 mM K^+ and back to 4 mM K^+ in HCO_3^--free solutions, gassing both sides with 100% O_2 and maintaining the pH constant.

Fig. 2 shows the principal changes in PD and resistance in HCO_3^--free solutions when the K^+ concentration is changed from 4 to 40 mM in the nutrient solution and returned to 4 mM. In the change from 4 to 40 mM K^+, there is an initial marked increase in PD above control levels (an anomalous response) followed by a decrease in PD below control levels (a normal response). As Fig. 2 shows, the control PD of about 8 mV rises to 15 mV and, after 10 min following the change to 40 mM K^+, the PD falls to about 4 mV below the control level. In the change to higher K^+, the resistance which just prior to the change was about 900 $\Omega \cdot cm^2$ fell rapidly to about 500 $\Omega \cdot cm^2$ and then increased more slowly in a period of 10 min to about 650 $\Omega \cdot cm^2$. These resistance changes in zero HCO_3^- are characteristic of all the experiments in zero HCO_3^-, that is, a sharp decrease in resistance occurs for a change from low to high K^- concentrations followed by a gradual increase in resistance but the final resistance in 40 mM K^+ after considerable time remains below the control level in 4 mM K^+. Furthermore, we observe that the high resistance just prior to the change to 40

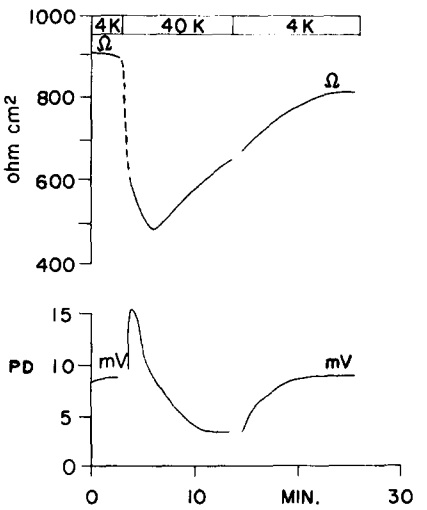

Fig. 2. Effect of changes in K^+ concentration on the nutrient side from 4 mM K^+ to 40 mM K^+ and back to 4 mM K^+ in 100% O_2 with PO_2 buffer to maintain pH constant. Resistance and PD vs. time.

mM K^+ is a condition, as mentioned in the Introduction, associated with the anomalous PD response. As we shall consider in the Discussion, the increase in transmembrane resistance is assumed as at least partially associated with an increase in the resistance of the leak pathway as compared to that of the $(Na^+ + K^+)$-ATPase pathway.

In the return from 40 to 4 mM K^+ in the nutrient solution, no minimum below control levels occurred. It is also evident from Fig. 2 that the resistance just prior to the change to 4 mM K^+ is considerably below control levels. In the figure, the resistance is about 650 $\Omega \cdot cm^2$ as compared to the control level of about 900 $\Omega \cdot cm^2$. Since the resistance is lowered, we assume that an anomalous PD response is less likely. The rest of Fig. 2 shows the increase in PD and resistance to control levels and the PD response is a normal response, attributable to K^+ pathways in the nutrient membrane.

As in previous studies [1,2], if the anomalous PD response due to K^+ is associated with the $(Na^+ + K^+)$-ATPase pump, it should be abolished by ouabain. In six experiments, ouabain in the nutrient solution at a concentration of $1 \cdot 10^{-3}$ M was found to eliminate the PD anomaly. In four of these experiments, ouabain was maintained for about 20 min or more in the nutrient solution before the K^+ concentration was increased from 4 to 40 mM in the HCO_3^--free nutrient solution and ouabain abolished the anomalous PD response completely. In two experiments ouabain was maintained for 10 min or so and the anomalous PD was reduced but not abolished. If then the antrum was returned to 4 mM K^+ in the nutrient solution with ouabain present for another period, the change to 40 mM K^+ now showed no anomalous PD. In an experiment in which ouabain was added to the 4 mM K^+ nutrient solution during the control period and was maintained throughout the experiment, the change in K^+ from 4 to 40 mM in the nutrient solution under zero HCO_3^- conditions did not evoke an anomalous PD response. Hence we concluded that the anomalous PD observed in changes from a non-zero K^+ concentration to a higher K^+ concentration in the nutrient is associated with the $(Na^+ + K^+)$-ATPase pump.

In Table I, both the initial maxima in PD and the PD effect 10 min after the changes in nutrient K^+ are considered for both 25 mM and zero

TABLE I

EFFECT ON PD AND RESISTANCE OF CHANGES IN K^+ CONCENTRATIONS ON THE NUTRIENT SIDE WITH AND 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^+.

No. of expts.	Original soln. $[K^+]$ (mM)	Final soln. $[K^+]$ (mM)	PD (mV)	ΔPD (mV)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
Max. PD, 25 mM HCO_3^-						
11	4	40	22.2 ± 5.8	1.7 ± 1.6 [a]	737 ± 224	–
7 [b]	4	40	22.2 ± 6.9	2.6 ± 1.3 [a]	707 ± 199	−171 ± 60 [a]
PD 10 min after control, 25 mM HCO_3^-						
13	4	40	21.7 ± 5.0	−13.0 ± 3.6 [a]	755 ± 227	−299 ± 94 [a]
13	40	4	7.7 ± 3.7	14.0 ± 4.9 [a]	418 ± 214	188 ± 92 [a]
Max. PD, zero HCO_3^-						
8	4	40	5.0 ± 3.7	7.8 ± 3.5 [a]	1007 ± 172	−343 ± 133 [a]
PD 10 min after control, zero HCO_3^-						
8	4	40	5.0 ± 3.7	−3.5 ± 1.8 [a]	1007 ± 172	−216 ± 124 [a]
8	40	4	1.4 ± 2.4	4.0 ± 1.8 [a]	783 ± 116	152 ± 96 [a]

[a] $P < 0.01$.

[b] In 7 of the 11 experiments, PD increased initially, reaching a maximum; in 4 other experiments, PD decreased from the beginning. The average of 11 experiments includes the 4 without initial PD increase i.e., ΔPD = zero (see Text).

HCO_3^-. In both cases, 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). While the initial increase in zero HCO_3^- occurred in all experiments, in 25 mM HCO_3^- the initial increase occurred in 7 of 11 experiments. Moreover, the initial increase was greater in zero HCO_3^- (7.8 mV), whether all 11 experiments are considered (1.7 mV) or only the 7 experiments with a maximum in PD (2.6 mV). The decrease in PD 10 min after the K^+ increase was greater in 25 mM HCO_3^- than in zero HCO_3^-, 13 mV vs. 3.5 mV. Upon return to 4 mM K^+, no minimum was obtained and the PD returned essentially to control levels in 10 min.

In regard to resistance, in both 25 mM and zero HCO_3^- the resistance decreased with an increase in nutrient K^+ and increased with a decrease in nutrient K^+. In 25 mM HCO_3^- the resistance continued to decrease with time. In zero HCO_3^- the resistance decreased considerably at the time of the initial PD maximum (343 $\Omega \cdot cm^2$) but then started to increase so that at the 10 min mark, the decrease in resistance was only 216 $\Omega \cdot cm^2$ below the control value. As previously stated, the absence of a PD minimum upon return to 4 mM K^+ may be attributed to the marked decrease in resistance (see Discussion).

Normal and anomalous PD responses due to changes in Na^+ concentration in 25 mM and zero HCO_3^- nutrient solutions

We turn our attention from concentration changes of K^+ to those of Na^+ (choline for Na^+) in nutrient solutions with and without HCO_3^-. Fig. 3 shows the principal effects. With 25 mM HCO_3^- in the nutrient solution and with 95% O_2/5% CO_2 gassing both sides of the antrum, the change from 102 to 6 mM Na^+ in the nutrient solution results in an initial small increase in PD of about 2 mV above the control level followed by a decrease of about 10 mV below the control level. Since the Na^+ concentration in the nutrient solution decreases, the first PD change is a normal response and the second PD change is an anomalous response. Upon return to 102 mM Na^+, there is no minimum in the PD response although in other experiments a minimum of about 1 mV occurred. The main effect is a return of the PD to the

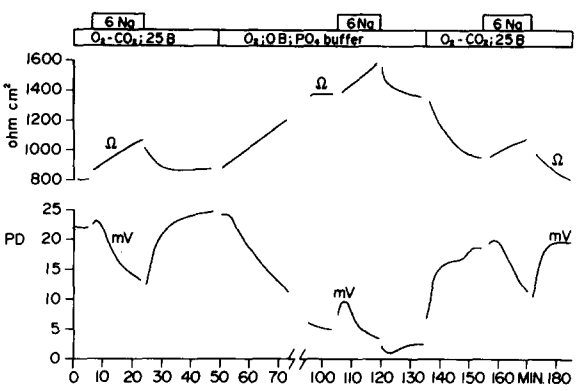

Fig. 3. Effect of changes in Na^+ concentration from 102 mM Na^+ to 6 mM Na^+ and back to 102 mM Na^+ both in 25 mM HCO_3^-, 95% O_2/5% CO_2 and in 0 HCO_3^-, 100% O_2. Resistance and PD vs. time.

control level. The resistance changes like the PD changes were similar to those obtained previously [11], an increase for the decrease to 6 mM Na^+ and a decrease for the change back to 102 mM Na^+. The nutrient solution was replaced with a HCO_3^--free solution and its pH was maintained constant with a PO_4 buffer. At the same time, the antrum was gassed on both sides with 100% O_2 for about 1 h. The resistance increased substantially. Then the change from 102 to 6 mM Na^+ accentuated the normal response, the PD increasing by about 5 mV above the control level, and depressed the anomalous response, the PD decreasing by about 3 mV below the control level. Upon return to 102 mM Na^+, there is a small minimum in the PD response and no recovery to the control level. The change from 102 to 6 mM Na^+ and back to 102 mM Na^+ resulted in similar changes in resistance to those in the first part of the experiment. Return to 25 mM HCO_3^- and 95% O_2/5% CO_2 gave the same responses to Na^+ concentration changes as in the first part.

The initial increase in PD due to a decrease in Na^+ concentration can be regarded largely as associated with the (Na^+ + K^+)-ATPase pump. Previous theoretical considerations [1] have shown that for the electrogenic (Na^+ + K^+)-ATPase pump more Na^+ is transported per cycle out of the cell than K^+ into the cell. Under these circumstances (see Discussion), a change in Na^+ concentration gives a normal PD response and a change in K^+

concentration, an anomalous response. If the PD peak is due to the $(Na^+ + K^+)$-ATPase pump, it should be abolished by ouabain. Such is the case except for a residual PD of about 2 mV. We attribute the residual response to the unmasking of a simple Na^+ conductance pathway whose conductance is so low that it is normally obscured by other PD responses due to Na^+. The decrease in PD following the initial increase is attributed to an electrogenic NaCl symport in which more Cl^- is transported per cycle than Na^+ [11]. Under these circumstances, the PD response is anomalous for Na^+ as indicated in Fig. 3 and normal for Cl^- as indicated later in Fig. 4.

In Table II, both the initial maxima and minima in PD and the PD effect 10 min after the changes in Na^+ concentration are considered for 25 mM

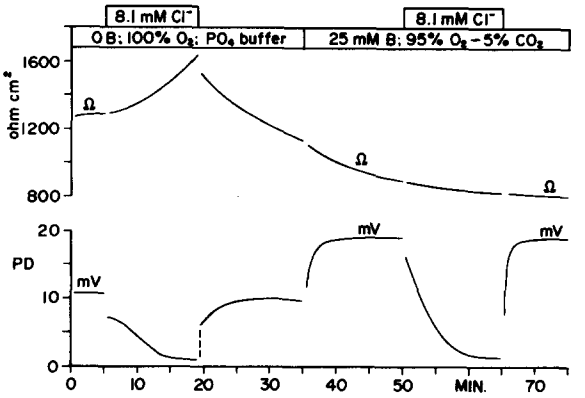

Fig. 4. Effect of changes in Cl^- concentration from 81 mM Cl^- to 8.1 mM Cl^- and back to 81 mM Cl^-. First part in zero HCO_3^-, 100% O_2 and second part in 25 mM HCO_3^-, 95% O_2/5% CO_2. Resistance and PD vs. time.

TABLE II

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

Information same as in Table I.

No. of expts.	Original soln. $[Na^+]$ (mM)	Final soln. $[Na^+]$ (mM)	PD (mV)	ΔPD (mV)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
Max. PD, 25 mM HCO_3^-						
15	102	8	19.5 ± 6.9	2.5 ± 1.9 [a]	751 ± 157	−30 ± 63 [c]
Min. PD, 25 mM HCO_3^-						
15	8	102	12.4 ± 5.1	−0.9 ± 1.1 [a]	908 ± 182	–
11 [d]	8	102	13.6 ± 5.0	−1.2 ± 1.1 [a]	922 ± 189	−45 ± 30 [a]
PD 10 min after control, 25 mM HCO_3^-						
15	102	8	19.5 ± 6.9	−6.8 ± 2.7 [a]	751 ± 157	120 ± 99 [a]
15	8	102	12.4 ± 5.1	7.5 ± 3.2 [a]	908 ± 182	−96 ± 80 [a]
Max. PD, zero HCO_3^-						
15	102	8	7.9 ± 6.7	6.2 ± 3.0 [a]	1073 ± 308	−73 ± 104 [b]
Min. PD, zero HCO_3^-						
15	8	102	5.7 ± 4.5	−2.0 ± 1.7 [a]	1201 ± 320	−87 ± 89 [a]
PD 10 min after control, zero HCO_3^-						
15	102	8	7.9 ± 6.7	−1.4 ± 3.3 [c]	1073 ± 308	95 ± 171 [b]
15	8	102	5.7 ± 4.5	1.4 ± 3.7 [c]	1199 ± 318	−103 ± 118 [a]
6 [e]	102	8	2.4 ± 1.9	1.7 ± 1.5 [b]	1190 ± 140	10 ± 200 [c]
6 [e]	8	102	2.9 ± 1.4	−1.9 ± 0.9	1246 ± 247	−24 ± 96 [c]
9 [f]	102	8	11.6 ± 6.1	−3.5 ± 2.3 [a]	996 ± 370	152 ± 130 [a]
9 [f]	8	102	7.5 ± 5.0	3.5 ± 3.3 [b]	1174 ± 384	−156 ± 103 [a]

[a] $P < 0.01$.
[b] $P < 0.05$.
[c] $P > 0.05$.
[d] Similar considerations to footnote in Table I on K^+ (See Text).
[e] 6 of 15 experiments with control PD < 5 mV.
[f] 9 experiments with control PD > 5 mV.

and zero HCO_3^-. In 25 mM HCO_3^-, for the decrease in nutrient Na^+ from 102 to 8 mM, the initial PD increase of 2.5 mV (normal response) was followed by a decrease of 6.8 mV below control values (anomalous response). In zero HCO_3^-, the initial PD maximum was enhanced, 6.2 mV compared in 25 mM HCO_3^- to 2.5 mV. However, the PD change 10 min after the change in Na^+ concentration was insignificant if all 15 experiments are lumped together. If the data were divided into two subgroups, those with a control PD in 6 experiments under 5 mV and those with a control PD in 9 experiments over 5 mV, the PD changed significantly in both cases. In the first case, the PD increased by 1.7 mV above control and, in the second case, the PD decreased by 3.5 mV below control. In any case, the magnitude of the 10 min change was greater in 25 mM HCO_3^- than in zero HCO_3^-. Upon return to 102 mM Na^+, there was an initial minimum in both cases, 0.9 mV in 25 mM HCO_3^- compared to 2.0 mV in zero HCO_3^-. In 25 mM HCO_3^-, the PD following the minimum returned essentially to control levels, whereas in zero HCO_3^-, the average change in PD in 15 experiments was not significant. For the division into 6 and 9 experiments, respectively, each subgroup returned to control levels.

The resistance changes at the maximum and minimum PD values were either relatively small or insignificant. The resistance after 10 min changed significantly for 25 mM HCO_3^-, and for the 9 experiments with control PD values above 5 mV for both increasing and decreasing Na^+ concentrations. The resistance increased with a decrease in Na^+ concentration and vice versa.

Normal PD responses due to changes in Cl^- concentration in zero and 25 mM HCO_3^- nutrient solutions

Fig. 4 shows, after the antrum was maintained for about 1 h in zero HCO_3^-, the PD responses resulting from a change of Cl^- in the nutrient solution from 81 to 8.1 mM and back to 81 mM. The decrease in Cl^- concentration decreased the PD in 10 min by 9 mV. The decrease in PD is slower than the increase in PD. The decrease, however, shows some deviation from the response one would expect if the only factor were the diffusion barrier adjoining the nutrient membrane. From Fig. 4, it is also evident that the decrease in Cl^- gave a marked increase in resistance and the increase in Cl^-, a marked decrease in resistance. Of special significance is that no anomaly in PD occurred, i.e., the decrease and increase in Cl^- concentration results in normal PD responses.

The antrum was next bathed with a nutrient solution containing 25 mM HCO_3^- and both sides were gassed with 95% O_2/5% CO_2. The PD results due to changes in Cl^- with 25 mM HCO_3^- differed from those with zero HCO_3^-, particularly in the

TABLE III

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

Information same as in Table I. Note: No maxima or minima.

No. of expts.	Original soln. $[Cl^-]$ (mM)	Final soln. $[Cl^-]$ (mM)	PD (mV)	ΔPD (mV)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
PD 10 min after control, 25 mM HCO_3^-						
7	81	8.1	18.9±6.4	−15.7±3.1 [a]	632±210	42± 53 [c]
7	8.1	81	2.9±4.5	15.8±3.0 [a]	674±190	−81± 37 [a]
PD 10 min after control, zero HCO_3^-						
7	81	8.1	9.9±8.9	−9.7±3.4 [a]	932±419	89± 83 [b]
7	8.1	81	−0.7±4.6	9.8±4.7 [a]	1034±484	−183±155 [b]

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

magnitude of the response. The decrease in Cl^- concentration decreased the PD by 17.5 mV and the increase in Cl^- concentration increased the PD by 17.5 mV. The resistance during the Cl^- changes in 25 mM HCO_3^- continued to decrease by a small amount. Here the rate of decrease or increase of PD can be explained in terms of the diffusion barrier. (Vide supra.)

Table III gives the average values of the PD changes in both 25 mM and zero HCO_3^-. With 25 mM HCO_3^-, the PD decreased by 15.7 mV for a 10-fold decrease in Cl^- concentration in the nutrient solution and increased by 15.8 mV for a 10-fold increase in Cl^- concentration. Similar changes occurred with zero HCO_3^- except that the magnitude of the PD was less for either an increase or a decrease in Cl^- concentration in the nutrient solution. The magnitude in each case was reduced by 38%. Moreover, the resistance changes in zero HCO_3^- were somewhat larger than in 25 mM HCO_3^-. The largest change occurred for the change from 8.1 to 81 mM Cl^- in zero HCO_3^-, an average decrease of 183 $\Omega \cdot cm^2$. As stated above, the PD responses were normal. These results are in accord with a model of a NaCl symport (in which more Cl^- than Na^+ is transported per cycle) in parallel with a simple, passive conductance pathway for Cl^-. For both of these limbs, the PD response to changes in Cl^- would be normal (see Discussion).

Discussion

In previous experiments [1,2], after keeping the fundic mucosa in zero K^+ for 30 min or more and the antral mucosa, in zero K^+ for 20 min or more, the change in concentration to 4 mM K^+ in the nutrient solution gave the anomalous response. In the fundus, bathing the mucosa with zero K^+ increased the resistance substantially and, thereby, established the condition necessary for an anomalous PD response. In the antrum, after bathing in zero K^+, the resistance did not always increase to any considerable extent. However, in the antrum the resistance is much higher than in the fundus and the anomalous response is easier to obtain [1].

In the antrum in 25 mM HCO_3^-, changes from 4 to 40 mM nutrient K^+ did not always produce an anomalous response. Even when it occurred, it averaged about 2.6 mV. Because it did not occur or was often small, we previously ignored this aspect of the PD response. However, in zero HCO_3^-, the resistance increased markedly and a change from 4 to 40 mM K^+ in the nutrient solution gave a considerably larger PD increase. The change to 40 mM K^+ caused the resistance to fall so that the return to 4 mM K^+ gave no PD anomaly. Why high resistance of the nutrient membrane is needed to elicit the anomalous response will be dealt with later, but first we consider briefly the theory which enabled us to account for the anomalous PD [1,2].

Since $1 \cdot 10^{-3}$ M ouabain or the lack of Na^+ in the nutrient solution abolished the anomalous PD response, it was reasonable to associate this response with the electrogenic ($Na^+ + K^+$)-ATPase pump. Application of thermodynamics [1,2] gave an expression for the e.m.f. for 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 sodium ions transported out of the cell into the nutrient and m the number of potassium ions transported from the nutrient into 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 (See Ref. 2 for details); and subscripts C and N refer to the cell and nutrient, respectively. If, then, $n > m$, it follows from Eqn. 1 that increasing the nutrient K^+ concentration increases E_P (an anomalous response) and increasing the nutrient Na^+ concentration decreases E_P (a normal response).

Since there are leak or simple conductance pathways as well as pump pathways, the anomalous response due to changes in K^+ concentration is evoked under special relationships between these two sets of pathways. From previous studies [1,2] and present studies, it appears that the anomalous PD response is associated with a high transmembrane resistance. Since the ($Na^+ + K^+$)-ATPase pump is located on the nutrient membrane, the increase of transmembrane resistance must pre-

sumably be at least in part the result of an increase of the resistance of pathways of the nutrient membrane other than that of the pump pathway. If the latter is assumed, we can next see why a high resistance produces an anomalous response.

For this purpose, let us consider the electrical circuit shown in Fig. 5. The circuit comprises three limbs, the first representing the $(Na^+ + K^+)$-ATPase pump pathway; the second, the NaCl symport pathway; and the third, the conductance or leak pathway for K^+, Cl^-, HCO_3^-, etc. In this circuit E_P, E_S and E_L represent the e.m.f. values of the pump, symport and leak pathways and R_P, R_S and R_L their respective resistances. The letters C and N refer to cell and nutrient, respectively.

The use of these circuits depends on the existence of a linear relationship between ΔPD and log of concentration of ion under consideration such as K^+ and Na^+ in a range of concentration values [1,8,9]. Under these circumstances, it is not unreasonable to assume as a first approximation the resistance as essentially constant. Moreover, in stomach unlike nerve, it has been demonstrated that the stomach is a linear, bilateral system with reference to voltage versus current [12]. Hence in the stomach unlike nerve the permeabilities are not a function of PD.

In considering the PD response to changes in nutrient K^+ concentration, the symport which involves Na^+ and Cl^- concentrations changes can be ignored. Then the PD due to the two limbs 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 under the assumption of constant R_L and R_P is

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

From Eqn. 4, when are the changes in PD normal or anomalous? If the changes in E_P and E_L were normal, then ΔPD would always be normal. From Eqn. 1, we note that the PD response for increases in K^+ concentration in the nutrient is anomalous. Then the orientation of the change in E_P will be opposite to that of E_L. Since ΔE_P is positive and ΔE_L is negative, then for an anomalous response, it follows that the magnitude of $R_L \Delta E_P$ is greater than the magnitude of $R_P \Delta E_L$. As an illustration, let us assume that the K^+ concentration in the nutrient solution changes from 4 to 40 mM. Let us for simplicity assume $n = 2$, $m = 1$. Then from Eqn. 1, $\Delta E_P \cong 60 \log(40/4) = 60$ mV. In addition, for a 10-fold change in K^+ concentration, ΔE_L from the Nernst equation is about -60 mV. With $\Delta E_P = -\Delta E_L$, an anomalous response occurs for $R_P < R_L$ and a normal response for $R_P > R_L$. Thus, if the leak resistance becomes reasonably high, the conditions are right for an initial anomalous response in the change from 4 to 40 mM K^+. The earlier work in which we went to zero K^+ gave or maintained a relatively high resistance. Then the change to 4 mM K^+ showed an anomalous PD response.

Associated with the anomalous PD response due to K^+ is the normal PD response due to Na^+. According to Eqn. 1, if the Na^+ concentration changes from 102 to 8 mM in the nutrient solution, then the PD increases (a normal response). In zero HCO_3^-, this effect largely due to the $(Na^+ + K^+)$-ATPase pump is heightened compared to the effect in 25 mM HCO_3^-. However, in order to explain these results, we need to consider first the NaCl symport and second the electrical circuit of Fig. 5 including the symport.

In previous studies [9,11], it was found that a

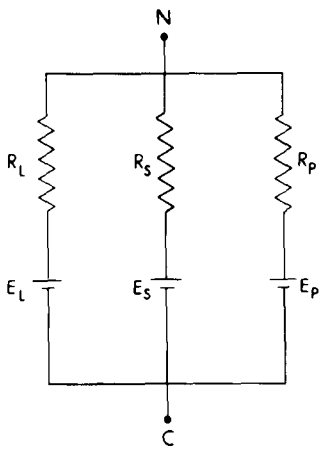

Fig. 5. Equivalent circuit for nutrient membrane comprising L limb representing the leak pathway, S limb representing the NaCl symport pathway and P limb representing the $(Na^+ + K^+)$-ATPase pump pathway. C refers to cell and N to nutrient.

decrease in Na^+ concentration gave a decrease in PD (an anomalous response) and a decrease in Cl^- concentration gave a decrease in PD (a normal response). To explain these results a NaCl symport was proposed. It was shown that the e.m.f. due to a passive, electrogenic symport was given by

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

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 $q > p$, the PD is anomalous for Na^+ and normal for Cl^-.

Next in considering why the normal PD effect due to nutrient Na^+ is heightened in zero HCO_3^-, we have the added complication that a change in Na^+ concentration in the nutrient solution affects all three limbs of the circuit of Fig. 5. However, if the Na^+ leak pathway is assumed to have a high resistance, we can give an approximate, simplified analysis based on the parameters of the other two pathways. For these two limbs, the PD is given by

$$PD = \frac{R_S E_P + R_P E_S}{R_P + R_S} \quad (6)$$

If now the Na^+ concentration is changed, then

$$\Delta PD = \frac{R_S \Delta E_P + R_P \Delta E_S}{R_P + R_S} \quad (7)$$

again under the assumption of constant resistances. From Eqn. 1, ΔE_P due to Na^+ is normal and, from Eqn. 5, ΔE_S due to Na^+ is anomalous. Hence the orientation of ΔE_P will be opposite to that of ΔE_S. Since for a decrease in Na^+ concentration ΔE_P is positive and ΔE_S is negative, it follows for a normal response that the magnitude of $R_S \Delta E_P$ is greater than the magnitude of $R_P \Delta E_S$. As an illustration, assume the Na^+ concentration changes from 100 to 10 mM in the nutrient solution. Moreover, for simplicity take $p = 1$, $q = 2$ and again take $m = 1$, $n = 2$. Then from Eqn. 1 we obtain $\Delta E_P = 120$ mV and, from Eqn. 5 $\Delta E_S = -60$ mV. Under these conditions, a normal PD response would occur if $2R_S > R_P$ and an anomalous PD response would occur if $2R_S < R_P$. With the increase in resistance in zero HCO_3^-, R_S would increase relative to R_P for the normal response. However, at best these considerations show that there are relationships between parameters that can account for normal and anomalous PD responses, but are limited by a lack of knowledge of these parameters. In actuality, from Table II, we see that in the change from 102 to 8 mM Na^+ in the nutrient solution, the resistance just before the change is higher in zero HCO_3^- than in 25 mM HCO_3^-. 10 min later the PD decreased below control levels (an anomalous response) and the decrease was greater in 25 mM HCO_3^- than in zero HCO_3^- if we consider in the latter case those experiments having an anomalous response. In each of these cases the resistance increased. One might infer from the discussion above that the reversal in PD in 10 min was connected with the resistance increase so that now R_P increased relative to R_S.

The changes in Cl^- concentration showed no anomalous PD response. The electrogenic NaCl symport and/or the simple conductance pathway for Cl^- could explain the normal PD response.

This work has demonstrated that removal of HCO_3^- from the bathing media modified the characteristics of the mucosa. As described above, the normal PD responses due to K^+ and Cl^- and the anomalous PD response due to Na^+ were considerably less in the absence than in the presence of HCO_3^-. On the other hand, the anomalous PD response due to K^+ and the normal PD response due to Na^+, both of which were associated with the $(Na^+ + K^+)$-ATPase pump, became prominent in the absence of HCO_3^-. With 25 mM HCO_3^- in the nutrient solution, the latter effects were at times absent and at other times barely suspect. The enhancement in zero HCO_3^- solutions was explained at least in part as dependent on the relative resistances of the $(Na^+ + K^+)$-ATPase pump pathway, the pathway of the NaCl symport and the leak pathway. However, how HCO_3^- and removal of HCO_3^- affect the conductance pathways of other ions and whether HCO_3^- itself has a conductance pathway in the antrum are not at present understood and deserve investigation.

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References

1 Schwartz, M., Kissel, D.E., Carrasquer, G. and Rehm, W.S. (1983) Biochim. Biophys. Acta 727, 45–55
2 Schwartz, M., Chu, T.C., Carrasquer, G. and Rehm, W.S. (1981) Biochim. Biophys. Acta 649, 253–261
3 Spenney, J.G., Flemstrom, G., Shoemaker, R.L. and Sachs, G. (1975) J. Gen. Physiol. 65, 645–662
4 Manning, E.C. and Machen, T.E. (1982) Am. J. Physiol. 243, G60–G68
5 Schwartz, M., Rehm, W.S. and Carrasquer, G. (1983) Fed. Proc. 42, 1352
6 Rehm, W.S. (1962) Am. J. Physiol. 203, 63–72
7 Durbin, R.P. and Heinz, E. (1959) J. Gen. Physiol. 41, 1035–1047
8 Spangler, S.G. and Rehm, W.S. (1968) Biophys. J. 8, 1211–1227
9 Carraquer, G., Chu, T.C., Schwartz, M. and Rehm, W.S. (1982) Am. J. Physiol. 242, G620–G627
10 Sanders, S.S., O'Callaghan, J., Butler, C.F. and Rehm, W.S. (1972) Am. J. Physiol. 222, 1348–1354
11 Carrasquer, G., Kissel, D.E., Rehm, W.S. and Schwartz, M. (1983) Am. J. Physiol. 245, G554–G561
12 Noyes, D.H. and Rehm, W.S. (1970) Am. J. Physiol. 219, 184–192