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NORMAL AND ANOMALOUS POTENTIAL RESPONSES DUE TO K^+ CHANGES IN BULLFROG ANTRUM

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The effect of changing the K^+ concentration in the bathing media was studied in the bullfrog antrum. Usually increasing K^+ on the nutrient side in standard Cl^--containing and Cl^--free solutions decreased the transmucosal potential difference (nutrient became more negative) – a normal effect. Similar results were obtained on the secretory side. Moreover, for K^+ changes on the nutrient side in Cl^- media, a plot of $|\Delta V|$ vs. log $[K^+]$ was linear for $[K^+] > 20$ mM with slope of 27 mV per 10-fold change in $[K^+]$. However, after bathing the mucosa in Cl^- media with zero K^+ for about 20 min, elevating the nutrient $[K^+]$ to 4 mM increased the potential difference (V) by 4.8 mV in 5 min and repeating the same sequence increased V by 6.9 mV in 5 min – both anomalous effects. Beyond 20 mM K^+ the response was normal. In SO_4^{2-} media, an anomalous potential difference of about 1 mV was obtained for changes from 0 to 3 or 6 mM nutrient K^+. Ouabain ($1 \cdot 10^{-3}$ M) in the nutrient solution abolished the anomalous response in Cl^- and SO_4^{2-} media. The normal response is attributed to passive, conductance pathways and the anomalous response because of the effect of ouabain, to a (Na^+ + K^+)-ATPase pump on the nutrient-facing membrane in which more Na^+ than K^+ ions are transported per cycle.

Introduction

In previous studies of the fundus of *Rana pipiens*, it was found that changing the K^+ concentration in the nutrient fluid in Cl^- and Cl^--free media gave two types of potential difference change. Generally, increasing the K^+ concentration in nutrient solution decreases the positivity of the nutrient. This type of change in V due to a change in K^+ concentration is regarded as a normal potential difference response. Specifically, in Cl^- media a 10-fold change in K^+ concentration in the nutrient solution changed V by about 36 mV [1] and a similar change in K^+ concentration in the secretory solution changed V by about 7 mV [2]. In contrast to the asymmetrical response in Cl^- media, Davis et al. [3] found in Cl^--free (SO_4^{2-}) media a symmetrical response of V to changes in K^+ concentration. They found that a 10-fold increase of K^+ on the nutrient side decreased the positivity of the nutrient by about 25 mV and a similar increase in K^+ concentration on the secretory side increased the positivity of the nutrient also by about 25 mV. Holloman et al. [4] confirmed that increases in K^+ concentration on the nutrient and secretory sides in SO_4^{2-} media gave symmetrical responses in V, although they obtained a value of 31 mV compared to 25 mV.

If, however, the fundus of the gastric mucosa were bathed with standard Cl^- solutions without K^+ for 30 min or more, then an increase in nutrient K^+ to 4 mM in Cl^- media increased the positivity of the nutrient, an effect opposite to that described above. This type of change in V due to a change in K^+ concentration has been referred to as an anomalous potential difference response [5–7]. In Cl^--free media, an increase of K^+ from zero to 1,2, or 3 mM K^+ in the nutrient solution gave an anomalous V response, but V was not significantly increased for higher K^+ concentrations. It was further found that ouabain abolished the anomalous V response, as did the absence of Na^+ (choline replacing Na^+) in the nutrient fluid [7].

These two V responses were previously explained as follows [1,7]. The normal V response was attributed to K^+ conductance channels in the nutrient or secretory membrane. The existence of K^+ conductance channels is supported by the experimental result that a plot of ΔV vs. log $[K^+]$ shows a good linear relation except for a small deviation from linearity for concentrations below 10 mM K^+. This logarithmic relation is readily explained in terms of passive conductance channels for K^+ [1]. As to the anomalous V response, since ouabain is known to inhibit the $(Na^+ + K^+)$-ATPase pump [8] and since ouabain abolishes the anomalous V response, these facts suggest that the anomalous V effect is associated with the $(Na^+ + K^+)$-ATPase pump. Choline replacing Na^+ also abolishes the anomaly, thereby providing further support for the $(Na^+ + K^+)$-ATPase pump and, in fact, the experimental data together with a theoretical model support the assumption of an electrogenic $(Na^+ + K^+)$-ATPase pump on the nutrient-facing membrane in which more Na^+ than K^+ is transported per cycle [7].

In the present work, we investigated whether the two types of V response to changes in K^+ concentration occurred for the physiological antrum of the bullfrog (i.e., no H^+ secretion with histamine). The former studies on the fundus have provided the experimental and theoretical background work. The question remained whether both types of V response would arise in the antrum and, if so, what might be the relative magnitudes of these effects. A preliminary report of this work has been published elsewhere [9].

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 [10]. 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 Cl^--free (SO_4^{2-}) experiments, the SO_4^{2-} nutrient solution contained (in mM): Na^+, 102; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; SO_4^{2-}, 41.3; HCO_3^-, 25; phosphate, 1; glucose, 10 and sucrose, 40; and the SO_4^{2-} secretory solution: Na^+, 102; K^+, 4; SO_4^{2-}, 53; and sucrose, 64. In zero-K^+ solutions, Na^+ replaced K^+ and in high K^+ solutions K^+ replaced Na^+.

In these studies, the transmembrane resistance and transmembrane potential difference (V) were measured. As a test that the antrum was being used, histamine was generally added to the nutrient solution to a concentration of $1 \cdot 10^{-4}$ M and no H^+ secretion occurred. Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring V. V 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 V per unit of applied current. Current (10–20 $\mu A \cdot cm^{-2}$) 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 [11]. The pH of the secretory solution was generally maintained between 4.6 and 5.0. Both sides of the mucosa were gassed with a mixture of 95% O_2 and 5% CO_2. Ouabain was used at a concentration of $1 \cdot 10^{-3}$ M in the nutrient solution for maximal effects [12].

For the normal V response on either the nutrient or secretory side of the gastric mucosa, an ion substitution method was used in which Na^+ is replaced by K^+ or K^+ by Na^+. In this technique [1], the concentration of a given ion is rapidly changed and the time course of the change in V is recorded. The fact that V 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 K^+ concentration, Spangler and Rehm [1] found that the absolute value of the change in V, $|\Delta V|$, versus time usually passed though a maximum. For a change from a high to a lower K^+ concentration, $|\Delta V|$ vs. time gave an inflection point. In the present studies, V at a maximum or a point of inflection was recorded together with the corresponding resistance. For those cases in which a maximum or point of inflection was not obtained, V 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 [1].

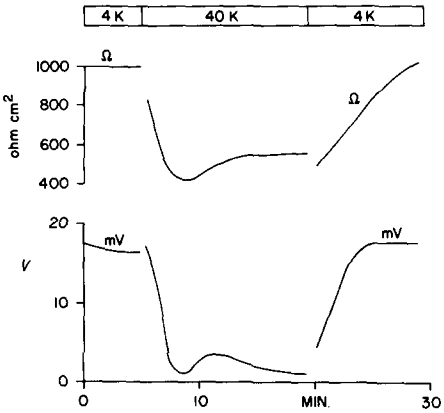


Fig. 1. Effect of changes in K^+ concentration on the nutrient side from 4 mM K^+ to 40 mM K^+ and back to 4 mM K^+. Resistance and V vs. time in Cl^- solutions.

Results

Normal V responses and resistance changes due to changes in K^+ concentration in the nutrient fluid in Cl^- media

The K^+ concentration was changed by replacement with Na^+ from 4 mM to 10 mM and back to 4 mM, then from 4 mM to 20 mM and back to 4 mM and likewise for concentrations of 40 and 80 mM K^+. Fig. 1 shows one part of the results, namely that obtained for changes from 4 to 40 mM K^+ and back to 4 mM K^+. In this figure both the resistance and V are plotted versus time. Let us first look at the V response.

The increase from 4 to 40 mM K^+ in the nutrient solution results in a rapid decrease in V and the decrease from 40 to 4 mM K^+ in a less rapid increase in V. It was previously found [1] 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 versus time which could represent both increases and decreases of the ion concentration in the bathing medium. Since V depends on the logarithm of the concentration ratio across the nutrient membrane, V at the nutrient membrane changes more markedly in going say from 4 to 8 mM on the way to 40 mM K^+ than from 40 to 36 mM on the way back to 4 mM K^+. The change from 4 to 8 mM K^+ gives a ratio of 2 : 1, a 2-fold change, whereas the change from 40 to 36 gives a ratio of 40 : 36, a much smaller change. The greater the change, the greater is the initial V response. (See Refs. 1 and 13 for a detailed analysis of the time constant of these responses.)

We now look at the resistance changes. The increase in K^+ concentration from 4 to 40 mM K^+ gives a rapid decrease in resistance, whereas the decrease in concentration from 40 to 4 mM K^+ gives a slower return of the resistance to control levels. Spangler and Rehm [1] previously found similar results for the secreting fundus. They found that, for changes from 4 to 79 mM K^+ and back to 4 mM K^+, the first change resulted in a rapid decrease in resistance and the second change in a slow increase in resistance to control levels. It would appear that with an increase in K^+ in the nutrient solution, the characteristics of the mucosa change (e.g., decrease in resistance [1,14]) so that the return of the resistance to control levels with 4 mM K^+ in the nutrient solution is longer than the time needed for the original decrease in resistance.

In Table I, the effect on V and resistance due to changes in K^+ concentration on the nutrient side in Cl^- solutions is shown. Increases in K^+ concentration gave decreases in resistance and in V. The resistance decreased as much as 328 $\Omega \cdot cm^2$ from a control level of 719 $\Omega \cdot cm^2$ for the increase from 4 to 40 mM K^+ and about the same for the increase from 4 to 80 mM K^+. The decrease in V

TABLE I

EFFECT ON V AND RESISTANCE OF CHANGES IN K^+ CONCENTRATIONS ON THE NUTRIENT SIDE IN Cl^- SOLUTIONS

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

Original soln. $[K^+]$ (mM)	Final soln. $[K^+]$ (mM)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)	V (mV)	ΔV (mV)
4	10	676±177	−88± 64	22.0±6.8	−0.30±2.3
4	20	725±154	−243±114 [a]	24.0±7.3	−5.8±2.8 [a]
4	40	719±168	−328±151 [a]	24.8±6.5	−15.0±3.1 [a]
4	80	700±169	−315±138 [a]	25.5±5.9	−22.4±5.0 [a]
10	4	641±151	9± 31	21.4±5.8	3.3±1.6 [a]
20	4	535±151	130± 63 [a]	17.7±3.6	7.4±3.4 [a]
40	4	417±114	169±148 [b]	6.9±3.5	16.8±4.3 [a]
80	4	357±110	147±155	−5.9±4.2	23.3±5.8 [a]

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

for a change in concentration from 4 to 10 mM K^+ was not significant but was significant for increases from 4 to 20, 40 and 80 mM K^+. The lower part of Table I shows the effect on V and resistance for changes from higher concentrations of K^+ to a final concentration of 4 mM K^+ in the nutrient solution. V increased significantly for all decreases in K^+ concentration. The increase in resistance was not significant for a decrease in concentration from 10 to 4 mM K^+ but was about 150 $\Omega \cdot cm^2$ for all larger decreases in K^+ concentration. The $|\Delta R|$ values in Table I for the increases in K^+ concentration are greater than for the decreases. Since these values were generally taken 10 min after the change in concentration, we assume, as indicated above, that this difference is due to the time taken for the elevated intracellular K^+ to return to its original level. In other words, elevation of intracellular K^+ lowers the resistance.

In Fig. 2 (based on the values of Table I), the absolute value of the change in V, $|\Delta V|$, is plotted versus the logarithm of the initial or final concentration. The closed circles are for a final concentration of 4 mM K^+ and the open circles for an initial concentration of 4 mM K^+ in the nutrient solution. Within the limits of experimental error (note the half lines for S.E.), there is a linear relationship for concentrations from 20 mM K^+ to higher values. In the case of the secreting fundus, a straight-line relationship exists for concentrations from about 10 mM K^+ to higher values [1]. For the antrum, for values below 20 mM K^+, the $|\Delta V|$ values deviate somewhat from the straight line. The slopes in the linear regions are 27 mV per

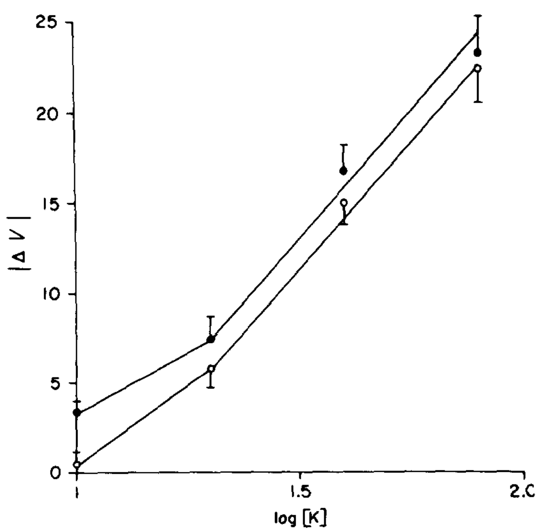


Fig. 2. Absolute value of the change in V, $|\Delta V|$, is plotted vs. log concentration for an initial concentration $[K^+]_i = 4$ mM (○) or a final concentration $[K^+]_f = 4$ mM (●). Half lines represent the standard errors of the mean.

10-fold change in K^+ concentration for both increases and decreases in K^+ concentrations.

Normal V responses and resistance changes in Cl^- and Cl^--free media on the nutrient and secretory side due to K^+ concentration changes

In these experiments, the K^+ concentration was changed from 4 to 40 mM K^+ and then back to 4 mM K^+. Changes in V and resistance were determined in Cl^- media first on the nutrient side and then on the secretory side. The solutions were next changed to Cl^--free (SO_4^{2-}) solutions and the same sequence of experiments was performed. The results similar to those shown in Fig. 1 were recorded generally at the 10 min mark.

The results summarized in Table II are as follows. With Cl^- solutions bathing both sides of the antrum, $|\Delta V|$ was about 16 mV for $\Delta[K^+]$ from 4 to 40 mM and also from 40 to 4 mM in the nutrient solution. These changes in V differed from those obtained from the slope of the straight line in Fig. 2, namely 27 mV per 10-fold $\Delta[K^+]$, since they include changes over the non-linear region (i.e., for 4 to 20 mM K^+ as shown in Fig. 2). In the acid secreting (non-resting) fundus, the values of $|\Delta V|$ are larger, namely 36 mV per 10-fold increase in $[K^+]$ for increases from 4 to 79 mM K^+. In both the antrum and the secreting fundus with Cl^- solutions, $|\Delta V|$ on the secretory side is only a few mV. In Cl^--free solutions, $|\Delta V|$ for the antrum is almost symmetrical for increases from 4 to 40 mM K^+ on the nutrient and secretory sides, i.e., 18.3 mV and 16.0 mV, respectively, and definitely symmetrical for decreases from 40 to 4 mM K^+, 11.8 mV and 11.6 mV, respectively. For the secreting fundus, Davis et al. [3] obtained in Cl^--free solutions symmetrical results of $|\Delta V| = 25$ mV and Holloman et al. [4] symmetrical results of $|\Delta V| = 31$ mV for increases in both cases from 4 to 40 mM K^+. Again comparable V values are less in the antrum than in the secreting fundus.

We note that in Cl^- solutions, the resistance of the antrum decreased for increasing K^+ concentrations and increased for decreasing K^+ concentrations irrespective of whether the changes in concentration were made on the nutrient or the secretory side. In the case of Cl^--free solutions, the changes in resistance were not significant except for the increase from 4 to 40 mM K^+. In this case, the resistance decreased substantially, 247 $\Omega \cdot cm^2$ from a control value of 1239 $\Omega \cdot cm^2$.

TABLE II

EFFECT ON V AND RESISTANCE OF CHANGES IN K^+ CONCENTRATIONS ON THE NUTRIENT AND SECRETORY SIDES IN Cl^- AND Cl^--FREE MEDIA

Values are means ±S.D. of six experiments. Heading below as Cl^- solutions, nutrient side means that both sides of antrum were bathed in Cl^- solutions and $\Delta[K^+]$ was made on nutrient side. Other information as in Table I.

Original soln. $[K^+]$(mM)	Final soln $[K^+]$(mM)	R ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)	V (mV)	ΔV (mV)
Cl^- solutions, nutrient side					
4	40	778±238	−239± 35 [a]	25.2± 4.2	−15.6±5.3 [a]
40	4	452±161	155±130 [b]	5.7± 4.2	15.8±4.7 [a]
Cl^- solutions, secretory side					
4	40	698±207	−112± 75 [a]	25.6± 3.6	4.8±1.2 [a]
40	4	577±187	191±106 [a]	30.8± 2.9	−5.2±1.5 [a]
SO_4^{2-} solutions, nutrient side					
4	40	1239±209	−247± 65 [a]	6.5± 5.3	−18.3±5.2 [a]
40	4	961±260	176±180	−4.0±12.7	11.8±4.1 [a]
SO_4^{2-} solutions, secretory side					
4	40	1079±272	−53±155	−0.9± 6.0	16.0±6.2 [a]
40	4	1066±253	91±191	14.4± 5.2	−11.6±5.7 [a]

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

Normal and anomalous effects in Cl^- solutions bathing the antrum

Fig. 3 shows a typical experiment exhibiting normal and anomalous *V* effects and the elimination of the anomalous effect but not the normal effect by ouabain. In this experiment the secretory solution was K^+-free. At about the 5 min mark, the nutrient solution containing 4 mM K^+ was replaced with one containing 79 mM K^+. The increase from 4 to 79 mM K^+ resulted in a normal *V* response and a decrease in resistance. Upon changing back to 4 mM K^+, *V* returned essentially to the control level (also a normal effect) and the resistance, after a continuing decline (due to the continued effects of 79 mM K^+ after a change back to 4 mM K^+), increased. The nutrient solution was changed to a K^+-free solution for about 20 min and thereafter the nutrient solution was changed back to 4 mM K^+. The first increase from 0 to 4 mM K^+ gave a small increase in *V* (an anomalous effect). Then a change to zero K^+ was again made for about 20 min followed by a return to 4 mM K^+. This time the change from 0 to 4 mM K^+ gave a more pronounced, anomalous *V* response. The increase in resistance on changing from 4 mM K^+ to zero K^+ is somewhat ambiguous in this experiment. In a series of other experiments, it was observed that the decrease to zero K^+ increased the resistance by about 100 $\Omega \cdot cm^2$ and the increase to 4 mM K^+ decreased the resistance by about the same amount. In the latter part of this experiment, the nutrient solution was again made K^+-free and eventually ouabain was added to this solution ($1 \cdot 10^{-3}$ M). The ouabain caused a decrease in *V* to about zero. In the presence of ouabain, an anomalous response was not observed in going from 0 to 4 mM K^+. Hence, ouabain abolished the anomalous response. The change to 79 mM K^+ resulted in a normal and marked decrease in *V* comparable to that at the beginning of the experiment. The resistance also decreased markedly.

From Table III, we note that in six experiments in going from 0 to 4 mM K^+ the first time (control (1)), the average *V* increased (the anomalous effect) by 2.4 mV in 2 min and by 4.8 mV in 5 min, and the second time (control (2)), by 2.3 mV in 2 min and by 6.9 mV in 5 min. The presence of

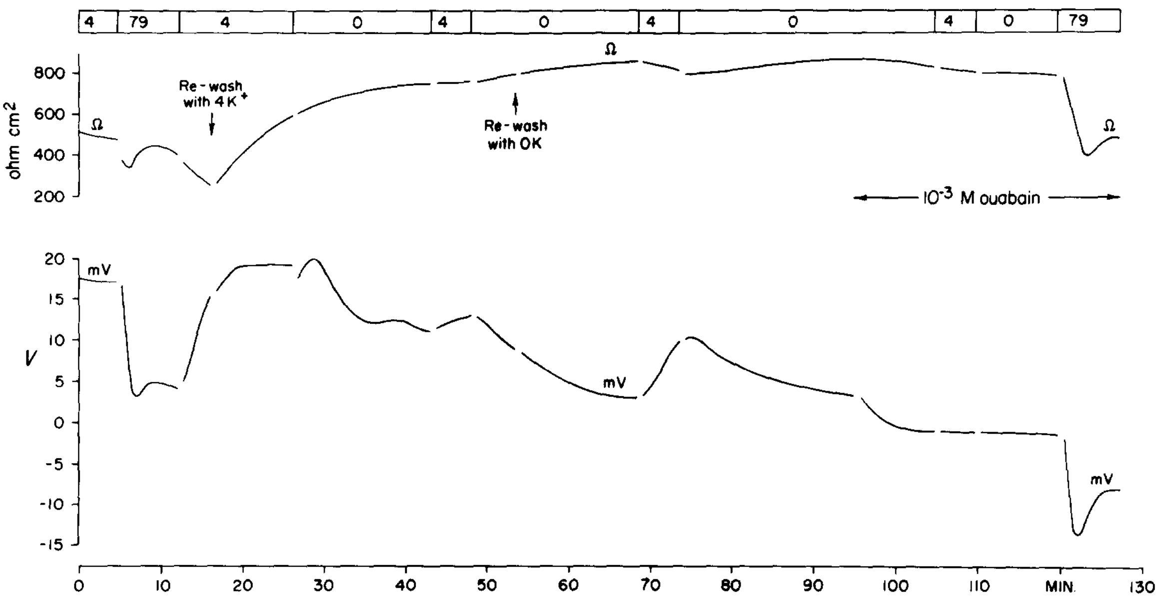


Fig. 3. Effect of changes in K^+ concentration (figures in upper block, $[K^+]$ in mM) on the nutrient side before and after addition of ouabain. Resistance and *V* vs. time in Cl^- solutions. K^+ was zero on secretory side at all times. The changes from 4 to 79 to 4 mM K^+ of the first part represent normal *V* responses. The changes to 4 mM K^+ following a period of about 20 min in zero K^+ represent anomalous *V* responses. The last part with ouabain ($1 \cdot 10^{-3}$ M) in nutrient solution shows elimination of anomalous but not normal *V* response. $4K^+ = 4$ mM K^+.

TABLE III

EFFECT ON V AND RESISTANCE OF ELEVATING K^+ FROM 0 TO 4 mM ON THE NUTRIENT SIDE IN Cl^- SOLUTIONS

Values are means ± S.D. of six experiments. V values are expressed in mV. Resistance values in $\Omega \cdot cm^2$. Student's t-test using paired observations was performed to determine the level of significance. Control (1) $[K^+] = 0$ for 20 min followed by $[K^+] = 4$ mM for 5 min. Control (2) A return to $[K^+] = 0$ for 20 min followed by $[K^+] = 4$ mM for 5 min. After ouabain in 4 mM K^+, $[K^+] = 0$ for 20 min followed by $[K^+] = 4$ mM for 5 min (see text).

	V before $0 \rightarrow 4\ [K^+]$	ΔV		R before $0 \rightarrow 4\ [K^+]$	ΔR	
		2 min	5 min		2 min	5 min
Control (1)	6.5 ± 5.3	2.4 ± 2.1 [b]	4.8 ± 3.6 [a]	737 ± 169	−1 ± 21	−9 ± 32
Control (2)	2.4 ± 2.9	2.3 ± 0.7 [a]	6.9 ± 3.8 [a]	816 ± 171	−4.2 ± 8.7	−18 ± 21
10^{-3} M ouabain	−0.5 ± 3.2	−0.25 ± 0.28	−0.77 ± 0.68 [b]	795 ± 152	−6.7 ± 7.6	−19 ± 24

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

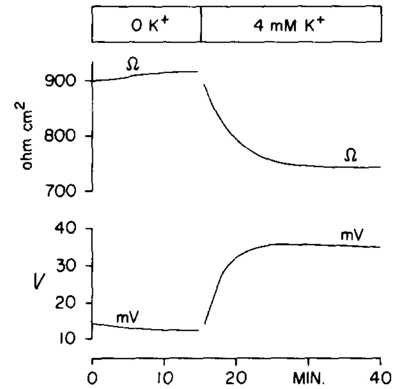
$1 \cdot 10^{-3}$ M ouabain in the nutrient solution abolished the anomalous V response and gave a small but significant decrease in V of 0.77 mV in 5 min. In these experiments, in a period of 5 min there was no significant change in resistance without and with ouabain.

We found that maximum V is obtained in about 7 min and the value is about 12 mV. Furthermore, the anomalous response to K^+ occurs when 4 mM K^+ is maintained on the secretory side. An example of the first statement is shown in Fig. 4, starting with the last 15 min of a 40 min period with zero K^+ solutions bathing both sides of the antrum. The return to a 4 mM K^+ nutrient resulted in an anomalous V response. In about 11 min V rose from 12.3 mV to a maximum of 35.8 mV, an increase in V of 23.5 mV. About one experiment in four gave maxima of this magnitude. The experiment also showed that, after a prolonged period in zero K^+, the V values returned essentially to the control level. On occasion, as in this experiment, the return to 4 mM K^+ caused the resistance to fall by almost 200 $\Omega \cdot cm^2$ although in general the decrease was far less (compare Tables I and III.).

Fig. 5 illustrates some special cases of normal and anomalous V responses. In the first part, changes from 4 mM to zero K^+ gave a small increase in V (a normal effect) and changes shortly thereafter from 0 to 4 mM K^+ gave a small decrease in V (also a normal effect). The changes from 4 mM to zero were at most about 4 mV and sometimes did not occur. In the fundus, these changes were quite marked, being quite often about 10 mV. In the next part of the experiment, changes were made from 0 to 20 mM K^+ for short and longer periods. The increase from 0 to 20 mM K^+ resulted initially in a small increase in V (an anomalous effect) followed thereafter by a rapid, marked decrease in V (a normal effect). The resistance decreased by about 200 $\Omega \cdot cm^2$ in a change from 0 to 20 mM K^+ and increased by this amount in a change from 20 mM to zero K^+. A concentration of 20 mM K^+ in the nutrient solution is about the limiting concentration for which some semblance of an anomalous V response is noticeable. Addition of ouabain to the nutrient solution ($1 \cdot 10^{-3}$ M) eliminated this response.

Anomalous effects in Cl^--free (SO_4^{2-}) solutions bathing the antrum

After a period of about 30 min or longer in K^+-free secretory and nutrient solutions, the nutrient solution was changed from 0 to 2, 3, 6, 12 or 24 mM K^+. The results for changes from 0 to either 2 or 12 mM K^+ were ambiguous in that sometimes anomalous V effects and at other times no changes in V occurred. For 24 mM K^+, the V response was normal. However, as shown in Table IV, significant anomalous V responses were obtained for changes from 0 to either 3 or 6 mM K^+. In four experiments, the increase from 0 to 3 mM K^+ gave a maximum increase of 0.7 mV and in



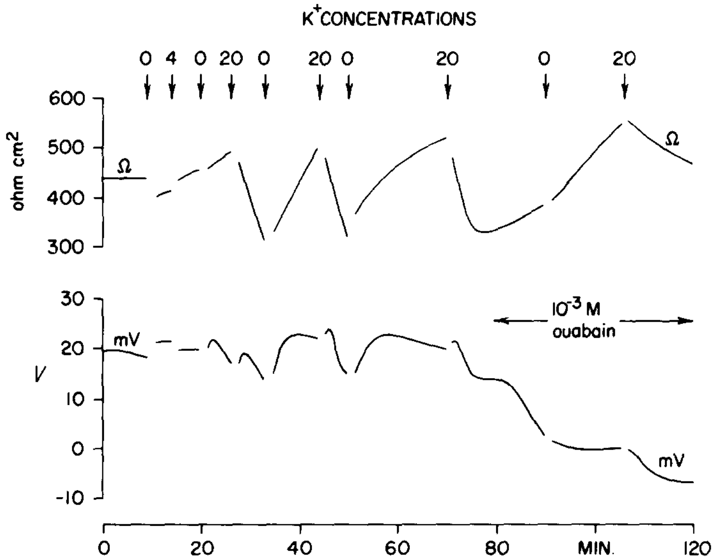


Fig. 4. (Left.) Effect of change from zero K^+ after a prolonged period to 4 mM K^+ on the nutrient side. Resistance and *V* vs. time in Cl^- solutions. This figure shows attainment of *V* maximum in about 11 min.

Fig. 5. (Right.) Effect of changes in K^+ concentration on the nutrient side from 4 to 0 to 4 and back to zero K^+. This part is followed by changes from 0 to 20 and back to zero K^+ for periods of varying length. Also effect of ouabain on latter change is shown. Resistance and *V* vs. time in Cl^- solutions.

five experiments the increase from 0 to 6 mM K^+ gave a maximum increase in *V* of 0.9 mV. The resistance changes were not significant. Addition of ouabain to the nutrient solution ($1 \cdot 10^{-3}$ M) abolished the anomalous *V* response.

Discussion

In the antrum and in the fundus, normal *V* responses arise on both the nutrient and secretory sides as a result of changes in the K^+ concentration. It was previously shown for the fundus that under certain circumstances, anomalous *V* responses resulted from K^+ changes on the nutrient side. In the work presented herein, for the antrum similar anomalous effects due to changes in K^+ concentration were also found. We consider first the normal *V* response.

A principal feature of the normal *V* response, as previously stated, is that above a certain K^+ con-

TABLE IV

MAXIMUM *V* INCREASES AND CORRESPONDING RESISTANCE CHANGES OF ELEVATING K^+ FROM ZERO TO HIGHER CONCENTRATIONS ON THE NUTRIENT SIDE IN Cl^--FREE (SO_4^{2-}) SOLUTIONS

Maxima for increases from 0 to 3 mM K^+ occurred in 1.5–2 min and from 0 to 6 mM K^+ in 1 min. Other information same as Table I.

$\Delta[K^+]$ (mM)	Number of experiments	*V* before increase (mV)	ΔV (mV)	*R* before increase ($\Omega \cdot cm^2$)	ΔR ($\Omega \cdot cm^2$)
0 → 3 mM	4	0.9 ± 5.2	0.7 ± 0.3 [b]	1600 ± 160	35 ± 68
0 → 6 mM	5	0.3 ± 4.6	0.9 ± 0.3 [a]	1574 ± 154	78 ± 88

[a] $P < 0.01$.
[b] $P < 0.02$.

centration a plot of $|\Delta V|$ versus the logarithm of the K^+ concentration is linear. For the fundus, the straight-line relationship exists for concentrations from about 10 mM K^+ to higher values, whereas, for the antrum, for concentrations from about 20 mM K^+.

In order to see more clearly why a part of the curve is linear, let us refer to the upper two limbs of Fig. 6. Then for these two limbs, V is given by

$$V = \frac{R_K}{R_K + R_{Cl}} E_{Cl} + \frac{R_{Cl}}{R_K + R_{Cl}} E_K \qquad (1)$$

If K^+ is changed in the nutrient solution, Eqn. 1 becomes

$$\Delta V = \frac{R_{Cl}}{R_K + R_{Cl}} \Delta E_K \qquad (2)$$

We assumed that R_K and R_{Cl} remain essentially constant. The change in E_K is determined from the Nernst equation, namely

$$\Delta E_K = \pm 58 \log C_f / C_i \qquad (3)$$

where C_i and C_f are respectively the initial and final concentrations of K^+ in the bathing medium. From Eqns. 2 and 3, it follows that a plot of ΔV versus the logarithm of a varying C_i or C_f under the condition of constant R_K and R_{Cl} gives a linear relation. From Table I, $|\Delta R|$ from about 20 mM K^+ to higher levels would result in relatively little resistance change. Hence, a linear relationship would be expected (see Fig. 2).

We next consider the anomalous V response of the antrum compared to the anomalous V response of the fundus. In the case of the fundus, a number of different V responses were obtained with a zero K^+ secretory solution [7]. For a zero K^+ nutrient solution lasting only a few minutes, the return to 4 mM K^+ gave a normal V response. For a very long period of time in zero K^+, the return to 4 mM K^+ gave an anomalous V response which was followed by a continued rise in V. For intermediate periods of time in zero K^+, the anomalous V response was followed by a decrease in V for a minute or so and then by a continued rise in V. In the case of the antrum, the anomalous V response levelled off for a considerable period for a change from 0 to 10 mM K^+. On the other hand, for a change from 0 to 20 mM K^+, the transient response was followed by a rapid decrease in V. For a prolonged period in zero K^+, the return to 4 mM K^+ gave an anomalous response followed by a continued rise in V (without a dip) generally to a maximum.

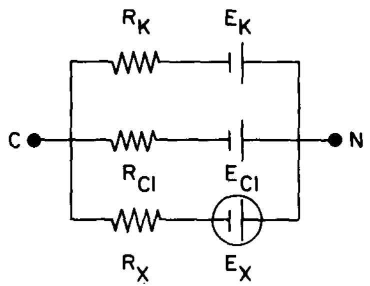


Fig. 6. Equivalent circuit for nutrient membrane comprising conductive limbs for K^+ and Cl^- and an X limb representing the ($Na^+ + K^+$)-ATPase pump. C refers to cell and N to nutrient.

As in the case of the fundus, the anomalous V response for the antrum can be explained on the basis of a ($Na^+ + K^+$)-ATPase pump which is represented by the X pathway in Fig. 6. Under usual circumstances, the K^+ pathway dominates over the X pathway and the V response is normal. For the fundus, it was found that, after a prolonged period of zero K^+ in the bathing media, the resistance of the K^+ pathway became very high and hence, the increase for 0 to 4 mM K^+ resulted in the Na^+, K^+ pump dominating over the K^+ pathway. In other words, the resistance of the K^+ pathway with zero K^+ in the nutrient fluid increased markedly and, when K^+ was changed from 0 to 4 mM K^+, the Na^+,K^+ pump dominated [1,7,15].

For the antrum, the resistance with 4 mM K^+ in the nutrient is generally very high (see Table I) while, for the fundus, the resistance is relatively low [1]. After a prolonged period of zero K^+ bathing both sides of the antrum, there was no significant increase in resistance (data from Table I compared to data from Table III), although on occasion the resistance changed by as much as 200 $\Omega \cdot cm^2$ (see Fig. 4). The ratio of the anomalous to normal $|\Delta V|$ is higher in the antrum than in the fundus so that the conductances of the R_K and R_X pathways would be expected to be more comparable in the antrum than in the fundus. Hence, in the antrum it is easier to demonstrate the anomalous response to changes in K^+.

To explain the continued rise in V, we note that the pump would increase the K^+ concentration in the continuous phase which, in turn, would increase E_K, and therefore, increase V. However, one might expect that the increase in K^+ in the cell would increase the positivity of the secretory side and this would cancel the increase in E_K. In this regard, changing the K^+ concentration on the secretory side in Cl^- media has a smaller effect on V than changing K^+ on the nutrient side (Ref. 2, and present results). Hence, the increase in K^+ in the cell would result in the nutrient becoming more positive.

In the antrum, as in the fundus, the anomalous effect in Cl^--free (SO_4^{2-}) media is small and, in fact, much smaller than in the fundus. By the replacement of Cl^- with SO_4^{2-}, the shorting effect of Cl^- is removed but, nevertheless, the anomalous V is less in SO_4^{2-}. A tentative consideration for the fundus was that in SO_4^{2-} the conductance of the K^+ limb did not decrease as much as in Cl^- media [7] and for this reason the effect of E_X in the parallel pathway was reduced. In the antrum, however, the conductance is very low in SO_4^{2-} media so that it is questionable whether the K^+ conductance pathway plays a significant role. It may well be that, in the antrum in SO_4^{2-} media, R_X is higher or, more significantly, E_X is lower than in Cl^- media.

Lastly, theoretical considerations (Ref. 7, see Appendix) give the expression for the emf (E_X of Fig. 6) for the ($Na^+ + K^+$)-ATPase pump under equilibrium conditions with $n \neq m$, namely

$$E_X = \frac{nRT}{(n-m)F}\ln\frac{[Na^+]_C}{[Na^+]_N} + \frac{mRT}{(n-m)F}\ln\frac{[K^+]_N}{[K^+]_C} + E^* \qquad (4)$$

where n is the number of sodium ions transported out of the cell into the nutrient and m the number of sodium ions transported from the nutrient into the cell in each cycle; R, T and F have their usual meanings; E^* is the contribution of the active transport energy to the emf (see Ref. 7 for details.); and subscripts C and N refer to the cell and nutrient respectively. If, then, $n > m$, it follows from this equation that increasing the Na^+ concentration decreases E_X (a normal response) while increasing the nutrient K^+ concentration increases E_X (an anomalous response). These considerations are also applicable to explain the anomalous response of the antrum.

Appendix

We previously developed Eqn. 4 (Ref. 7) but we present for the convenience of the reader a simpler development. The decreases in free energy ($-\Delta G$) for Na^+, K^+ and ATP are as follows:

$$-\Delta G_{Na} = nRT\ln\frac{[Na^+]_C}{[Na^+]_N} + nF(\psi_C - \psi_N)$$

$$-\Delta G_K = mRT\ln\frac{[K^+]_N}{[K^+]_C} + mF(\psi_N - \psi_C)$$

$$-\Delta G_{ATP} = RT\ln k\frac{[ATP]}{[ADP][P_i]}$$

where k = equilibrium constant for ATP → ADP + P_i and ψ is the potential. Let $E_X = \psi_N - \psi_C$ where E_X is the emf given in Eqn. 4. Since the system is at equilibrium (no completed circuit), the sum of the free energies must be zero, i.e.:

$$-\Delta G_{Na} - \Delta G_K - \Delta G_{ATP} = 0$$

After rearrangement, we obtain Eqn. 4 where

$$E^* \equiv \frac{RT}{(n-m)F}\ln k\frac{[ATP]}{[ADP][P_i]}$$

If $n = m$, then the electrical terms cancel and the sum of the free energies is not generally equal to zero. When the sum equals zero with $n = m$, then

$$\frac{[Na^+]_C}{[Na^+]_N}\frac{[K^+]_N}{[K^+]_C} = \left(\frac{[ADP][P_i]}{k[ATP]}\right)^{1/m}$$

and when the right-hand side equals unity, then

$$\frac{[Na^+]_C}{[Na^+]_N} = \frac{[K^+]_C}{[K^+]_N}$$

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