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ANOMALOUS POTENTIAL RESPONSE AND ($Na^+ + K^+$)-ATPase IN IN VITRO FROG GASTRIC MUCOSA

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In general, increasing K^+ on the nutrient side decreases the transmucosal PD (nutrient becomes more negative) but after bathing the mucosa in zero K^+ media for about 30 min, or longer, elevation of K^+ on the nutrient side increases the PD, an anomalous effect. In Cl^- media, increasing nutrient K^+ from zero to 4 mM produces an increase in PD (an anomalous response) of 3.1 and 5.3 mV in 2 and 5 min, respectively. Ouabain (10^{-3} M) to the nutrient side abolished the anomalous response as did removal of Na^+ (choline for Na^+) from bathing media. In SO_4^{2-} media (SO_4^{2-} for Cl^-), a significant anomalous PD response was observed when K^+ on the nutrient side was increased from zero to 1, 2 or 3 mM but not to higher K^+ concentrations. In this case, ouabain also abolished the anomalous response. It is postulated, on the basis of the effects of ouabain and the use of choline media, that an electrogenic ($Na^+ + K^+$)-ATPase pump is present on the nutrient-facing membrane in which more Na^+ than K^+ are transported per cycle.

Introduction

With 4 mM K^+ on the nutrient side, and either 4 mM or zero K^+ on the secretory side in standard Cl^- solutions, reduction of K^+ on the nutrient side from 4 mM to zero results in an increase in PD and within 10 min a return to 4 mM K^+ in the nutrient side decreases the PD to about the original level. However, if zero K^+ is maintained on both sides for about 1 h or more, then increasing the K^+ on the nutrient side results in an increase rather than a decrease in PD [1, 2]. This latter response has been referred to as an anomalous response [1,2]. Parenthetically, after 1 h with zero K^+ on both sides, the H^+ secretory rate decreases, eventually to zero, and the resistance markedly increases.

The effect on PD is regarded as anomalous since, generally, elevation of K^+ on the nutrient side decreases the positivity of the nutrient side and vice versa. This decrease in positivity will be referred to as a normal or as a conductive-limb response since it can be explained on the assumption of K^+ conductance channels in the nutrient membrane [3]. Further evidence for the existence of K^+ conductance channels is provided by experiments involving step changes of the K^+ concentration in the nutrient solution from standard conditions. It is found that decreasing the K^+ concentration increases the positivity of the nutrient and vice versa. A plot of PD vs. log [K^+] shows a good linear relation except for a small deviation from linearity for concentrations below 10 mM K^+ [3]. This logarithmic relation is readily explained in terms of passive conductance channels for K^+.

A similar anomalous effect which was abolished by ouabain was found by Petersen [4] using microelectrodes in mouse pancreatic acinar cells and by Graf and Petersen [5] using microelectrodes in mouse liver parenchymal cells. These investigators explained their findings in terms of an electrogenic ($Na^+ + K^+$)-ATPase pump. The anomalous effect in the frog gastric mucosa was previously explained on the assumption that the increase of K^+ from 0 to 4 mM activated an electrogenic Cl^- mechanism transporting Cl^- from nutrient to secretory [2]. However, studies

under short-circuiting conditions of the frog gastric mucosa in the absence of acid secretion [6–8] and other tissues (e.g., frog cornea [9]) suggest that active Cl^- transport is secondary to active Na^+-K^+ transport. It is assumed that a $(Na^+ + K^+)$-ATPase is present in the basolateral membrane and that in one cycle more Na ions are transported from cell to bathing solution than K ions in the opposite direction, for example, 3 Na^+–2 K^+ [10–12]. However, evidence for coupling ratios different than 3 Na^+/2 K^+ have been presented (see Ref. 13 for a review). In view of these considerations, we decided to determine whether the anomalous effect for the frog gastric mucosa could be explained in terms of an electrogenic $(Na^+ + K^+)$-ATPase pump. A preliminary report of this work was published elsewhere [14].

Methods

Experiments were performed on gastric mucosae of *Rana pipiens* by an in vitro method in which the mucosae are mounted between a pair of cylindrical chambers [15]. All experiments began with standard Cl^- solutions on both sides of the mucosa. The standard Cl^- nutrient (or 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 standard Cl^- secretory (or mucosal) solution contained: Na^+, 102; K^+, 4; and Cl^-, 106. In Cl^--free (SO_4^{2-}) experiments, the standard 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 standard SO_4^{2-} secretory solution contained: Na^+, 102; K^+, 4; SO_4^{2-}, 53; and sucrose, 64. Several experiments were also performed in which sodium isethionate replaced NaCl and Mg^{2+} and Ca^{2+} were added as $MgSO_4$ and $CaSO_4$. In zero K^+ solutions, Na^+ replaced K^+ and in high K^+ solutions K^+ replaced Na^+.

In this work, the transmembrane resistance, the transmembrane potential difference (PD) and the H^+ secretory rate were measured. 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 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 H^+ secretory rate was measured by the pH-stat method introduced by Durbin and Heinz [16]. 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_2/5% CO_2. Histamine was added in the first stage of the experiment to the nutrient solution to a concentration of 10^{-4} M and thereafter histamine was usually not added to subsequent solutions. Ouabain was used at a concentration of 10^{-3} M in the nutrient solution for maximal effects [12].

To avoid artefacts resulting from changing nutrient solutions, elevating the $[K^+]$ was achieved by pipetting appropriate volumes of a 79 mM K^+ solution into the K^+-free solution, e.g., 0.5 ml of a 79 mM K^+ solution added to a 9.5 ml K^+-free solution gave a $[K^+]$ of approx. 4 mM. For changes from 0 to a large concentration of K^+, say 79 mM K^+, the nutrient side was drained and rapidly replaced with the new solution. In the latter case, K^+ replaced Na^+ in the standard nutrient solution.

Results

Conductance and anomalous effects in Cl^- solutions bathing the frog gastric mucosa

Fig. 1 shows a typical experiment exhibiting the conductance effect, the anomalous effect and the elimination of the anomalous effect by ouabain. During the period shown in Fig. 1, the secretory solution was K^+-free. At the 5 min mark, the nutrient solution containing 4 mM K^+ was replaced with a K^+-free nutrient solution. The change from 4 mM K^+ to 0 K^+ resulted in an increase in PD (a K^+ conductive-limb effect) which was followed by a gradual decrease in PD. Upon rewashing with zero K^+ nutrient solution, the PD continued to decrease and the resistance increased. The H^+ secretory rate goes to zero with no K^+ in both solutions and is not plotted [1]. At about the 36 min mark, changing the nutrient solution from 0 K^+ to 4 mM K^+ gave an immediate increase in PD (an anomalous effect) and a decrease in resistance. After 3 min, the nutrient was changed back to 0 K^+ and the PD responded in an anomalous fashion, i.e., the PD instead of increasing, decreased to about the

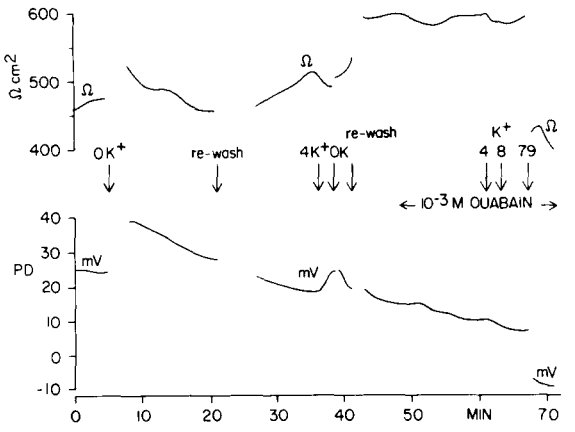


Fig. 1. Effect of changes in K^+ concentration on the nutrient side before and after ouabain. Resistance and PD vs. time in Cl^- solutions. K^+ was zero on the secretory side at all times. At first arrow (5 min), K^+ removed from nutrient, at second arrow, both sides rewashed with zero K^+, at third arrow, K^+ added to nutrient to 4 mM; at fourth arrow, nutrient replaced with zero K^+; at fifth arrow, both sides washed with zero K^+. Ouabain was added to nutrient at 47 min and then K^+ was added to nutrient to 4, 8 and 79 mM.

TABLE I

EFFECT ON PD AND RESISTANCE OF ELEVATING K FROM 0 TO 4 mM ON THE NUTRIENT SIDE IN Cl SOLUTIONS

Values are means ± S.E. of 24 experiments. PD 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.

	PD before 0 → 4 K	ΔPD	
		2 min	5 min
Control	19.1 ± 1.7	3.1 ± 0.5 [a]	5.3 ± 0.7 [a]
10^{-3} M ouabain	9.0 ± 0.9	−0.9 ± 0.9	−1.4 ± 0.2 [a]
	Resistance before 0 → 4 K	ΔR	
		2 min	5 min
Control	443 ± 22	−23 ± 6 [a]	−24 ± 11 [b]
10^{-3} M ouabain	486 ± 6	−23 ± 6 [a]	−16 ± 4 [a]

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

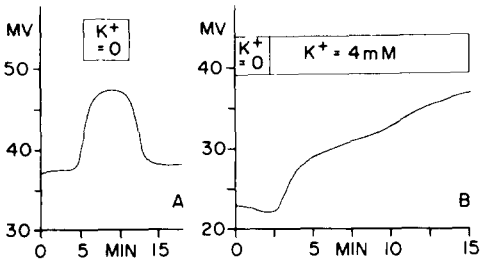
previous level and the resistance increased. Addition of ouabain to the nutrient solution (10^{-3} M) essentially abolished the anomalous response (see Table I). Note that changes of the nutrient to 4 and then to 8 mM K^+ had essentially no effect on PD, indicating a high resistance of the K^+ conductance pathway. However, changing the nutrient side to a 79 mM K^+ nutrient solution decreased the PD (a K^+ conductive-limb effect) and markedly decreased the resistance.

From Table I, we note that in 24 experiments in going from 0 to 4 mM K^+ in the nutrient solution, the average PD increased (the anomalous effect) by 3.1 mV in 2 min and by 5.3 mV in 5 min. The presence of 10^{-3} M ouabain in the nutrient solution abolished the anomalous response of the PD and resulted in a small but significant decrease in PD of the order of 1 mV (a K^+ conductive-limb effect).

In addition, three experiments in the absence of ouabain were followed long enough to obtain a maximum increase of the PD; their average change, attained in about 25 min, was 13.2 mV, confirming previous results [1,2]. Previous work [1] and unpublished results demonstrate that the anomalous response to K^+ occurs when 4 mM K^+ is maintained on the secretory side.

As shown in Table I, the resistance at the 2 and 5 min marks decreased by a small (about 5%) but significant amount both before and after the addition of ouabain. Parenthetically, the resistance decreases to approximately the original level (i.e., level before the removal of K^+ from the media) in 20–30 min after elevating the nutrient K^+ from 0 to 4 mM [1]. The reduction in resistance occurs concurrently with the reestablishment of H^+ secretion and a major portion of the decrease is undoubtedly associated with the reestablishment of secretion [1,2].

Since ouabain is known to inhibit the $(Na^+ + K^+)$-ATPase pump [17] and since ouabain abolished the anomalous effect of PD, these facts suggest that the anomalous effect is associated with the $(Na^+ + K^+)$-ATPase pump. As a further test of this hypothesis, Na^+ was replaced with choline in both the secretory and nutrient solutions. Under these circumstances, the addition of K^+ to the K^+-free nutrient solution, after a prolonged period with zero K^+ in the secretory and nutrient solutions, did not elicit the anomalous effect. Three such experiments were performed, thereby providing further support of the hypothesis that the $(Na^+ + K^+)$-ATPase pump is responsible for the anomalous effect.



I ig 2 (A) Effect on PD of changing K^+ in nutrient from 4 to zero and back to 4 mM in Cl^- solutions (4 mM K^+ on secretory side). (B) Effect on PD of changing K^+ in nutrient from zero to 4 mM in Cl^- solutions (zero K^+ on secretory side)

Variations of the anomalous effect

The PD responses depicted in Figs. 2A, B and 3A, B arise after the following treatment of the gastric mucosa. In Fig. 2A, the standard Cl^- solutions containing 4 mM K^+ were used and at the time indicated, the nutrient solution was replaced with a zero K^+ solution and in about 10 min the nutrient solution was changed back to the 4 mM K^+ solution. The PD response from 4 mM K^+ to 0 K^+ and the response from 0 K^+ to 4 mM K^+ are K^+ conductive-limb effects since the positivity of the nutrient increased for a decreased K^+ concentration and decreased for an increased K^+ concentration. In Fig. 2B and Fig. 3A and B, there was always zero K^+ in the secretory solution and one or more prolonged periods of zero K^+ in the nutrient solution. Fig. 2B shows a typical anomalous effect arising from the elevation of the K^+ from 0 to 4 mM. The initial increase in PD (the anomalous effect) is followed by a continuing increase during the entire 13 min (see Discussion). Fig. 3A shows an intermediate PD response. There is an initial increase in PD (an anomalous effect) followed by a decrease in PD which is then followed by an increase in PD. Fig. 3A and B show results obtained from the same stomach, those of Fig. 3B being obtained subsequent

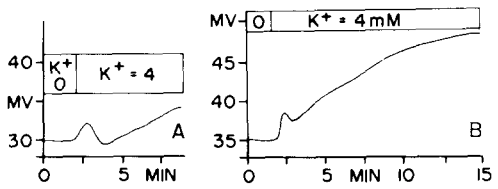


Fig. 3. (A, B) Effect on PD of changing K^+ in nutrient from zero to 4 mM in Cl^- solutions (zero K^+ on secretory side).

to the response shown in Fig. 3A. In Fig. 3B, there is only a small dip in the PD. Later in this same stomach a response similar to that seen in Fig. 2B was obtained and subsequent addition of ouabain abolished the anomalous response.

Anomalous effect in SO_4 solutions bathing the frog gastric mucosa

Experiments were performed to determine whether the anomalous effect of the PD would be enhanced (see Discussion) with SO_4^{2-} solutions bathing both sides of the gastric mucosa. In these experiments, both sides of the frog gastric mucosa were first bathed with standard Cl^- solutions with histamine at a concentration of 10^{-4} M in the nutrient solution. Then the Cl^- solutions were replaced with standard SO_4^{2-} solutions and the nutrient side became negative [18–20]. Next, the standard SO_4^{2-} solutions containing 4 mM K^+ were replaced with K^+-free SO_4^{2-} solutions for about 30 or more min, the H^+ rate decreased to zero, the resistance increased and the PD became positive. Changing the nutrient solution from 0 to 4 mM K^+ resulted either in no anomalous response or a small one. We then tried different concentrations of K^+ and found consistent anomalous responses for K^+ concentrations less (but not greater) than 4 mM in the nutrient solution.

Fig. 4 illustrates these results (see also Table II). Throughout the part of the experiment shown in Fig. 4, there was no H^+ secretion. In Fig. 4A, the PD was initially 14.7 mV (the nutrient had become positive). At the 5 min mark, K^+ was added to the nutrient solution to a concentration of 1 mM. The PD increased (an anomalous effect) to a maximum value of 1.7 mV above the control of 14.7 mV. Fig. 4B, C and D show the effects at later times (see x-axis) resulting from the addition, respectively, of 2, 3 and 4 mM K^+ to the nutrient solution following a prolonged period of a zero K^+ nutrient solution prior to each addition. The time scales along the x-axis indicate the length of the periods of zero K^+. In Fig. 4 (A, B, C and D) the maximum increases in PD above the K^+-free control levels were, respectively, 1.7, 2.7, 4.3 and 1.4 mV. It is also to be noted that with increasing concentrations of K^+ in the nutrient solution, the PD after it attains a maximum value starts to fall gradually for low concentrations and then more and more rapidly for higher concentrations. The rate of

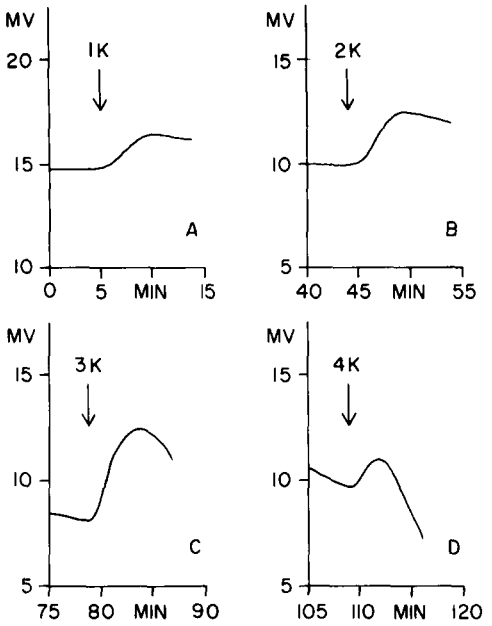


Fig 4. (A, B, C and D) Effect on PD of changing K^+ in the nutrient from zero to 1, 2, 3 or 4 mM in sulfate solutions (zero K^+ on secretory side).

decrease for 4 mM K^+ in the nutrient solution is quite pronounced. The decrease in positivity of the nutrient can be attributed in part to a K^+ conductive-limb effect. Also the decrease in PD, eventually to negative values would be expected to occur, since the presence of K^+ in the nutrient SO_4^{2-} solution activates the electrogenic proton pump, presumably located in the secretory membrane [1,19,20].

Table II gives a summary of the anomalous PD response for the SO_4^{2-} experiments. In these experiments, the mucosa had been exposed to zero K^+ in the bathing media and the H^+ rate was zero. It can be seen that there was a significant increase in PD (the anomalous effect) at both 2 and 5 min for 1 and 2 mM K^+ and at 2 min for 3 mM K^+. With 4 mM K^+, the PD decreased (nutrient becoming more negative) but only significantly at 5 min. In four of these experiments, ouabain (10^{-3} M) was added to the nutrient SO_4^{2-} solutions and the anomalous response of the PD was abolished.

In three experiments, isethionate replaced Cl^-. With isethionate, the anomalous effect on the PD was observed ([K^+] from 0 to 2 mM) and the average change was the same as that of SO_4^{2-}, i.e., 1.5 mV. Again, ouabain abolished the anomalous response.

In contrast to the result with Cl^- media, the resistance with SO_4^{2-} media (see Table II) did not show a significant decrease; in fact with 1 mM K^+, there was a small (2%) but significant increase in resistance at

TABLE II

EFFECT ON PD AND RESISTANCE OF ELEVATING K CONCENTRATION ON THE NUTRIENT SIDE FROM 0 TO 4 mM IN SO_4 SOLUTIONS

Symbols and abbreviations same as in Table 1.

K increase (mM)	No. of expts	PD before K change from 0	ΔPD		
			2 min	5 min	max
1	13	4.1 ± 1.7	0.7 ± 0.2 [a]	1.5 ± 0.4 [a]	1.6 ± 0.4 [a]
2	13	4.1 ± 1.9	1.4 ± 0.3 [a]	1.5 ± 0.4 [a]	1.7 ± 0.3 [a]
3	4	9.8 ± 1.6	2.3 ± 0.5 [b]	2.3 ± 1.3	3.0 ± 0.8 [b]
4	11	−0.2 ± 3.0	−3.3 ± 1.5	−6.1 ± 1.6 [a]	–
		Resistance before K change from 0	ΔR		
			2 min	5 min	
1	13	1082 ± 86	34 ± 10 [a]	41 ± 22	
2	13	1029 ± 85	35 ± 39	71 ± 28 [b]	
4	11	861 ± 81	2 ± 21	−26 ± 23	

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

the 2 min mark and with 2 mM K^+, there was a small (7%) but significant increase in resistance at the 5 min mark. There was no significant change in resistance with 4 mM K^+ at either the 2 or 5 min mark.

Discussion

In order to analyze our findings, we use the equivalent circuit diagram for the nutrient membrane shown in Fig. 5. The K^+ and Cl^- conductance pathways in the nutrient membrane are represented by e.m.f. values of E_K and E_{Cl} and resistances R_K and R_{Cl} The pathway X represents an active e.m.f. E_X and a resistance R_X. Previously the X referred to an active electrogenic Cl^- pump [2]. It was assumed that an increase in the K^+ concentration in the nutrient solution from 0 to 4 mM K^+ activated the Cl^- pump. The findings presented herein, that is, the abolition of the anomalous response by ouabain and in the absence of Na^+ are evidence for an electrogenic $(Na^+ + K^+)$-ATPase pump. These findings are essentially the same as those of Thomas on the snail neuron [21]. Other findings [6–8] support the concept of a $(Na^+ + K^+)$-ATPase in the nutrient membrane. On the basis of our findings, the X pathway is now considered to be the $(Na^+ + K^+)$-ATPase pathway which, because of its electrogenicity, can also be represented as a conductive pathway with an active e.m.f.

Since little is known about the parameters depicted in Fig. 5, a qualitative explanation only can be given. It can be seen that, if the X pathway dominates over the K^+ pathway, this would explain the anomalous effect and vice versa. Under standard conditions (4 mM K^+ on both sides), changes in

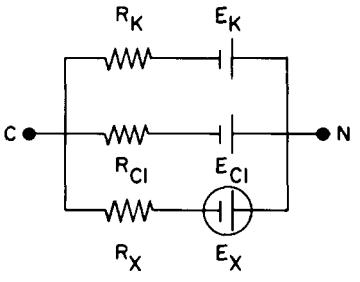


Fig 5 Equivalent circuit for nutrient membrane comprising conductive limbs for K^+ and Cl^- and an X limb representing $(Na^+ + K^+)$-ATPase pump. C refers to cell and N to nutrient.

nutrient K^+ produce changes in PD which can be explained on the basis of the K^+ pathway dominating over the X pathway.

The question arises as to why the X pathway dominates under zero K^+ conditions. Under zero K^+ conditions, the Na^+ and K^+ concentrations in the continuous phase of the cytoplasm would be expected to change (i.e., Na^+ increasing and K^+ decreasing) so that reactivation of the Na^+-K^+ pump by restoring K^+ to the nutrient side could result in a larger E_X and/or a smaller R_X than under standard conditions. Hence, changes in the parameters of the X pathway may be a factor in the anomalous response. Then again, changes in the K^+ pathway may be the most important factor in the anomalous response.

In this regard, we note that the conductance of the in vitro frog gastric mucosa depends on the K^+ concentration in the nutrient solution [3,22,23]. Increasing the $[K^+]$ in the nutrient solution increases the conductance, it attains a maximum with about 30 mM K^+. On the other hand, decreasing the K^+ concentration in the nutrient to zero with zero K^+ on the secretory side decreases the conductance eventually to very low levels [1]. In time, acid secretion ceases and at this point the K^+ in the continuous phase of the cytoplasm is very low [1,2]. Although the total cellular K^+ is still high [1,24], we assume that it is released very slowly from its stores to the continuous phase

With a prolonged period of zero K^+ in the bathing media, we assume from the above discussion that the conductance of the K^+ limb is very low (i.e., the resistance R_K is very high). Now in going from 0 to 4 mM K^+ on the nutrient side, the K^+ diffusion potential E_K would be changed so as to decrease the positivity of the nutrient, however, because the K^+ resistance is high, the Na^+-K^+ effect predominates. In Fig. 5, we assume that, with an activated Na^+-K^+ pump (an increased E_X), the X pathway dominates and the PD of the nutrient becomes more positive. However, to account for the continued rise in PD, we need to consider another factor. The pump would increase the K^+ concentration in the continuous phase which would increase the value of E_K thereby resulting in an increase in positivity of the nutrient with respect to the cell. The question arises as to whether an elevation of K^+ in the cell would not result in an increase in the positivity of the secretory fluid with respect to

the cell and hence cancel out the effect on the PD of a change in E_K in the nutrient membrane. Pertinent to this question is the fact that changing the K^+ concentration of the secretory fluid (in Cl^- media) has a much smaller effect on the transmucosal PD than changing the K^+ on the nutrient side [25]. Therefore, the increase in K^+ in the cell would be expected to increase the positivity of the nutrient side.

The two extremes of the response to changing K^+ on the nutrient side are illustrated in Fig. 2 (A and B). In going from the conditions of Fig. 2A to those of 2B, it is not surprising to find intermediate responses such as illustrated in Fig. 3, i.e., an initial increase in PD followed by a dip in PD and a subsequent increase in PD.

In Cl^- media, the anomalous response due to the increase in E_X is shunted by the Cl^- conductance limb in the nutrient membrane. One would predict that SO_4^{2-} replacing Cl^- would result in a greater anomalous response since ΔE_X would not be shunted by the Cl^- conductance limb. This result has been found by Rang and Ritchie [26] for the posttetanic hyperpolarization response in nerve; with Cl^- media the response is a few mV while with SO_4^{2-} media, about 20 mV.

In actuality, the magnitude of the anomalous response of frog stomach was not increased in SO_4^{2-}; in fact the response was more difficult to demonstrate in SO_4^{2-} than in Cl^-. At present we can at best offer some tentative considerations for this difference.

It is possible that in SO_4^{2-} or isethionate the conductance of the K^+ limb does not decrease as much as in Cl^- media. Hence, in increasing K^+ from zero to 4 mM, the E_K limb dominates over the E_X limb. With smaller increases in K^+ (0 to 1 or 2 mM), ΔE_K is smaller than with zero to 4 mM K^+ so that the E_X limb predominates.

Another consideration is that in contrast to the effects on PD in Cl^- media, changes of K^+ in SO_4^{2-} media reveal a symmetrical effect on the PD, i.e., $|\Delta PD|$ across the nutrient membrane is about the same as that across the secretory membrane [27,28]. Hence, one would not expect the PD to continue to increase in positivity in SO_4^{2-} media as it does in Cl^- media. A further point is that with 4 mM K^+ on the nutrient side, the absence of K^+ on the secretory side makes little difference; during H^+ secretion, with Cl^- media the PD is positive while with SO_4^{2-} media, due to the predominance of the proton pump, the PD is negative. Thus in SO_4^{2-} media, restoration of K^+ on the nutrient side quite rapidly produces a negativity of the nutrient due to the turning on of the proton pump.

Implication of the anomalous effect

Implicit in the foregoing explanations is the concept that the $(Na^+ + K^+)$-ATPase system is electrogenic and that more Na^+ is transported from the cell to the nutrient fluid than K^+ in the opposite direction. Substantial evidence [11,17,29] supports this concept. Some workers suggest that three Na^+ ions are transported for 2 K^+ ions but others believe that the ratio for particular tissues is different from 1.5, e.g., Candia and Reinach [9] suggest a ratio of 4 or higher for the corneal epithelium.

If more Na^+ than K^+ is transported per cycle, then in the absence of parallel pathways the system would be in equilibrium in order not to violate electroneutrality. The e.m.f. (E_X of Fig. 5) would then be given by (see Appendix):

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

where n is the number of Na ions and m the number of K ions transported per cycle; R, T and F have their usual meanings; E^* is the contribution of the active transport energy to the e.m.f. and subscripts C and N refer to the cell and nutrient, respectively.

Examination of Eqn. 1 reveals that, with $n > m$, increasing the nutrient Na^+ concentration would decrease E_X (the direction for a simple Na^+ conductive pathway) while increasing the outside K^+ would increase E_X (the opposite direction (an anomalous one) to a simple K^+ conductive pathway).

Therefore, within the framework of our assumptions, the results presented herein constitute electrophysiological evidence for more Na^+ than K^+ transport for the $(Na^+ + K^+)$-ATPase of the frog gastric mucosa.

Appendix

In this section, Eqn. 1 is developed in a similar fashion to previous developments for electrogenic proton and other electrogenic ion pumps [30–32]. See also Spangler and Goodall [33] and Rapoport [34,35].

The model consists of an ATP-driven Na^+-K^+ pump in which for 1 cycle n mol Na^+ move from the cell (C) to the nutrient (N), m mol K^+ from N to C and 1 mol ATP is hydrolyzed to 1 mol ADP and 1 mol P_i. We define k as

$$k = \frac{[Na]_C^n [K]_N^m [ADP] [P_i]}{[Na]_N^n [K]_C^m [ATP]} \quad (2)$$

We do not include the activity of H_2O in Eqn. 2 since (see Eqn. 6) we assume it to be invariant.

For K to be the equilibrium constant, either (1) $n = m$ or (2) $n \neq m$ (the situation of interest to us) and the circuit has to be completed. We consider the case $n \neq m$. Under these circumstances, the system is electrogenic. Therefore, because of the electroneutrality principle, the circuit has to be completed. Otherwise, any pattern of concentrations could be used and these concentrations would give no measurable change.

We note that Eqn. 2 assumes chemical and not electrochemical equilibrium. Hence we need a completed circuit of which the most convenient would be one in which the pump was short-circuited, i.e., the PD across the active membrane would be maintained at zero. The concentrations would change until the e.m f. of the pump (E_X) was zero.

The value of K would then be the same as that obtained by placing the enzyme in an appropriate aqueous solution, without vesicle formation, in which there were only single Na^+ and K^+ concentrations. In other words, with the pump short-circuited and with the Na^+ and K^+ ratios both equal to unity, K would be given by

$$K = \frac{[ADP]' [P_i]'}{[ATP]'} \quad (3)$$

which is the equilibrium constant for the ATP reaction. The primes indicate the concentrations at equilibrium. It is to be noted that with the Na^+ and K^+ ratios not being unity, the concentrations of ATP, ADP and P_i would be shifted, since K would obviously be constant under short-circuit conditions.

The decrease in Gibbs' free energy ($-\Delta G$) would then be given by:

$$-\Delta G = RT \ln \boldsymbol{k} + RT \ln \frac{[Na]_C^n [K]_N^m [ATP]}{[Na]_N^n [K]_C^m [ADP] [P_i]} \quad (4)$$

The decrease in ΔG with an appropriate completed circuit would be:

$$-\Delta G = (n-m) F E_X \quad (5)$$

so that from Eqn. 4 and 5 E_X would be given by Eqn. 1 and E^* by.

$$E^* = \frac{RT}{(n-m)F} \ln \frac{\boldsymbol{k} [ATP]}{[ADP] [P_i]} \quad (6)$$

Inspection of Eqn. 1 reveals that, when the Na^+ and K^+ ratios are unity, $E_X = E^*$.

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