Cationic Conductances of Nutrient and Secretory Membranes of Frog Stomach in Cl^--free Solutions (40148)

T. L. HOLLOMAN, M. SCHWARTZ AND G. CARRASQUER

Departments of Applied Mathematics, Physics and Medicine University of Louisville, Louisville, Kentucky 40208

In Cl^--free media the existence of an electrogenic pump in the frog gastric mucosa has been clearly established (1, 2). Davis *et al.* (3) showed that there exists a K^+ pathway on the secretory membrane which constitutes a return pathway associated with the H^+ pathway for current flow under open-circuit conditions. Recently Holloman *et al.* (4) showed that there exists a Na^+ return pathway. In the absence of Na^+ and K^+ in the secretory solution, the H^+ secretory rate although reduced was not abolished. This fact suggested the existence of a return pathway other than that of K^+ and Na^+, presumably SO_4^{2-}.

While the experiments above gave results concerning conductive pathways on the secretory membrane associated with H^+ secretion, they did not provide information about pathways on the nutrient membrane. The relative conductance of an ion on this membrane could be assessed by determining the effect on the transmucosal potential difference (PD) by rapid changes of the ionic composition of the nutrient fluid. Such a technique is also applicable to the secretory fluid, if so desired. Using this technique, Davis *et al.* (5) found that an increase of K^+ on the nutrient side of the gastric mucosa in SO_4^{2-} media increased the negativity of the PD of the nutrient side relative to the secretory side with a PD-log [K^+] slope of about 25 mV and that an increase of K^+ on the secretory side increased the positivity of the nutrient side relative to the secretory side with a PD-log [K^+] slope of about 25 mV. Thus in Cl^--free media the relative conductance of K^+ became the same on both sides of the gastric mucosa.

Spangler and Rehm (6) performed an extensive set of experiments using this pulse technique of changing concentration to study relative conductances of K^+ and Cl^- in Cl^- media on the nutrient side. They found that the time course of the absolute value of the change in PD following a change from a low to a high concentration of a permeant ion differed from that following the reverse change. To explain the difference, they showed that the time course for the rapid change in each case was essentially that predicted on the basis of an idealized model consisting of a membrane with fixed conductance in series with a diffusion barrier.

In the light of these considerations of Spangler and Rehm, it was decided for the gastric mucosa of Rana pipiens in Cl^--free solutions to re-examine the K^+ and Na^+ conductance on both the nutrient and secretory membranes and to study, moreover, the effect of changes of the divalent ions such as Mg^{2+} and Ca^{2+} on both membranes. The results of this investigation are described herein. A preliminary report is given elsewhere (7).

Methods. The experiments were performed on gastric mucosae of Rana pipiens with an in vitro method described in detail elsewhere (8). Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The resistance was obtained as the change in PD per unit of applied current. The H^+ secretory rate was determined by the pH stat method introduced by Durbin and Heinz (9). The pH of the secretory side was maintained at 4.90. The regular nutrient bathing solution contained (in m*M*): Na^+, 101; 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 regular secretory bathing solution contained: Na^+, 100; K^+, 4; SO_4^{2-}, 52; and sucrose, 64. Both sides of the mucosa were gassed with 95% O_2 and 5% CO_2. Histamine was added to the nutrient solution to a concentration of 10^{-4} *M*. In modified nutrient solutions Na^+ was replaced by K^+, Mg^{2+} or Ca^{2+} and K^+ by Na^+. On the secretory side Na^+ was replaced by K^+, Mg^{2+} or Ca^{2+}. In secretory experiments involving divalent ions the initial concentration of that ion was 1 m*M* in the secretory solution. For experiments with divalent ions, the sucrose concentration was altered to

0037-9727/78/1581-0096$01.00/0

insure isosmolality. Generally the nutrient solution was drained and washed once with the new solution. The first PD reading could be taken about 15 sec after the change. Further details are provided elsewhere (6). For each specific concentration, six experiments were performed except that for the first set of experiments, in which the concentration of the nutrient solution was changed from 4 m*M* K^+ to 79 m*M* K^+, eight experiments were performed.

Results. Replacement of Na^+ by K^+ and vice versa. Figure 1 shows the effects of changing the K^+ concentration on the nutrient side from 4 to 79 m*M* and then back again to 4 m*M*. At the 30 min mark, the K^+ concentration in the nutrient solution was changed to 79 m*M*. The PD at first decreased markedly, attained a minimum in the first few minutes, and then commenced to increase slowly. The resistance behaved quite similarly to the PD although this result was not typical of the series of experiments. The H^+ secretory rate decreased a small extent. At the 60 min mark, the K^+ concentration in the nutrient solution was changed back to 4 m*M*. The parameters returned partially to control levels.

Eight experiments of the type described immediately above were carried out. The results at the end of the 30 min period involving 79 m*M* K^+ in the nutrient solution were

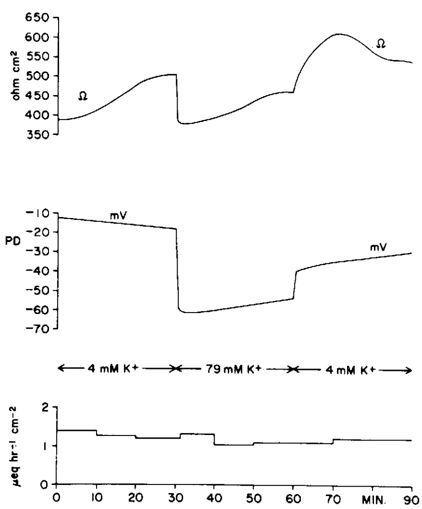

FIG. 1. Effect of changing 4 m*M* K^+ in nutrient solution to 79 m*M* K^+ in nutrient solution and back to 4 m*M* K^+ on resistance, PD and H^+ secretory rate versus time. K^+ replaced Na^+.

compared with those at the end of the control period with 4 m*M* K^+ in the nutrient solution. For these experiments, the resistance did not change significantly ($P > 0.10$) but the PD and H^+ secretory rate changed significantly ($P < 0.01$). The PD decreased by 38 mV (± 6 SD) and the H^+ secretory rate decreased by 0.25 μeq $hr^{-1}cm^{-2}$ (±0.14 SD). The latter represented a 22% (±9 SD) decrease in the H^+ rate. These results agreed with those of Davis *et al.* (3) as far as the resistance and PD changes were concerned but differed from theirs in that our results gave a significant decrease in the H^+ secretory rate. For similar experiments in which 79 m*M* K^+ in the nutrient solution was changed to 4 m*M* K^+ in that solution, our results agreed with those of Davis *et al.* in that only the PD changed significantly ($P < 0.01$). The PD increased by 30.7 ± 6.7 mV compared to their increase of 17.5 ± 6.5 mV.

However, it is with the changes that occur in the first few minutes that we are mainly concerned since from the analyses of Spangler and Rehm (6) it appears that the characteristics of the mucosal cells do not alter essentially during this period. For a change from 4 m*M* K^+ to 79 m*M* in a Cl^- nutrient solution, they showed that the absolute value of the change in PD, |ΔPD|, versus time passed through a maximum. For a change from 79 m*M* K^+ to 4 m*M* K^+ in a Cl^- nutrient solution, |ΔPD| versus time gave an inflection point. In actual practice the PD sometimes levelled off before it again decreased. In our studies we applied these criteria, recording data at maxima and inflection points.

Table I presents the results for changes in resistance and PD resulting from the change in concentration of 4 m*M* K^+ in the nutrient solution to 40 m*M* and 79 m*M* K^+ in that solution. In both cases the PD decreased significantly ($P < 0.01$) while the resistance decreased significantly ($P < 0.05$) only for the higher change in concentration. The decrease in resistance was at most 11% compared to the very marked changes in PD.

A plot of |ΔPD| versus log C_f/C_i was made where C_i represents the initial concentration of K^+ in the bathing medium and C_f the final concentration of K^+. For this purpose the two points in Table I were used together with the point $\Delta PD = 0$ for $C_f = C_i$. As shown in Fig.

TABLE I. SHORT-TIME EFFECTS ON RESISTANCE AND PD DUE TO REPLACEMENT OF Na^+ BY K^+ IN NUTRIENT SO_4^{2-} SOLUTION BATHING FROG GASTRIC MUCOSA.

Nutrient sol.					
Orig.	Final	R Ω-cm²	ΔR Ω-cm²	PD mV	ΔPD mV
Na 102[a] K 4	Na 66 K 40	540 ± 52[b]	−3.2 ± 65 ($P > 0.80$)	−31 ± 11	−33 ± 13 ($P < 0.01$)
Na 102 K 4	Na 27 K 79	613 ± 112	−67 ± 74 ($P < 0.05$)	−40 ± 16	−39 ± 4 ($P < 0.01$)

[a] Concentrations are in mM.
[b] Values are means ± SD. Columns labelled R and PD refer to original solution. Columns labelled ΔR and ΔPD refer to final solution, values corresponding to the maximum |ΔPD|.

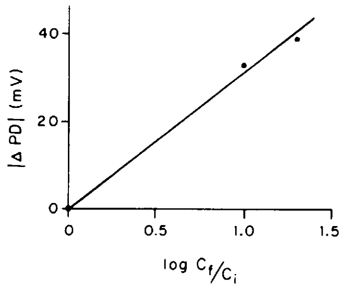

FIG. 2. Relationship of ΔPD versus log C_f/C_i. The straight line relationship is for data in which 4 mM K^+ was the reference concentration, C_i.

2, the three points lie essentially on a straight line of slope 31 mV for a tenfold change in K^+ concentration in the nutrient solution.

For nutrient solutions starting with 79 mM K^+ and changing to lower concentrations, there was an insignificant change in resistance and a significant increase in PD ($P < 0.01$). The slope was 27.4 mV. The fact that the slope is smaller in this case than in the previous case agrees with the findings of Spangler and Rehm (6).

For secretory solutions starting with 4 mM K^+ and changing to higher concentrations, there was a significant decrease in resistance of about 14% for a concentration change to 100 mM K^+ and, in general, a significant increase in PD ($P < 0.01$). The PD change in this case was opposite to that occurring for similar concentration changes on the nutrient side (Table I). The slope was 30.7 mV. It is to be noted that the slopes obtained for both the nutrient and secretory membranes with initial K^+ concentrations of 4 mM and with final higher K^+ concentrations are essentially the same for both membranes. This result agrees with that of Davids *et al.* (5). There is some difference, however, in absolute values.

Replacement of Na^+ by Mg^{2+} or Ca^{2+}. The pulse technique described above for monovalent ions was used to investigate the relative conductances of divalent cations, Mg^{2+} and Ca^{2+}, by replacing Na^+ with one of these ions in either the nutrient or secretory solution. Table II shows the results. It is evident that in all cases the replacement of Na^+ by a divalent cation gave a significant increase in resistance and a significant decrease in PD. In these experiments 4 mM K^+ was present at all times in both the nutrient and secretory solutions bathing the frog gastric mucosa.

Discussion. Figure 3 illustrates a simple approximate model which we found to be adequate to explain the results for the nutrient membrane in general and for the secretory membrane for those cases in which the resistance changes were minimal. In particular, this model readily accounts, as shown below, for the straight line relationships. The pathway comprising the emf due to a K^+ gradient and the resistance R represents the K^+ pathway across the membrane under consideration, either nutrient or secretory. This pathway is shunted by R_p which, in the absence of detailed information about ionic pathways, suffices for our present considerations. From these experiments one cannot determine whether R_p is transcellular or intercellular. However, one might speculate that R_p is transcellular since the high resistance obtained with inhibitory agents and anoxia suggests a very high resistance for the intercellular pathways. It is to be noted that, as long as the resistance changes are minimal, the H^+ pathway which is in parallel with the K^+ pathway on the secretory membrane (3, 4) may be ignored.

Let us, for the sake of the discussion, assume that the slope for experiments starting

TABLE II. SHORT-TIME EFFECTS ON RESISTANCE AND PD DUE TO REPLACEMENT OF Na^+ BY Mg^{2+} OR Ca^{2+} IN NUTRIENT AND SECRETORY SO_4^{2-} SOLUTIONS BATHING FROG GASTRIC MUCOSA.[a]

Solution		R	ΔR	PD	ΔPD
Orig.	Final	Ω-cm²	Ω-cm²	mV	mV
Nutrient sol					
Na 102 Mg 0.8	Na 27 Mg 38.3	601 ± 46	109 ± 26 ($P < 0.01$)	−35 ± 2	−10 ± 1 ($P < 0.01$)
Na 102 Ca 1	Na 80 Ca 12	490 ± 116	215 ± 57 ($P < 0.01$)	−35 ± 4	−28 ± 6 ($P < 0.01$)
Secretory sol					
Na 100 Mg 1	Na 4 Mg 49	419 ± 27	240 ± 80 ($P < 0.01$)	−29 ± 9	−8.4 ± 1.9 ($P < 0.01$)
Na 100 Ca 1	Na 80 Ca 12	534 ± 156	102 ± 63 ($P < 0.02$)	−29 ± 15	−8.6 ± 5.1 ($P < 0.01$)

[a] Same considerations as Table I. K^+ concentrations were 4 mM in nutrient and secretory solutions.

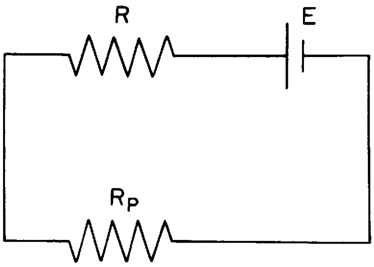

FIG. 3. Equivalent electric circuit representing the K^+ pathway on either nutrient or secretory membrane in parallel with a resistance R_p.

with 4 m*M* K^+ is approximately 30 mV per tenfold change in K^+ concentration in either the nutrient or secretory solution. Let us further suppose that the Nernst equation yields the approximate expression for a change in emf, namely

$$\Delta E = \pm 60 \log C_f/C_i \quad (1)$$

where C_i and C_f are respectively the initial and final concentrations of K^+ in the bathing solution of the membrane under consideration. From the circuit it is evident that

$$PD = R_p E/(R + R_p). \quad (2)$$

Since the change from 4 m*M* K^+ to higher concentrations of K^+ gave either no change or at most a small change in resistance, we can assume as a first approximation that R and R_p are constant. Hence Eq. (2) becomes after a concentration change in K^+

$$\Delta PD = R_p \Delta E/(R + R_p). \quad (3)$$

From Eq. (3) considering the fact that ΔE is proportional to $\log C_f/C_i$ we note that a graph of ΔPD versus $\log C_f/C_i$ should be a straight line. Moreover, for a tenfold change in K^+ concentration across the nutrient membrane, ΔE from Eq. (1) equals −60 mV and, from experiment ΔPD equals −30 mV. It follows from Eq. (3) that $R_p = R$. This accounts for the fact that ΔPD in SO_4^{2-} solutions in accordance with Eq. (3) never gets close to 60 mV.

For the divalent ions, the increases in resistance were always significant, as indicated in Table II. In the case of the nutrient membrane, if we attribute the increase in resistance to the divalent ion blocking the K^+ pathway, we can explain the decrease in PD. The K^+ gradient on the nutrient membrane is oriented with the nutrient side positive relative to the cell (6). If now the resistance R increases due to the divalent ion blocking the K^+ pathway, it follows from Eq. (2) that the PD decreases. Since the PD of the H^+ pump is oriented in the opposite direction to the PD associated with the K^+ gradient across the nutrient membrane, a decrease in the latter will make the PD across the cell more negative, that is, the nutrient side will become more negative relative to the secretory side.

In the case of the secretory membrane, we might also expect that the divalent ions would block the K^+ pathway and that, as a consequence, the resistance R would increase. Here the situation is complicated by the fact that there exists a H^+ pathway in parallel with the K^+ pathway and under the conditions of a variable resistance R, we cannot neglect this pathway. Let E_H and R_H represent respectively the emf and resistance of the H^+ pump. If we consider the appropriate orientation of the emf's in a circuit comprising the H^+ pathway in parallel with the two pathways shown in Fig. 3, we obtain for the PD across the secretory membrane

$$PD = -(R_H R_p E + R R_p E_H)/(R_p R + R R_H + R_p R_H). \quad (4)$$

Since R occurs in both numerator and denominator of Eq. (4), it is not possible without more adequate knowledge of the parameters to determine with certainty whether ΔPD would decrease with an increase in *R*.

It might be remarked that it was found previously that 1 m*M* Ba^{2+} in Cl^- nutrient solutions produced a marked increase in resistance (10) and that this increase was associated with Ba^{2+} blocking the K^+ pathway (11). The present results for the nutrient membrane suggest that Mg^{2+} and Ca^{2+} behave similarly if not as dramatically as Ba^{2+}.

Summary. The K^+ conductance in Cl^--free media of the frog gastric mucosa of Rana pipiens was found to be relatively high and essentially the same for both the nutrient and secretory membranes. The replacement of Na^+ with either Mg^{2+} or Ca^{2+} in either the nutrient or secretory solution and in the presence of 4 m*M* K^+ in both solutions increased the resistance and decreased the PD. An analysis of the results for the nutrient membrane suggests that Mg^{2+} and Ca^{2+} block the K^+ pathway.

1. Rehm, W. S., and LeFevre, M. E., Amer. J. Physiol. **208**, 922 (1965).
2. Schwartz, M., and MacKrell, T. N., Proc. Soc. Exp. Biol. Med. **130**, 1048 (1969).
3. Davis, T. L., Rutledge, J. R., and Rehm, W. S., Amer. J. Physiol. **205**, 873 (1963).
4. Holloman, T. L., Schwartz, M., Dinno, M. A., and Carrasquer, G., Amer. J. Physiol. **231**, 1649 (1976).
5. Davis, T. L., Rutledge, J. R., and Rehm, W. S., Physiologist **7**, 115 (1964).
6. Spangler, S. G., and Rehm, W. S., Biophys. J. **8**, 1211 (1968).
7. Holloman, T. L., Schwartz, M., and Carrasquer, G., Proc. Int. Union Sci. **13**, 960 (1977).
8. Rehm, W. S., Amer. J. Physiol. **203**, 63 (1962).
9. Durbin, R. P., and Heinz, E., J. Gen. Physiol. **41**, 1035 (1958).
10. Schwartz, M., Pacifico, A. D., MacKrell, T. N., Jacobson, A., and Rehm, W. S., Proc. Soc. Exp. Biol. Med. **127**, 223 (1968).
11. Pacifico, A. D., Schwartz, M., MacKrell, T. N., Spangler, S. G., Sanders, S. S., and Rehm, W. S., Amer. J. Physiol. **216**, 536 (1969).

Received October 12, 1977. P.S.E.B.M. 1978, Vol. 158.