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Microelectrode study of effects of luminal K^+ on surface cells of frog stomach

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Microelectrode studies were conducted to determine whether increasing the K^+ concentration from 4 to 80 mM in the secretory solution affected the surface cells of the frog gastric mucosa (fundus) of *Rana pipiens*. The short-circuit current (I_{sc}) increased by 10% and the conductance (G_t) increased by 19%. The potential difference (V_{sc}) from secretory solution to cell and the fractional resistance (F_o) remained essentially unchanged. On the basis of the constancy of the latter two parameters, it was inferred that the change in K^+ concentration did not affect the surface epithelial cells.

Introduction

The frog gastric mucosa has primarily two kinds of cells. These are surface epithelial cells and tubular cells, the latter being located deep in the gastric pits. The question arises whether changes of ionic concentration in the secretory fluid induce comparable changes in the lumina of the tubules. On the basis of an analysis of a distributed parameter model of the gastric mucosa [1], the concentration in the lumina constitutes a substantial fraction (60 to 80%) of that in the secretory fluid.

In attempting to decide among the various models for HCl secretion, electrogenic versus neutral models, the actual concentration in the lumina compared to the bulk concentration becomes an important consideration. Forte and Reenstra (personal communication) are of the opinion that changes in the concentration of the secretory fluid result only in small changes in the concentration in the lumina contrary to the model [1] mentioned above. Specifically we have recently shown [2] that increasing the K^+ concentration in the secretory fluid from 4 to 80 mM produces a marked decrease in the transmucosal resistance. An interpretation of this decrease is that the K^+ in the lumina is increased substantially and thereby produces the observed decrease in resistance by acting on the tubular cells. However, one might argue that this decrease in resistance could be due to an effect on the surface cells.

The primary purpose of this paper is to determine whether the surface cells are affected by the increase in K^+ concentration. In order to examine whether the observed changes in resistance occur in the surface cells, microelectrodes studies of the surface cells were conducted.

Methods

Experiments were performed on the gastric mucosa (Fundus) of *Rana pipiens* by an in vitro method in which the mucosa was mounted in a modified Ussing chamber for studies with microelectrodes [3]. On the top of the lower half chamber lies a metallic grid upon which the stripped

mucosa is placed. The upper half chamber, placed over the mucosa, has a side window for viewing the gastric mucosa with a microscope. Microelectrodes were prepared from omega-dot microfiber capillary tubes (Frederick Haer, Ann Arbor, MI) using a Narishige microelectrode puller. Afterwards the capillaries were filled with 3 M KCl from behind. The microelectrodes were driven into the surface epithelial cells by using a piezoelectric driven micromanipulator (Burleigh). The microelectrodes had input resistances of 25–75 MΩ.

Standard chloride nutrient and secretory solutions [1,4] were used to perfuse continuously the gastric mucosa at a rate of 1 ml/min for the lower compartment and about 5–10 ml/min for the upper compartment. The solutions were continuously gassed with 95% O_2/5% CO_2. The standard nutrient solution contained (in mM): 102 Na^+, 4 K^+, 1 Ca^{2+}, 0.8 Mg^{2+}, 81 Cl^-, 0.8 SO_4^{2-}, 25 HCO_3^-, 1 phosphate, and 10 glucose. The standard Cl^- secretory solution contained (in mM): 156 Na^+, 4 K^+, and 160 Cl^-. For a secretory solution with increased K^+ concentration, K^+ was substituted for Na^+, the Cl^- remaining at 160 mM. The exchange of solutions were done by a four way valve.

The short-circuit current (I_{sc}), the membrane conductance (G_t), the intracellular potential (V_{sc}) from secretory solution to cell and the fractional resistance [$F_o = (\Delta V_{sc}/\Delta V_t)$] were measured. The gastric mucosae were short-circuited by means of an automatic voltage clamping device (E. Nagel Biomedizinische Instrumente, Germering, Munich, F.R.G.). The short-circuited system was pulsed approximately every 1.2 s with a pulse of magnitude 10 mV across the mucosa and of 150 ms duration. The conductance was determined as ($\Delta I/\Delta V$) where ΔI is the change in the current and $\Delta V = 10$ mV. The intracellular potential was measured using a higher impedance microelectrode amplifier and the fractional resistance was then calculated as the change of the intracellular potential from secretory fluid to cell divided by the change of potential across the mucosa. All the parameters I_{sc}, G_t, V_{sc} and F_o were recorded simultaneously on a four-channel recorder (Linseis) and at the same time a parallel output was fed to an Apple computer where the data were digitized and printed.

Results

As in previous work with microelectrode impalements of gastrointestinal epithelia [5–8] and due to the complex morphology of these epithelia in which more than one type of cell exists, it is essential to establish proper criteria for a good impalement. In the present study, our criteria for acceptable impalement were similar to those established by others [9,10]. Our objective was to impale the surface cells and, therefore, no effort was made to evaluate potentials appearing at a depth lower than 20 μm after the first appearance of negative potential. Specifically, the electrode was moved under the view of a microscope in steps of 10 μm. After the first impalement into a cell in which negative potential was recorded, the microelectrode was moved back out of the cell and was then laterally very slightly. Then the microelectrode was advanced again in steps of 2 μm and as soon as a stable negative potential appeared in which no significant change of the microelectrode resistance occurred, the microelectrode was not advanced further. Typically, for a good and stable impalement, no electrical noise

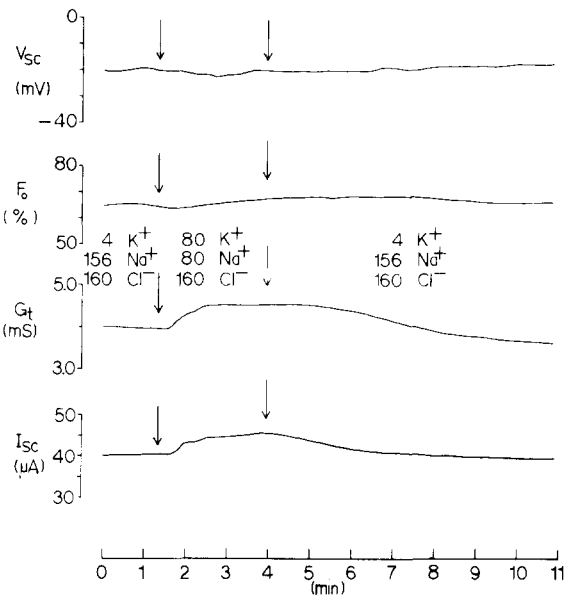

Fig. 1. Actual tracing of the short-circuit current (I_{sc}), the conductance (G_t), the fractional resistance (F_o), and the intracellular membrane potential (V_{sc}) versus time.

TABLE I

THE EFFECT ON TRANSCELLULAR AND INTRACELLULAR PARAMETERS DUE TO INCREASES IN K^+ CONCENTRATIONS ON THE SECRETORY SIDE

Values are means ± S.E. for eight experiments. Student's *t*-test using paired observations were used to determine the level of significance. The columns labeled I_{sc}, G_t, V_{sc} and F_o refer to the control values of the short-circuit circuit, transmembrane conductance, potential difference from secretory solution to cell and fractional resistance respectively. The columns labeled ΔI_{sc}, ΔG_t, ΔV_{sc} and ΔF_o refer to changes in the four parameters following the change to the final concentration of K^+. Control $[K^+] = 4$ mM. Final $[K^+] = 80$ mM.

Expt.	I_{sc} (μA)	ΔI_{sc} (μA)	G_t (mS)	ΔG_t (mS)	V_{sc} (mV)	ΔV_{sc} (mV)	F_o (%)	ΔF_o (%)
1	46	7	2.95	0.25	48	−6	53	−3
2	80	6	2.40	0.92	22	4	40	10
3	84	12	3.05	1.60	46	−2	44	2
4	41	5.5	4.00	0.60	20	−1	65	3
5	85	17	3.37	1.13	20	0	20	−2
6	80	3	3.90	0.20	43	−2	23	0
7	75	5	4.10	0.24	40	2	22	−1
8	87	3	3.45	0.25	43	−1	28	−3
Mean	72.2	7.3	3.4	0.65	35.2	−0.75	36.9	0.75
S.E.	6.4	1.7 [a]	0.21	0.18 [a]	4.4	1.0	5.8	1.50

[a] Indicates $P < 0.01$.

was observed on the microelectrode signal (monitored on a Tektronix memory oscilloscope).

From a good impalement, the intracellular potential under short-circuit conditions varied from 20 to 50 mV while the fractional resistance varied from 20 to 70%. These variations were observed in eight different mucosae with a large number of impalements in each mucosa. The variations, however, in any single tissue showed much less scatter. It is to be noted that a low fractional resistance does not imply corresponding low intracellular potential as one would expect if the sealing of the cell membrane around the microelectrode was inadequate, that is, it was found that there is no correlation between the value of the fractional resistance and that of the intracellular potential. Fig. 1 depicts a tracing of the data as recorded by the strip chart recorder. At the position of the first arrow the secretory solution was changed from (4 K^+, 156 Na^+, 160 Cl^-) to (80 K^+, 80 Na^+, 160 Cl^-). At the second arrow the original solutions were restored.

Table I summarizes the principal results in eight experiments in which the K^+ concentration in the secretory solution was changed from 4 to 80 mM. It is evident that the short-circuit current and the conductance increases significantly by 7.3 μA and 0.65 mS, respectively. The other two parameters, namely the intracellular potential V_{sc} and the fractional resistance F_o, do not change significantly.

We shall now compare the results obtained here

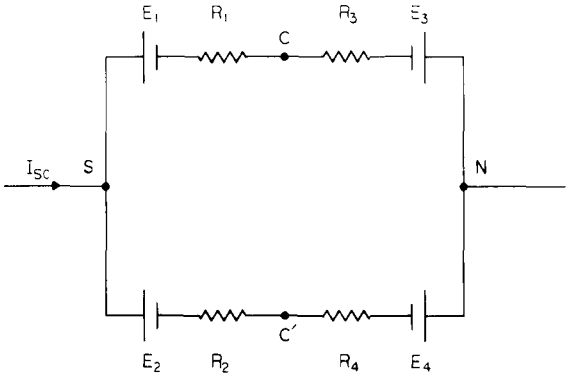

Fig. 2. A simple electrical circuit showing the surface cells and the tubular cells. R_1, E_1 refer to the apical resistance and emf, respectively, of the surface cells and R_3, E_3 to the basolateral resistance and emf of these cells. R_2, E_2 and R_4, E_4 are corresponding resistances and emf's of the tubular cells. S refers to secretory side and N to nutrient side. C and C′ refer, respectively, to the surface and tubular cells.

with those determined by us in a previous study of transepithelial measurements with macroelectrodes [2]. In that paper, the short-circuit current was not determined but the direction of change could be inferred from calculations, i.e. $I_{sc} = (PD/R_t)$ where R_t is the total or equivalent resistance across the mucosa. Since it was found that the resistance decreased by about 37% while the PD after a transient increase of 6 mV decreased to control levels, it follows that the short-circuit current, I_{sc}, increased. Qualitatively this result concurs with the increase in I_{sc} observed in Table I.

In the previous paper the conductance increased by 37% whereas in the present study, the conductance increased by 19%. It should be noted that short-circuiting increases the resistance [11] and hence changes in resistance under short-circuiting conditions would generally not be expected to correspond quantitatively with resistance changes under open-circuit conditions.

Discussion

Let us refer to the circuit shown in Fig. 2 in which R_1 and R_3 are the respective resistances of the apical and basolateral parts of the surface epithelial cells and R_2 and R_4 are the respective resistances of the apical and basolateral parts of the tubular cells. The E's are the corresponding emf's. Under short-circuit conditions, it follows that for the surface cells

$$V_{sc} = E_1 + \frac{E_3 - E_1}{1 + R_3/R_1} \quad (1)$$

We now show that the resistances R_1 and R_3 are unlikely to be the locus of the observed changes in conductance. Since experimentally G_t was found to increase, then the resistance either in the surface cells or tubular cells or both decreases. If the decrease occurred in R_1, then according to Eqn. 1, V_{sc} would decrease and, if the decrease occurred in R_3, V_{sc} would increase. Both of these results are contrary to the experimental finding that V_{sc} remains constant. It might happen that R_1 and R_3 change proportionately as, for example, if both of these resistances were inversely proportional to the concentration. However, such proportionalities seem unlikely since the K^+ concentration changes are made in the secretory fluid and would have to induce comparable changes of cellular K^+ which in turn would have to cause a proportional change in R_3.

In addition, if the surface cells had a finite partial K^+ conductance, then one would expect a marked change in the diffusion potential and hence in E_1. In other words, in going from 4 to 80 mM K^+ in the secretory solution, the ratio of the concentrations across the apical membrane would decrease and hence E_1 would decrease. From Eqn. 1, it is evident that the dominating term is the first term on the right side of the equation, namely E_1, and hence V_{sc} would markedly decrease contrary to the experimental finding.

According to the above analysis, R_1 and R_3 do not change and hence the fractional resistance does not change in agreement with the experimental results. It follows that the observed changes in conductance and short-circuit current induced by the changes in K^+ concentration do not occur in the surface epithelial cells. This conclusion supports the previous interpretations [2] that elevation of K^+ in the secretory solution produces a significant increase in the K^+ concentration in the lumina.

In changing from 4 to 80 mM K^+, the Na^+ concentration in the secretory solution changed from 156 to 80 mM. Unpublished experiments with choline replacing K^+ and with constant Na^+ gave similar results to the present study.

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