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NON-STEP INTRACELLULAR VOLTAGE RESPONSE OF EPITHELIAL AND OXYNTIC CELLS IN FROG GASTRIC MUCOSA

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Summary

Intracellular analyses of the electrical potential response to current pulses were performed in epithelial and oxyntic cells of the frog gastric mucosa. Independently of the use of Cl^- or SO_4^{2-} solutions and of the transmucosal potential difference measured, the resistance and slow potential changes observed across the mucosal surface of epithelial cells are not different from those registered across the serosal surface. In the oxyntic cells the resistance and slow potential changes are significantly higher across the mucosal than across the serosal surface, when the solution in contact with the serosal surface is positive with respect to the solution in contact with the mucosal surface. These differences were not observed when the transmucosal potential difference was reversed. It is proposed that the ionic concentration changes, by effect of current flow across the mucosa, occur in the oxyntic cells and that these changes are the origin of the slow transmucosal potential change. The epithelial cells act as a passive shunt.

Introduction

The passage of steps of current across isolated frog gastric mucosa results in non-step changes in transmucosal potential difference. The time courses of the electrical potential changes have been described by curves with one or two exponential terms and one term independent of time [1—6]. A single exponential with a long time constant may describe the potential transient changes obtained with current densities up to $10\,\mu A/cm^2$ [1]. Some departures from the values calculated using the single exponential model have been obtained during the first few seconds, when higher current densities were used. Under these circumstances a second exponential term is required to fit the observed potential changes [2,3]. The step potential change, corresponding to the

term independent of time, represents mainly the contribution of the resistance to the final potential change. The occurrence of the slow potential change has been explained by alterations in the ionic concentration in a region comprised between two membranes of different characteristics [1,3]. This region between two membranes must correspond to the continuous cell layer separating the nutrient from the secretory solution. However, this cell layer is basically formed by two different cells types, i.e. epithelial and oxyntic cells [7]. They maintain different intracellular potentials [8] and show different response to current pulses [9].

Since analyses of transmucosal potential changes are unable to elucidate the cellular origin of the potential transient, a study of the role of each cell type on the generation of the long time-constant potential change seems reasonable.

For this purpose, the intracellular potential response of the oxyntic and epithelial cells, to transmucosal current flow, is studied in this paper. This analysis is based on the observation that in chloride solution oxyntic and epithelial cells have different intracellular electrical potentials and that therefore they may be located and identified by their electrical values [8]. In addition electrogenic Cl^- transport [10] has been located at the mucosal surface of the oxyntic cells, while the serosal surface of the oxyntic cells and both surfaces of the epithelial cells are thought to play passive roles in the maintenance of the transmucosal potential difference [8,11]. Potential changes were measured across both surfaces of each cell type, within 4.0 s of step current changes performed in Cl^- and SO_4^{2-} solutions. A 4-s observation time was chosen since, in this relatively short period, 50% of the slow potential change has been completed [3].

Methods

Frogs (*Rana pipiens*) used in these experiments were pithed, their stomachs removed and opened along the small curvature and the mucosa stripped by blunt dissection.

Composition of the solutions. Two buffered solutions, Cl^- and SO_4^{2-}, were used in the experiments. The Cl^- solutions used has the following composition [8] (in mM): NaCl, 84.6/KCl, 3.2/$CaCl_2$, 1.8/KH_2PO_4, 0.8/$MgSO_4$, 0.8/$NaHCO_3$, 17.8/glucose, 22.0. The SO_4^{2-} solution was made by replacing Cl^- by SO_4^{2-} and adding sucrose to keep the osmolality between 215 and 220 mosM. Histamine diphosphate was added to solutions to a final concentration of 10^{-4} M. The solutions were always bubbled with 95/5, O_2/CO_2. The pH of the solution in equilibrium with the gas phase containing 5% CO_2 was 7.4. The pH of the solutions used was always checked before and after incubation of the mucosa.

Experimental procedure. Each mucosa was mounted between two chambers, as previously described [8]. The area of the mucosa exposed between the chambers was 0.79 cm^2. Both chambers were filled with the same solution and each contained two agar-Ringer bridges. One bridge, located close to the mucosa, in each chamber was connected to a calomel electrode. A glass microelectrode, less than 0.5 μm in diameter and filled with 3 M KCl, was implanted in the cell from the mucosal surface. The microelectrode was connected to a

third calomel electrode by an agar-KCl bridge. The potential differences from the calomel electrodes were measured into two high input impedance digital multimeters and registered into printers at time intervals of 0.4 s. One meter registered the transmucosal potential difference, between calomel electrodes connected to the two external agar-Ringer bridges in the chambers. The other meter recorded the potential difference between the microelectrode tip and the bridge located in the solution in contact with the mucosal surface. The remaining two agar bridges, located as far as possible (approximately 10 mm) from the mucosa, were connected to two Ag-AgCl electrodes. These electrodes were used to pass current across the mucosa from a battery-operated source.

Each experiment was performed in the following sequence: the mucosa was mounted between the chambers and equilibrated for 30 min with Cl^- solution in both chambers. After equilibration, cells were impaled to obtain cellular potential measurements. Sequences of positive and negative current pulses were then passed through the preparation. In some cases 50 μA positive pulses were passed first, followed by negative pulses. In other cases negative pulses were passed first, followed by positive pulses. Then the solution in both chambers and in the circulating system was changed 5 times by SO_4^{2-} solution. After 15 min equilibration, cellular measurements with their corresponding pulse sequences were obtained again. Finally, enough current (from the external source) was passed through the preparation so that the transmucosal potential difference reached a value close to that measured in Cl^- solution. After 15 min equilibration, cellular measurements with their corresponding pulse sequences were obtained.

From both the transmucosal and intracellular electrical potential records, the following measurements were used: (a) the electrical potential difference, i.e., the last reading obtained before pulse application; (b) the initial transmucosal potential change, i.e., the difference between the reading obtained before pulse application and the (second) potential reading taken 0.8 s after pulse application; (c) the slow potential change, i.e., the difference between the (second) 0.8 s-reading and the (tenth) 4.0 s-reading after pulse application. Comparisons between the potential changes obtained with positive and negative current pulses show no differences. In consequence, the two values for the potential difference changes obtained from each impaled cell were pooled. Values from cells in which the intracellular potentials before pulse application and 10 s after show a difference smaller than 3 mV were used in the calculation. If the difference was larger than 3 mV the measurements were rejected.

Results

Fig. 1 shows the frequency of the distribution of the intracellular potentials recorded in Cl^- and in SO_4^{2-} solutions. The top graph represents results of the 407 intracellular potentials registered during incubation of 32 mucosae in Cl^- solution. The mean transmucosal potential was 26 ± 2 mV. The middle graph corresponds to the 219 intracellular potentials measured during incubation of the same mucosae in SO_4^{2-} solution and open circuit condition. The spontaneous transmucosal potential was −10 ± 3 mV. The bottom graph corresponds to the 406 intracellular potentials measured during incubation of the

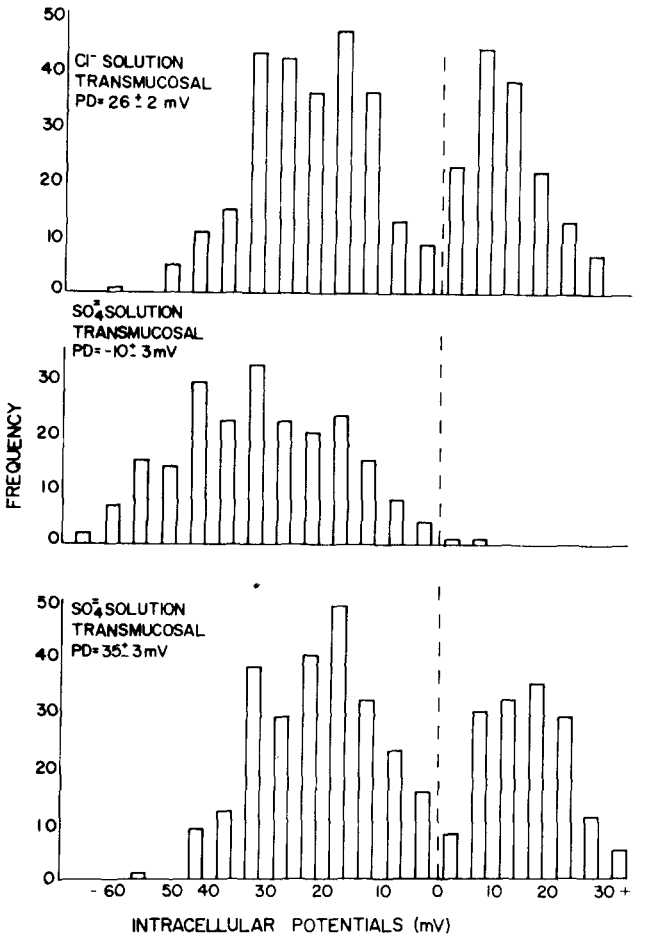

Fig. 1. Frequency of distribution of intracellular potentials as referred to the solution in contact with the mucosal surface. The top graph corresponds to mucosae incubated in Cl^- solution. Middle and bottom graph correspond to the same mucosae during incubation in SO_4^{2-} solution. Measurements in the top and middle graph obtained with mucosae maintaining spontaneous transmucosal potential difference. In the lower graph values obtained when transmucosal potential difference was reversed by passing a current across the mucosa.

same mucosae in SO_4^{2-} solution, when the transmucosal potential difference was restored to a mean value of 35 ± 3 mV by passing across the mucosa current from the external circuit. When the transmucosal potential difference was positive (top and bottom graphs) the intracellular potentials are grouped in two populations, one of positive and another of negative cells. In the middle graph, which corresponds to negative transmucosal potential, only negative intracellular potential were registered.

In Table I the means of the potential values obtained are presented. In Cl^- solutions the two cells populations were averaged separately. The positive cells which should correspond to the oxyntic cells [8,11] represent 1/3 of the total number of measurements. The negative cells found in 2/3 of the impalement should correspond to the epithelial cells [8,11]. In SO_4^{2-} solution only

TABLE I

INTRACELLULAR POTENTIALS REFERRED TO THE SOLUTION IN CONTACT WITH THE MUCOSAL SURFACE

Each value is the mean ± S.E. The number given in parenthesis is the number of measurements obtained in 27 mucosae.

Solution	Transmucosal potentials (mV)	Intracellular potentials (mV)	
		Positive cells	Negative cells
Cl^-	26 ± 2 (407)	12.4 ± 0.6 (148)	−24.1 ± 0.6 (259)
SO_4^{2-}	−10 ± 3 (219)	— (2)	−33.0 ± 1.0 (217)
SO_4^{2-} *	35 ± 3 (406)	17.7 ± 0.7 (159)	−21.8 ± 0.7 (247)

* Potential induced by passing current across the mucosae from an external circuit.

negative intracellular potentials were registered. This posed the problem of determining when the microelectrode tip was in an oxyntic or in an epithelial cell. Records from both cells were expected from the depth of the microelectrode tip position. When external current was applied to restore the original sign of the transmucosal potential difference, observed in Cl solutions, negative and positive intracellular potentials were again obtained in SO_4^{2-} solutions.

Table II represents the ratio of the cell membrane resistances calculated from the difference between the reading obtained before pulse application and the reading taken 0.8 s after pulse application, registered from the same cells reported in Table I. The positive cells, in Cl^- solution show a R_{muc}/R_{ser} ratio significantly higher than that of the negative cells. When Cl^- is replaced by SO_4^{2-} in the solution, the mean R_{muc}/R_{ser} presumably represents both oxyntic and epithelial cells. The mean R_{muc}/R_{ser} in SO_4^{2-} solution is smaller than that obtained separately for oxyntic and epithelial cells in Cl^- solution. Even this suggests that cell resistances become symmetrical when the electrogenic Cl^- transport was abolished and transmucosal potential reversed. When the transmucosal potential is restored to the level measured in Cl^- solution, by passing a current from an external souce, the membrane resistance ratio of the positive

TABLE II

CELL MEMBRANE RESISTANCES RATIO (R_{muc}/R_{ser}) CALCULATED FROM THE INITIAL (90.8 s) POTENTIAL CHANGES

Each value is the mean ± S.E. The number given in parenthesis is the number of measurements obtained in 27 mucosae.

Solution	Transmucosal potentials (mV)	R_{muc}/R_{ser}	
		Positive cells	Negative cells
Cl^-	26 ± 2 (407)	2.82 ± 0.14 (148)	1.29 ± 0.05 (259)
SO_4^{2-}	−10 ± 3 (219)	— (2)	1.06 ± 0.05 (217)
SO_4^{2-} *	35 ± 3 (406)	1.82 ± 0.09 (159)	0.99 ± 0.04 (247)

* Potential induced by passing current across the mucosae from an external circuit.

TABLE III

SLOW (0.8—4.0 s) INTRACELLULAR POTENTIAL CHANGES

Each is the mean ± S.E. The number given in parenthesis is the number of measurements obtained in 27 mucosae.

Solution	Intracellular potential changes (mV)			
	Positive cells		Negative cells	
	Cell-mucosal	Cell-serosal	Cell-mucosal	Cell-serosal
Cl^-	1.12 ± 0.05	0.10 ± 0.02 (148)	0.63 ± 0.06	1.18 ± 0.06 (259)
SO_4^{2-}	—	—	3.65 ± 0.09	0.80 ± 0.06 (217)
SO_4^{2-} *	4.35 ± 0.12	0.09 ± 0.06 (159)	2.99 ± 0.10	1.44 ± 0.11 (247)

* Potential induced by passing current across the mucosae from an external circuit.

cells is significantly higher than the ratio measured in the negative cells in SO_4^{2-} solutions.

Table III present the slow potential change registered between 0.8 and 4.0 s after the current pulses were passed. In Cl^- solution the slow potential changes which developed across the mucosal and serosal surfaces of the negative cells are not significantly different from each other ($P > 0.20$). In the positive cells more than 90% of the slow potential change occurs across the mucosal surface. The difference between average slow potential changes recorded across mucosal and serosal cell surfaces is significant to a level to $P < 0.001$. When Cl^- is replaced by SO_4^{2-} (all cells are negative), the difference between mucosal and serosal average potential changes is significant to the level of $P < 0.001$ and both values are also significantly higher than those obtained in positive and negative cells during Cl^- solution incubation. During current flux across the mucosa incubated in SO_4^{2-} solutions the slow potential develops at both surfaces of the negative cells and predominantly in the mucosal surface of the positive cells, as observed during incubation in Cl^- solution.

Discussion

In the present work intracellular analyses of the potential response to current pulses were intended. Recordings were performed in the two cell types that constitute the continuous cell layer separating the two external solutions, i.e., the epithelial and the oxyntic cells.

The epithelial cells cover the surface and the pits region and play a passive role in the transmucosal ionic fluxes [8]. They manifest always negative electrical potentials with respect to solutions in contact with both mucosal and serosal surfaces [8,11]. The present work shows that independently of the solution used and of the transmucosal potential difference registered, they show a cell-mucosa to cell-serosal membrane resistance ratio (R_{muc}/R_{ser}) approaching 1. Also independently of solution compositions and of transmucosal potential difference they develop symmetrical slow potential changes at both cell surfaces by effect of square current pulses.

The oxyntic cells occupy almost all the oxyntic gland and seem to be

responsible for the electrogenic Cl^- transport [10] and for the H^+ secretion. They show a negative cellular potential with respect to the solutions in contact with both surfaces only in the absence of Cl^-, when the serosal side is negative [8]. When the solution in contact with the serosal surface is positive with respect to the solutions in contact with the mucosal side, either spontaneously in Cl^- solution, (by effect of the electrogenic chloride transport) or in SO_4^{2-} solutions (when a current is passed across the mucosa (from an external circuit)), oxyntic cells are positive with respect to the solution in contact with the mucosal surface. Under these circumstances the cell-mucosal resistance is always significantly higher than the cell-serosal resistance. Current pulses develop a long-term constant transient potential significantly higher in the mucosal than in the serosal surface. In SO_4^{2-} solution, when the transmucosal potential difference is negative and all cells have negative potentials, the mean value of slow potential at the mucosal surface is also higher than that at the serosal surface, probably by the contribution of the oxyntic cells.

A difference in membrane resistance is required to change the intracellular composition subsequent to passage of a current flow [1,3]. Therefore, in the oxyntic cells, which are the cells that show different membrane resistances, but not in the epithelial cells, current flow must induce changes in their ionic content. The gradual change in ionic content may originate the slow potential changes recorded across the two membranes limiting oxyintic cells. The differences between those two potentials created in the mucosal and serosal surfaces is manifested as the slow transmucosal potential change. This potential difference is partially shunted across the epithelial cells. In short: (a) in the absence of electrogenic chloride transport, when the original transmucosal potential was restored, negative and positive cell electrical potentials were again obtained in SO_4^{2-} solutions, (b) long-term constant potential change manifested across the mucosa originates in the oxyntic cells and is shunted by epithelial cells.

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