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INTERCELLULAR SPACE CONDUCTANCE IN FROG GASTRIC MUCOSA

LEOPOLDO VILLEGAS AND LAURA SANANES

Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, I.V.I.C., Apartado 1827, Caracas (Venezuela)

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SUMMARY

The effects of urea or sucrose hyperosmotic solutions and the effect of abolishing the transmucosal potential difference on the electrical conductance of the mucosa were investigated. The results are interpreted considering the intercellular space as the third pathway for the ionic movement in addition to the oxyntic and epithelial cells.

INTRODUCTION

In frog gastric mucosa there are at least two active mechanisms: one for the transport of Cl^- and the other for the H^+ secretion[1,2]. Both active mechanisms are located in the oxyntic cells[3,4]. The current created by these mechanisms is dissipated by passive ionic shunting and it is manifested as the spontaneous potential difference. This passive ionic shunting was located at the epithelial cells[3,4]. In addition, a third conductance pathway has been proposed more recently[5,6]. This pathway may represent the intercellular spaces. The present paper deals with the contribution of this third pathway to the transmucosal conductance. The transmucosal conductance was measured in mucosae incubated in solutions of given tonicities which produce known changes in the cellular and extracellular volumes[7].

EXPERIMENTAL AND DISCUSSION

Four groups of experiments were performed: groups A and B in open circuit conditions, using hyperosmotic solutions prepared with urea and sucrose. In Groups C and D, the mucosae were maintained in short circuit conditions with the same hyperosmotic solutions. The isosmotic solution[3] had the following composition 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; and glucose, 22.0. Sucrose and urea hyperosmotic solutions were prepared by adding 100 mosmoles of the test molecule per l of isosmotic solution. Each experiment was performed as follows: the mucosa was mounted between two chambers[8] with two calomel electrodes for measuring the potential difference and two Ag–AgCl electrodes for sending current through the mucosa. Both chambers were filled with isosmotic solution and were stirred and oxygenated with O_2–CO_2 (95:5, v/v). After 30 min equilibration,

Abbreviation: PD, potential difference.

two pulses of 2 s duration, 50 μA positive followed by 50 μA negative, were applied each min during the rest of the experiment. The changes in potential (ΔPD) induced by these current pulses were recorded. Conductance was obtained from the ratio between potential changes and current pulses. The mucosa was incubated in this way for 60 min in isosmotic solution. Then it was switched during the last 30 min, to the test hyperosmotic solution. In the case of experiments in short circuit conditions, after the initial 30 min equilibration in open circuit conditions, the mucosa was clamped at zero potential. Again current pulses were applied each min. At the end of each experiment of the four groups, K^+ and water content were measured in the mucosa. Water content was obtained by weight difference, after drying in an oven for 24 h at 95 °C. The K^+ content was determined by flame photometry in an extract obtained in 1 M HNO_3.

Fig. 1 shows the results obtained in one experiment of each group. The applied current (I) is shown at the top and the potential difference (PD) at the bottom of each graph. After switching to hyperosmotic urea solution (A and C) there was a small increase in the Δ PD in the open circuit (A) or in the short circuit conditions (C). Under open circuit conditions the addition of sucrose (B) increases the Δ PD. This

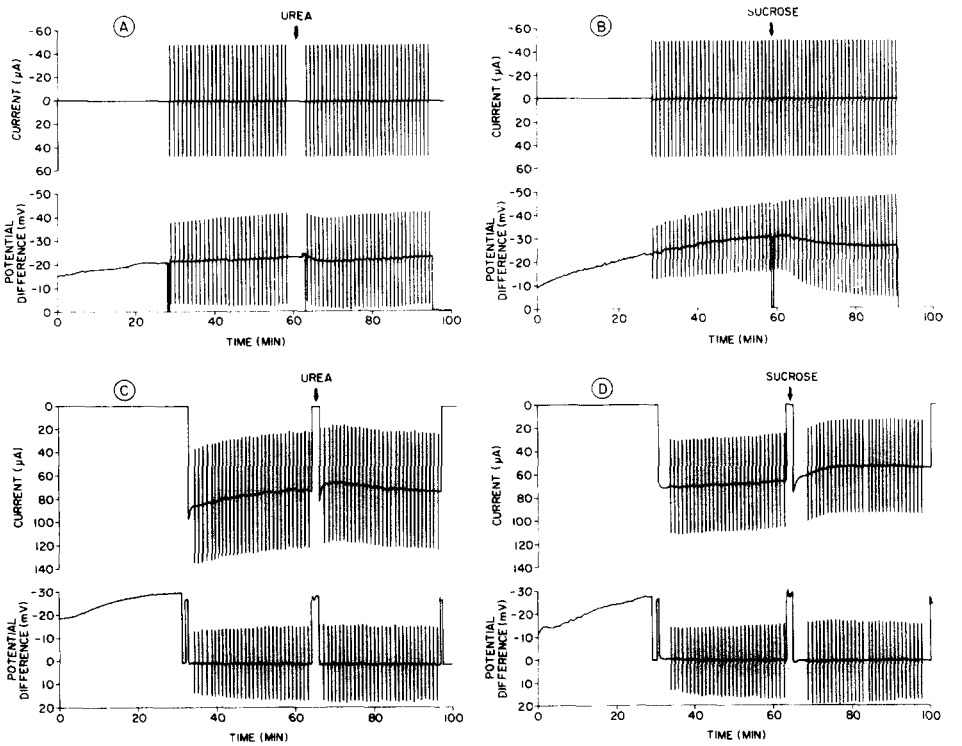

Fig. 1. Effect of the osmolality of the solution on the electrical conductance of the frog gastric mucosa. Applied current and transmucosal potential as function of time: (A) mucosa incubated in open circuit with hyperosmotic urea solution; (B) mucosa incubated in open circuit with hyperosmotic sucrose solution; (C) mucosa incubated in short circuit with hyperosmotic urea solution; and (D) mucosa incubated in short circuit with hyperosmotic sucrose solution.

increment requires about 15 min to be completed and corresponds to a decrease in the transmucosal conductance. This effect of sucrose is smaller when the experiment is performed in short circuit conditions (D). This smaller effect indicates that the change in conductance produced by the hyperosmotic solution was associated in some way to the presence of the transmucosal potential difference.

Table I gives values of the conductance, equivalent to the inverse of the initial resistance[9], measured before the long transient produced by a dc transmucosal current flow[10]. The first column shows the values obtained in isosmotic solutions (g_1). The second column corresponds to the values obtained in the final 15 min of incubation in hyperosmotic solutions (g_2). The last column corresponds to the mean values of the conductance ratios between g_2 and g_1 estimated for each mucosa. In all cases conductance in the hyperosmotic solution is smaller than in the isosmotic solution. Sucrose has a larger effect on the conductance than urea. Sucrose is expected to have a larger osmotic effect than urea. Reflection coefficients[11] of 0.99 and 0.86, respectively, can be estimated from the values previously reported for $A/\Delta x$[12]. This suggests a relationship between the change in conductance and the osmotic effect of the test molecule. The relative permeability of the cellular membrane to K^+ is higher than to other ions[3,13]. Thus, the K^+ concentrations were measured in order to use them as indicators of the conductance through the cells.

TABLE I

EFFECT OF THE OSMOLALITY OF THE SOLUTION ON THE CONDUCTANCE OF THE FROG GASTRIC MUCOSA

Each value is the mean ± S.E. Number of experiments are given in parentheses.

	Conductance ($m\Omega^{-1}/cm^2$)		g_2/g_1
	Isosmotic (g_1)	*Hyperosmotic* (g_2)	
Urea, open circuit (10)	3.37 ± 0.34	3.10 ± 0.28	0.93 ± 0.03
Sucrose, open circuit (7)	3.99 ± 0.35	2.80 ± 0.21	0.71 ± 0.04
Urea, short circuit (10)	3.27 ± 0.17	3.14 ± 0.19	0.96 ± 0.03
Sucrose, short circuit (10)	2.74 ± 0.11	2.41 ± 0.11	0.88 ± 0.02

TABLE II

EFFECT OF THE OSMOLALITY OF THE SOLUTION ON THE POTASSIUM CONTENT OF THE FROG GASTRIC MUCOSA

Each value is the mean ± S.E. from 10 experiments. Sucrose and urea solutions were prepared by adding 100 mosmoles of the test molecule per 1 isosmotic solution.

	μequiv K^+/g dry wt	*μequiv K^+/ml water*
Open circuit		
Isotonic	248 ± 9	48 ± 2
Urea	217 ± 7	35 ± 3
Sucrose	220 ± 5	48 ± 2
Short circuit		
Isotonic	215 ± 5	40 ± 1
Urea	224 ± 4	39 ± 2
Sucrose	211 ± 3	44 ± 1

Table II shows the K^+ content measured after incubation. In the first column, K^+ contents are expressed per unit dry weight. In the second column, the same results are expressed as concentrations per unit water of the mucosa. At the top of the table are the values obtained from the mucosae incubated in open circuit conditions. A reduction of the potassium content per unit dry weight is observed with urea and sucrose. Because of the water loss, after 30 min incubation in the sucrose hyperosmotic solutions, the potassium concentration per unit water reaches values similar to those obtained in isosomotic solution. After 30 min incubation in hyperosmotic urea solution mucosa has recovered its water content. This is manifested in a reduction in the potassium concentration per unit water content of the mucosa. At the bottom of the table are the values obtained from mucosae incubated in short circuit conditions. Under these circumstances, hypertonicity by either sucrose or urea produces a smaller effect on the K^+ and water content. No change in the K^+ concentration per unit water content is observed in either case.

The conductance is likely to vary with the ionic concentrations and with the membrane potential[14]. No change was observed either in potentials or in K^+ concentrations (K^+_i and K^+_0). Thus the contribution of the cellular fraction to the conductance of the mucosa should remain constant. However, the transmucosal conductance decreases with sucrose. In open circuit conditions, the volume of the extracellular space increases from 0.41 ± 0.02 in isosmotic solution to 0.50 ± 0.02 in hyperosmotic sucrose solution[7]. This increment of intercellular space width, and cross sectional area, should result in an increase of the conductance. The observed reduction in the conductance can be interpreted as being due to a reduction of the ionic concentration gradient created in this space by the bulk solution flow[15]. Apparently the geometric change does not compensate totally the composition change resulting in a reduced conductance through the intercellular space. In short circuit conditions, the electrical potential difference through the intercellular space has been abolished. Ionic composition of this space must also change. The extracellular space is reduced from 0.34 ± 0.07 to 0.21 ± 0.04 by short circuiting[16]. This should result in a reduced contribution of intercellular space and the transmucosal conductance. As a consequence, the transmucosal conductance should be smaller and the effect of sucrose hyperosmotic solution on this conductance should be reduced. This interpretation implies that all the intercellular space, and not only the junctional complex, offers a restriction to the ionic flux. This implication is in good agreement with the low width (200 Å) to length (20 μm) ratio reported for the intercellular space pathway[15]. In short, the change in the transmucosal conductance produced by the hyperosmotic solutions can be explained as a consequence of the changes produced in the intercellular spaces. In that case, the conductance through this pathway represents an important fraction of the transmucosal conductance.

REFERENCES

1 C. A. M. Hogben, *Am. J. Physiol.*, 180 (1955) 641.
2 E. Heinz and R. P. Durbin, *Biochim. Biophys. Acta*, 31 (1959) 246.
3 L. Villegas, *Biochim. Biophys. Acta*, 64 (1962) 359.
4 J. G. Forte, P. H. Adams and R. E. Davies, *Nature*, 197 (1963) 874.
5 L. Villegas and L. Sananes, *Acta Cient. Venez.*, 22, Suppl. 2 (1971) 77.
6 L. Villegas, *Proc. 1st Eur. Biophys. Congr.*, 5 (1971) 347.
7 L. Villegas and L. Sananes, *Proc. Int. Union Physiol. Sci.*, 7 (1968) 453.

8 H. H. Ussing and K. Zerahn, *Acta Physiol. Scand.*, 23 (1957) 110.
9 E. Heinz and R. P. Durbin, *J. Gen. Physiol.*, 41 (1957) 101.
10 G. W. Kidder and W. S. Rehm, *Biophys. J.*, 10 (1970) 215.
11 O. Kedem and A. Katchalsky, *J. Gen. Physiol.*, 45 (1961) 143.
12 L. Villegas, *Biochim. Biophys. Acta*, 75 (1963) 131.
13 L. Villegas, *Proc. 4th Inter-Am. Symp. Peaceful Appl. Nuclear Energy, Pan-Am. Union*, 2 (1962) 93.
14 A. L. Hodgkin and P. Horowitz, *J. Physiol.*, 148 (1959) 127.
15 L. Villegas, L. Sananes and F. Michelangeli, *Am. J. Physiol.*, 220 (1971) 1282.
16 L. Sananes and L. Villegas, *Acta Cient. Venez.*, 21, Suppl. 1 (1970) 17.