

BBA 71854

NON-ELECTROLYTE DIFFUSION ACROSS THE FROG GASTRIC MUCOSA UNDER OSMOTIC GRADIENTS

LEOPOLDO VILLEGAS *

Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, IVIC, Apartado 1827, Caracas 1010A (Venezuela)

(Received May 6th, 1983)

Key words: *Non-electrolyte transport; Water diffusion; Osmotic gradient; (Frog gastric mucosa)*

Serosal-to-mucosal and mucosal-to-serosal diffusion of ^{14}C -labelled inulin, sucrose, erythritol and propionamide was compared with ^3HHO diffusion in mucosae incubated with isosmotic solutions at both surfaces, as well as isosmotic solution at serosal surface and hyperosmotic solution at the mucosal surface. The use of a hyperosmotic solution at the mucosal surface significantly increases unidirectional diffusion fluxes of inulin and of sucrose. To a nonsignificant extent, it affects the fluxes of erythritol and propionamide and significantly reduces the ^3HHO diffusion. A size increment of the diffusion path utilized by the larger molecules is proposed.

Introduction

The apical border of the oxyntic cells in the stomach is the site of active transport and therefore the locus of the endogenous osmotic gradient responsible for the spontaneous net water flow [1]. This endogenous osmotic gradient is accessible to the external solution, at least in frog gastric mucosa [2].

If active transport and osmotic flows are distributed uniformly over this apical surface, a modest osmotic gradient should be sufficient to drive the observed spontaneous net water flux [3–5]. In addition, the fluxes obtained in response to positive or negative osmotic gradients occur in the direction predicted by the tonicity of the solutions [6] and are asymmetric in magnitude [7]. Simultaneously with these osmotic fluxes, changes in the tonicity of the solutions alter the cell water content, the spontaneous net water fluxes [8] and also

the transmucosal electrical conductance of the frog gastric mucosa [9].

The main aim of the present series of experiments is to investigate to what extent changes in the osmolality of the solution at the mucosal surface alter the permeability of mucosa to graded-size, low-lipid-soluble non-electrolytes.

Methods

Frogs, *Rana pipiens*, were used in the experiments. Diffusion of ^{14}C -labelled inulin ($M_r = 5000\text{--}5500$), sucrose, erythritol and propionamide was measured by placing the isolated mucosa between two chambers. The volume of each chamber was 4 ml. The area of the mucosa exposed between the chambers was 1.13 cm^2 . After 15 min equilibration in isosmotic buffered solution [1], diffusion fluxes were measured over two successive 90 min periods. To measure the unidirectional diffusion fluxes, one of the chambers was filled with radioactive solution prepared with $0.5\text{ }\mu\text{Ci}$ of ^{14}C -labelled molecule per ml of solution. The solution in the other chamber was removed every 10 min

* The author is also Professor at the Instituto Internacional de Estudios Avanzados, Caracas, Venezuela.

thereafter for 90 min and counted for ^{14}C activity. Initial and final 10 μl samples from the labelled solution were counted in each period. In some of the experiments of each group, carrier-free tracer was used. The final concentration ranged from 0.11 mM for erythritol to 0.21 mM for sucrose. In other experiments, carrier was added to a final concentration of 1 mM. No significant relation was observed between tracer concentration and diffusion, and, consequently, results were pooled for each test molecule. Isosmotic (220 mosmol/kg water) solutions were used in both chambers during the initial 90 min period. During the second period, the osmolarity of the solution in contact with the mucosal surface was increased to 520 mosmol/kg water by adding sucrose to the isosmotic solution. Isosmotic solution was used at the serosal surface also during the second period. The solutions in both chambers were always oxygenated and stirred by bubbling with O_2/CO_2 (95/5, v/v). Simultaneously, the transmucosal electrical potential difference was continuously recorded through two calomel electrodes connected by 3% agar buffered close to the mucosa. The means of the potentials obtained were not significantly different from those previously reported [7].

Results

Fig. 1 presents the results obtained in a typical serosal-to-mucosal inulin diffusion experiment. The ordinates represents the quotient obtained by dividing the total activity recovered in the unlabelled solution (cmp) by the area of the mucosa (cm^2) times the specific activity of the labelled solution (cmp/ μl). The abscissa represents the elapsed time (min) after tracer addition. Two straight lines were fitted by least-squares: one to the values obtained in the first period, with isosmotic solution at both surfaces, and the other to the values obtained during the second period, after the change to hyperosmotic solution at the mucosal surface. Slopes of these lines have units of permeability coefficient, cm/min. Initial values were discarded and lines were fitted to the points obtained after the tracer fluxes reached a steady value. As expected, the time required for the flux to reach the steady value was shorter for the smaller molecules than for the larger molecules. In the case of

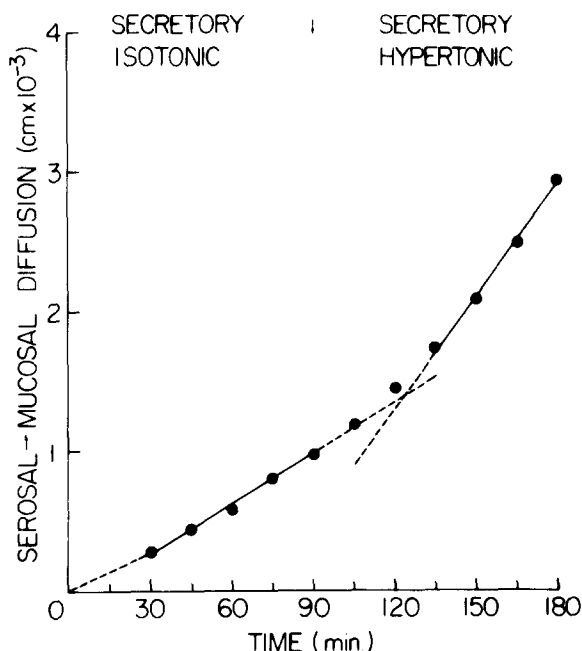


Fig. 1. Results of a serosal-to-mucosal diffusion experiment. The lines were fitted by least-squares to the points between 30 and 90 min and between 135 and 180 min. Tonicity of the solution at the mucosal surface was changed from 220 to 520 mosmol/kg after 90 min diffusion measurement. Isosmotic, 220 mosmol/kg solution was used at the serosal surface during the 180 min.

inulin, the two initial points of each period were discarded and lines were fitted to the remaining points.

The means of the slopes obtained from groups of five diffusion experiments performed with each of the test molecules are given in Table I, as well as similar values for ^3HHO diffusion taken from a previous paper [10]. Columns 1 and 2 present the values obtained with isosmotic solution at both surfaces, and with isosmotic solution at the serosal surface and hyperosmotic solution at the mucosal surface. The last column presents the means of the differences between each one of the values used in the calculation of the means shown in columns 1 and 2. The upper half of the table corresponds to the serosal-to-mucosal fluxes and the lower half corresponds to the mucosal-to-serosal fluxes.

In the case of resting and stimulated mucosae, an endogenous spontaneous serosal-to-mucosal net water flux is present [6,11]. In the presence of this

TABLE I
TRANSMUCOSAL DIFFUSION UNDER OSMOTIC GRADIENTS

Permeability coefficients are expressed in $\text{cm} \cdot \text{s}^{-1} (\times 10^7)$. S-to-M, serosal-to-mucosal; M-to-S, mucosal-to-serosal. Each value is the mean \pm S.E. from five experiments.

	220	220	Differences
Serosal tonicity (mosmol/kg)			0
Mucosal tonicity (mosmol/kg)	220	520	300
Permeability coefficients			
S-to-M			
Inulin	2.0 ± 0.4	3.4 ± 0.5	1.4 ± 0.2
Sucrose	4.7 ± 1.0	8.0 ± 1.2	3.3 ± 0.9
Erythritol	10.8 ± 0.6	13.7 ± 0.8	2.9 ± 1.4
Propionamide	132.8 ± 12.0	170.1 ± 11.9	37.3 ± 13.1
^3HHO	1019.4 ± 19.4	816.7 ± 17.7	-202.7 ± 25.0
M-to-S			
Inulin	2.4 ± 0.2	2.9 ± 0.2	0.5 ± 0.2
Sucrose	3.1 ± 0.4	4.9 ± 0.6	1.8 ± 0.6
Erythritol	11.6 ± 1.3	13.8 ± 3.0	2.2 ± 2.3
Propionamide	115.1 ± 24.1	126.2 ± 42.2	11.1 ± 17.0
^3HHO	983.3 ± 16.7	730.6 ± 13.9	-252.7 ± 22.2

net water flux, solutes going in the same direction will be speeded up, whereas solutes moving in the opposite direction will be slowed down, thus distinguishing the ratio between the unidirectional fluxes from the ratio between the solutes activities (solvent drag) [12]. Similarly, a sweeping effect of net water flow can enhance the solute concentration in the unstirred layer adjacent to the serosal surface or deplete the concentrations in the unstirred layer at the mucosal surface. In this way, water flow can alter the local concentration gradient across the mucosa and thereby change the solute fluxes (solute polarization) [13]. In the presence of spontaneous net water flux, the serosal-to-mucosal solutes fluxes are not significantly different ($P > 0.05$) from the mucosal-to-serosal fluxes of the same molecules. The use of hyperosmotic solution at the mucosal surface induces an increment of the serosal-to-mucosal net water flux. Both solvent drag and solute polarization, which presumably produce separate effects, tend to increase the serosal-to-mucosal solute fluxes. However, this increment in net water flux does not significantly increase the difference between the unidirectional fluxes of the test molecules.

It must be pointed out that the comparison is made between values obtained in different muco-

sae: one group of mucosae was used to measure the serosal-to-mucosal flux of each one of the test molecules, and a different group of mucosae was used to measure the mucosal-to-serosal flux of the same molecules. On the other hand, the comparison between fluxes in the same direction within Table I, corresponds to different periods of measurements of the same group of mucosae. Consequently, the last column of Table I presents the means of the differences produced by increments of the tonicity of the solution at the mucosal surface. These differences are significant ($P < 0.01$) for inulin, sucrose and ^3HHO : the solute fluxes increase, while the ^3HHO flux decreases when a hyperosmotic solution is used at the mucosal surface. Differences for erythritol and propionamide are not significant ($P > 0.05$). For all solutes, the difference between serosal-to-mucosal and mucosal-to-serosal increment induced by hyperosmotic solution at the mucosal surface should be explained by a solvent drag effect or a solute polarization. However, the increment in the mucosal-to-serosal solute fluxes does not respond to a solvent drag, since mucosal-to-serosal fluxes should have been reduced by the increment in the net water flux moving in the opposite direction. To explain this flux increment, a change in the per-

TABLE II

ESTIMATION OF THE RATIO $A/\Delta x$ AND FRACTIONAL DIFFUSIONAL AREA (A_s/A_w) FOR THE LOW-LIPID-SOLUBLE NON-ELECTROLYTIC MOLECULES

$$\frac{A_s}{A_w} = \frac{A/\Delta x \text{ test molecule}}{A/\Delta x \text{ water}}$$

	$D(\times 10^5)$ (cm ² /s)	Mucosal isosmotic			Mucosal hyperosmotic			Hyperosmotic/ isosmotic A_s/A_w (%)
		$P(\times 10^7)$ (cm/s)	$A/\Delta x$ (cm)	A_s/A_w (%)	$P(\times 10^7)$ (cm/s)	$A/\Delta x$ (cm)	A_s/A_w (%)	
Inulin	0.15	2.2	0.15	3.7	3.2	0.21	6.7	181
Sucrose	0.48	3.9	0.08	2.0	6.5	0.13	4.2	210
Erythritol	0.98	11.2	0.11	2.7	13.8	0.14	4.5	167
Propionamide	1.20	124.0	1.03	25.2	148.2	1.23	39.4	156
³ HHO	2.45	1001.3	4.09	100.0	763.7	3.12	100.0	100

meability of the mucosa to the test molecule is required. The larger increment of the fluxes of the test molecules from the serosal-to-mucosal surface could result from the presence of both a change in permeability of a positive drag effect. On the other hand, the smaller increment in the fluxes from the mucosal-to-serosal surface could be the result of the increment in permeability and the simultaneous negative effect of solvent drag and solute polarization.

Although the asymmetry between serosal-to-mucosal and mucosal-to-serosal fluxes is an effect of solvent drug or solute polarization, the means of the unidirectional fluxes were arbitrarily used to calculate as a first approximation the permeability coefficients for this molecule. Table II, first column, presents the average permeability coefficients, and the second column presents the diffusion coefficients in free solutions. According to Fick's equation, dividing the permeability coefficient by the diffusion coefficient, a value for the ratio ($A/\Delta x$) is obtained, where A represents the effective area available for diffusion, and Δx the length of the diffusion path.

The fraction of the water diffusional area that could be used for the diffusion of the test molecule was estimated by dividing $A/\Delta x$ for each test molecule by $A/\Delta x$ for water. The values obtained in isosmotic and hyperosmotic solution are also shown in Table II. The last column of this table presents the ratio between the fractional area for

the diffusion of the test molecule in hyperosmotic solution and the fractional area in isosmotic solution. No errors or significant levels are given in the table, since (a) the unidirectional diffusion permeabilities for each molecule have been averaged, (b) comparison has been established between results from different groups of experiments, and (c) diffusion in free water has been used for molecules with different oil/water partition coefficients. However, it is clear that the use of hyperosmotic solution at the mucosal surface significantly increases the fractional diffusional area utilized by the test molecules. In addition, the ratio observed between the size of the molecule and the fractional diffusional area suggests that the increment in the area could be achieved by increasing the size of the pathways more than by increasing the number of paths. This may occur across the paracellular pathway without alteration of the cellular membrane, thus keeping constant the transcellular ionic conductivity [9].

In summary, simultaneous with the reduction in water diffusion, the use of hyperosmotic solution at the mucosal surface produces an increment in the fluxes of non-electrolyte, low-lipid-soluble molecules. The relation between the size of the test molecule and the diffusion effect suggests an increment in the size of the diffusion path mainly utilized by the larger molecules to cross the isolated frog gastric mucosa.

Acknowledgements

The author thanks Dr. G. Whitembury for his comments on the manuscript and Dr. Laura Sananes for her co-operation in the completion of the experiments. The valuable technical assistance of Mrs. Aurora Vila-Rios is appreciated.

References

- 1 Villegas, L. (1962) *Biochim. Biophys. Acta* 64, 359–367
- 2 Durbin, R.P. (1979) *Am. J. Physiol.* 236, E63–E69
- 3 Helander, H.F. and Hirschowitz, B.I. (1972) *Gastroenterology* 63, 951–961
- 4 Durbin, R.P. and Helander, H.F. (1978) *Biochim. Biophys. Acta* 513, 179–181
- 5 Helander, H.F. (1977) *Gastroenterology* 73, 956–957
- 6 Durbin, R.P., Frank, H. and Solomon, A.K. (1956) *J. Gen. Physiol.* 39, 535–551
- 7 Villegas, L. (1980) *Am. J. Physiol.* 238, G298–G302
- 8 Villegas, L. (1975) *Am. J. Physiol.* 228, 738–741
- 9 Villegas, L. (1983) in *Ion and Water Transport* (Vasilescu, V., ed.), Plenum, London, in the press
- 10 Villegas, L. (1982) *Biochim. Biophys. Acta* 685, 249–252
- 11 Rehm, W.S., Butler, C.F., Spangler, S.G. and Sanders, S.S. (1970) *J. Theoret. Biol.* 27, 443–453
- 12 Andersen, B. and Ussing, H.H. (1957) *Acta Physiol. Scand.* 39, 228–239
- 13 Smulders, A.P., Tormey, J.McD. and Wright, E.M. (1972) *J. Membrane Biol.* 7, 164–197