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WATER DIFFUSION UNDER OSMOTIC GRADIENTS IN FROG GASTRIC MUCOSA

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The asymmetry of the osmotic response of the frog gastric mucosa has been further analyzed by studying the effect of external tonicity changes on the water diffusion fluxes. Hyperosmotic solution at the serosal surface does not affect the water diffusion fluxes. Hyperosmotic solution at the mucosal surface, with isosmotic solution at the serosal surface, significantly reduces ($P<0.001$) the serosal-to-mucosal and the mucosal-to-serosal water diffusion. An increment in the restriction offered by the mucosa to water diffusion by effect of hypertonicity at the mucosal surface is proposed.

Introduction

In frog gastric mucosa in vitro the osmotically induced net water flux is asymmetric, independent of the tonicity of the solutions, and is not significantly affected by the sweep of solutes at the mucosal surface [1]. To further analyze this asymmetry, the effect of external tonicity changes on the water diffusion was studied. Hyperosmolality was created by adding glucose to isosmotic buffered solution [2]. The effect of the use of hyperosmotic solution at each surface and also at both surfaces of the mucosa simultaneously on the water diffusion fluxes were measured.

Methods

Frogs, *Rana pipiens*, were used in the experiments. Diffusion of ^{3}HHO was measured by placing the isolated mucosa between two chambers. The volume of each chamber was 4 ml. The area of the mucosa exposed to the solution was 1.13 cm^2. After 15 min equilibration in isosmotic buffered solution, the diffusion fluxes were measured in three successive 1-h periods. To measure the ^{3}HHO diffusion, one of the chambers was filled with solution prepared in water labelled with 0.2 μCi of ^{3}H per ml. The solution in the other chamber was removed and replaced every 10 min thereafter for 1 h and counted for ^{3}H activity. Initial and final 10-μl samples from the labelled solution were counted in each period. Isosmotic solutions were used in both chambers during the initial and the final periods. During the second period the osmolality of the solution, in one or in both chambers, was changed as indicated in Tables I to III. 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 solution bridges close to the mucosa. The means of the potentials obtained were not significantly different from those previously reported [1].

Results

Tables I to III present the water diffusion fluxes in μl/cm^2 per h calculated from the activities recovered in the unlabelled solution after 10 min in the chamber, the activities of the labelled solu-

TABLE I

EFFECT OF THE SIMULTANEOUS TONICITY CHANGE AT BOTH SURFACES ON THE WATER DIFFUSION FLUXES

Isosmotic (220 mosM/kg water) solution was used at both surfaces during the first and the third periods. Each value is the mean ± S.E. from ten mucosae.

Tonicity 2nd period (mosM/kg water)		Water fluxes (μl/cm^2 per h)		
		1st period	2nd period	3rd period
Serosal	Mucosal			
Serosal-to-mucosal water flux				
320	320	366 ± 6	369 ± 5	354 ± 5
420	420	370 ± 5	352 ± 5	345 ± 5
518	518	306 ± 10	298 ± 8	277 ± 11
Mucosal-to-serosal water flux				
320	320	380 ± 6	384 ± 7	357 ± 7
420	420	373 ± 7	377 ± 7	345 ± 7
518	518	375 ± 8	375 ± 5	361 ± 4

tions and the area of the mucosa exposed between the solutions. Activity of the labelled solution at each time was calculated from the initial and the final activities counted, assuming that the tracer disappearance was lineal. Within 10 min of replacing solutions, water flow reaches a steady value. In 10 min, mucosa maintained in hyperosmotic media has completed its volume change, and potassium concentrations lowers to 46.1 ± 2.1 mequiv./kg tissue water which is not significantly ($P > 0.1$) different from the 43.9 ± 1.5 mequiv./kg tissue water measured in isosmotic solution [3]. Water flux in each period is the average of the five measurements obtained between 10 and 60 min of incubation. No correction was made for the back flow since the specific activity of the unlabelled solution after 10-min incubation was always in the order of 1% of the specific activity of the labelled solution.

Table I corresponds to the effect of simultaneous and symmetrical osmolality changes at both surfaces of the mucosa, and during the second period, on the water diffusion fluxes. The upper half of the table presents the serosal-to-mucosal fluxes. The lower half of the table presents the mucosal-to-serosal fluxes. No significant ($P > 0.5$)

TABLE II

EFFECT OF TONICITY CHANGES AT THE SEROSAL SURFACE ON THE WATER DIFFUSION FLUXES

Isosmotic (220 mosM/kg water) solution was used at both surfaces during the first and the third periods. Each value is the mean ± S.E. from ten mucosae.

Tonicity 2nd period (mosM/kg water)		Water fluxes (μl/cm^2 per h)		
		1st period	2nd period	3rd period
Serosal	Mucosal			
Serosal-to-mucosal water flux				
320	220	356 ± 7	349 ± 7	358 ± 5
420	220	349 ± 10	350 ± 10	351 ± 10
520	220	377 ± 8	379 ± 8	396 ± 7
Mucosal-to-serosal water flux				
320	220	339 ± 6	337 ± 7	332 ± 6
420	220	365 ± 7	368 ± 7	368 ± 10
520	220	361 ± 6	380 ± 7	370 ± 6

change is observed in the serosal-to-mucosal or in the mucosal-to-serosal fluxes when isosmotic solutions were changed to hyperosmotic solutions ($P > 0.01$ for the serosal-to-mucosal flux in 440/440 mosM solutions). In the third period when isosmotic solution was restored in both chambers, the reductions of the mucosal-to-serosal water diffusion fluxes are significant. In general the total reductions from the initial to the final periods, both measured in isosmotic solutions, are significant ($P < 0.01$) for the serosal-to-mucosal and for the mucosal to serosal water diffusion fluxes, independently of the tonicity of the solutions used in both chambers during the second hour.

Table II corresponds to the effect of changes in the osmolality of the solution at the serosal surface during the second period on the water diffusion fluxes. In these experiments an isosmotic solution was used at the mucosal surface during the three successive periods, as indicated in the table. No changes in water diffusion fluxes ($P > 0.5$) was observed when the solution at the serosal surface was changed from iso- to hyperosmotic, in the second period, or when the isosmotic solution was restored at the serosal surface during the third hour.

Table III corresponds to the effect of change in the osmolality of the solution used at the mucosal surface, on the water diffusion fluxes during the second period. In these experiments an isosmotic solution was used at the serosal surface during the three successive periods as indicated in the table. Both the serosal-to-mucosal and the mucosal-to-serosal water diffusion fluxes are significantly ($P < 0.001$) reduced when the solution at the mucosal surface was changed from iso- to hyperosmotic during the second period. These reductions represent from 17 to 20 per cent of the water diffusion fluxes measured during the initial period and is higher than the spontaneous net water flux previously reported [4,5]. The changes observed in the water diffusion fluxes are independet of the hypertonicity of the solution at the mucosal surface. On restoration of isosmotic solution at the mucosal surface, during the third period, water diffusion does not recover the values obtained during the initial period, measured also in isosmotic solution.

Reductions are observed in the flux originated from the solution with reduced water activity (mucosal-to-serosal) and also in the flux originated from the unaltered isosmotic solution (serosal-to-mucosal), only when hyperosmolality was used at the mucosal surface. The reduction in mucosal-to-serosal water diffusion is in the direction predicted from the reduction in the water activity at the mucosal surface, considering the mucosa as a single barrier for the water diffusion. Considering the existence of extracellular restricted compartments arranged in series with the mucosa [6,11], accumulation of solutes at the mucosal surface could

TABLE III

EFFECT OF TONICITY CHANGES AT THE MUCOSAL SURFACE ON THE WATER DIFFUSION FLUXES

Isosmotic (220 mosM/kg water) solution was used at both surfaces during the first and the third periods. Each value is the mean±S.E. from ten mucosae.

Tonicity 2nd period mosM/kg water		Water fluxes (μl/cm^2 per h)		
		1st period	2nd period	3rd period
Serosal	Mucosal			
Serosal-to-mucosal water flux				
220	320	327±8	265±5	259±3
220	420	370±5	309±4	321±6
220	524	367±7	294±6	296±6
Mucosal-to-serosal water flux				
220	320	369±7	294±5	292±5
220	420	319±5	257±4	274±6
220	524	354±6	263±5	286±8

produce reductions in the mucosal-to-serosal water diffusion fluxes larger than those expected from the externally applied hyperosmolality. On the contrary, reductions observed in the serosal-to-mucosal water diffusion could not be explained by solvent/solute interactions, considering the mucosa as a single barrier for water diffusion. If local solute gradients in the restricted extracellular compartments was the true explanation, the solutes would have to be lowered at the mucosal surface or increased at the serosal surface in order to reduce the serosal-to-mucosal water diffusion. Reductions in solutes at the mucosal surface as a source of the reduction in the serosal-to-mucosal water diffusion could be ruled out since a simultaneous reduction in the mucosal-to-serosal diffusion was observed, rather suggesting a reduction in the water activity by increasing solutes at the mucosal surface. The possible accumulation of solutes at the serosal surface is unlikely: The transmucosal ion fluxes do not appear to be altered by addition of glucose at the mucosal surface since transmucosal electrical potential difference, short circuit current and acid secretion remain unchanged (Table VI, Ref. 1) and glucose is large enough to be retained at the mucosal surface [12]. Even in the extreme case of using isosmotic solution at the serosal surface and hyperosmotic 520 mosM solution at the mucosal surface during the second hour, the short circuit current and acid secretion of 4.86 ± 0.56 and 4.29 ± 0.21 μequiv./cm^2 per h measured in isosmotic solution during the first hour, do not change significantly ($P > 0.1$) being 4.18 ± 0.42 and 4.27 ± 0.18, respectively, during the second hour. In the absence of solute/solvent interaction able to explain the reduction in serosal-to-mucosal water diffusion, a change in the total restriction offered by the mucosa to water diffusion is required to explain the results observed. This reduction in water conductivity across the mucosa, by effect of hyperosmolality at the mucosal surface, could also be responsible for at least part of the reduction in the mucosal-to-serosal water diffusion. Hyperosmolality at the serosal surface does not produce any effect on the total restriction offered by the mucosa to water diffusion when the solution at the mucosal surface was isosmotic and cancel the effect of the use of hyperosmotic solution at the mucosal surface. Simultaneously with this reduction in water conductivity by hyperosmolality at the mucosal surface, glucose added to the solution either at the mucosal or the serosal surface, reduces the water activity in the external media. This reduction in water activity at either one of the surfaces creates a net water flux toward the hyperosmotic media. The asymmetry in the osmotic response at each surface [1] could be due to the difference in water conductivity when hyperosmotic solution was used at the serosal or at the mucosal surface. The effect of hyperosmotic solution at the mucosal surface seems to be independent of the concentration of the solutions used in the present experiments, 320 to 520 mosM/kg water. This latter observation is also in favor of an effect on the conductivity of the mucosa itself and not on solute/solvent interaction originating the driving force for the water diffusion.

In short the water diffusion fluxes reported in the present communication supply sufficient evidence to propose a change in water conductivity across the frog gastric mucosa by effect of hyperosmolality at the mucosal but not at the serosal surface.

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