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Effect of osmolarity on the epithelial paracellular permeability in rat jejunum

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Studies *in vivo* have shown an important increase in the substrate passive permeability across small intestine when Na^+-cotransported substrates, like galactose, are present at the luminal side. The influence of the solution osmolarity on the passive absorption of mannitol or 2-deoxy-D-glucose across rat jejunum and on the galactose-increasing effect is now studied both *in vivo* and *in vitro*. *In vivo*, luminal perfusion with 400 or 500 mosm/L solutions does not affect passive absorption of 10 mmol/L 2-deoxy-D-glucose, although a net fluid secretion towards lumen is observed. Luminal hyperosmolarity, however, prevents the stimulatory action of 25 mmol/L galactose on the passive absorption, a stimulation that is well manifested in the same intestinal segment when the perfusion is made with isoosmotic solutions. However, *in vitro* results with everted intestinal sacs or with preparations of intestinal wall in Ussing chambers, indicate that hyperosmolarity (500 mosm/L) of the solutions clearly increases net passive mucosal to serosal flux of D-mannitol or 2-deoxyglucose. With low osmolarity solutions (180 or 160 mosm/L by diminution of NaCl) the *in vivo* passive mannitol absorption is not affected and the stimulatory action of 25 mmol/L galactose is not observed, although net water absorption is enhanced. Moreover, the passive absorption stimulation by the galactose cotransport in isoosmotic solutions is dependent on Na^+ levels requiring at least 80 mmol/L. Results suggest that passive paracellular absorption across the small intestine may be modified by changes in the luminal content composition.

Key words: Intestinal epithelium, Tight junction, Paracellular permeability, Water flow, Osmotic loads, Cotransporter activation.

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The major route for the non mediated passive transfer of hydrophilic solutes across small intestinal epithelium appears to be paracellular (10, 22). The epithelial tight junctions between adjacent cells form a continuous permeability barrier that determines and regulates the molecule flux through the paracellular space and allows the existence of the two biochemically and functionally distinct, apical and basolateral plasmatic membranes, enabling the cell to carry out polarized transport (4, 5).

There is now substantial evidence that the structure of these tight junctions exhibits a remarkable degree of plasticity and can be modulated by physical, pathological and physiological events, a fact that supports regulation of the passive paracellular fluxes of fluid and solutes (9).

Intestinal absorption of nutrients such as sugars and amino acids may take place not only by the transcellular pathway using specific membrane carriers, but also through the paracellular pathway. In postprandial conditions, the membrane hydrolase activity may originate monosaccharide and amino acid concentrations just at the enterocyte luminal interfase highly in excess of those required for total saturation of the transporters (18) favoring passive paracellular absorption. Conditions are known where glucose absorption seems to markedly exceed the maximum rate that could be removed by active transport, suggesting an important significance of the paracellular pathway under those situations (17).

Moreover, in a previous paper (20) the presence of actively Na^+-cotransported substrates, like galactose, has been shown to elicit a rise in the *in vivo* absorption of the non transportable passively transferred substrates 2-deoxy-D-glucose and D-mannitol through the paracellular pathway. As osmotic loads may provoke changes in the tight junction structure and in the paracellular permeability (9), the present work reports the effects of osmolarity changes on the passive substrate permeability in rat small intestine. The *in vitro* experiments reveal an increase in the passive flux of mannitol and 2-deoxyglucose when the medium is hyperosmolar (500 mosm/L). *In vivo* however, when the intestinal perfusion was made with hyperosmolar (400 or 500 mosm/L) or hypoosmolar (160 or 180 mosm/L) solutions, changes in passive absorption of 2-deoxyglucose were not appreciated but the stimulation of that passive absorption by the presence of galactose is lost.

Materials and Methods

The animals were obtained from C.I.F.A. (University of Navarra), reared and kept under GLP, and handled according to the EC rules.

IN VIVO TECHNIQUE

Male Wistar rats weighing 180-200 g and 24 hours fasted were anesthetized with pentobarbital sodium s.c. at 60 mg/Kg. An about 20 cm segment of proximal jejunum was perfused (peristaltic pump) according to the *in vivo* PONZ *et al.*, method (21). Ten ml of perfusion solution containing labelled (^{14}C) 10 mmol/L 2-deoxy-D-glucose were recycled at 5.6 ml/min during 5 or 10 min absorption periods. Previous results have shown that with isoosmotic solution the absorption rate remains constant throughout the successive periods for at least 120 min. In each animal, the passive absorption of 2-deoxy-D-glucose was measured under different consecutive conditions and three absorption periods were usually carried out under each one of these conditions. At the end of each absorption period, air was pumped through the perfusion system to collect the residual fluid, which was rapid-

ly weighed to determine the final volume and then 15 ml saline solution and afterwards air were also pumped to rinse the intestinal lumen and to collect any possible labelled substrate still adhered to the intestinal mucosa.

Absorption of 2-deoxyglucose was calculated from the difference between the amount of solute initially present in the 10 ml of perfusion solution and that recovered in the intestinal perfusate volume at the end of each experimental period plus that collected in the washing solution. Absorption rates are expressed in micromol of solute per cm length of the intestine (21) per 5 or 10 min.

The control 300 mosm/L isoosmotic solution contained (in mmol/L): 140 NaCl, 5.6 KCl, 3 $CaCl_2$, 2.8 $MgSO_4$. 7 H_2O, 2.8 KH_2PO_4, 6.1 TRIS, 4.88 HCl; pH 7.4. The 500 mosm/L hyperosmotical solution was obtained by addition of 200 mmol/L sorbitol. Other solutions were prepared by changing NaCl and sorbitol concentrations: Isoosmotical (300 mosm/L), Na^+-low: 80 or 70 mmol/L NaCl plus 120 or 140 mmol/L sorbitol. Hyperosmotical (400 mosm/L), Na^+-low: 80 mmol/L NaCl plus 220 mmol/L sorbitol. Hypoosmotical (180 or 160 mosm/L), Na^+-low: 80 or 70 mmol/L NaCl.

IN VITRO TECHNIQUES

Everted intestinal sacs.– Male Wistar rats weighing 180-200 g, were fasted and anesthetized as for *in vivo*. Sacs of everted jejunum of about 3-3.5 cm in length were prepared (26), filled with serosal substrate-free solution and incubated during 15 min at 37 °C in an oxygenated mucosal medium containing the labelled substrate. The control isotonic incubation medium was the same as for *in vivo* experiments. Hyperosmotical solutions 500 mosm/L were obtained by addition of 200 mmol/L mannitol. The substrates 2-deoxy-D-glucose or D-galactose at 20 mmol/L concentration were used. After the incubation time the sacs were emptied, the serosal content collected and the tissue extracted in 0.1 M HNO_3. Then, the labelled sugar transferred from the mucosal to the serosal side and that retained in the tissue were evaluated.

For comparison, the sacs obtained from the jejunum of an animal were distributed at random between the isoosmotical and the hyperosmotic media.

Ussing chambers.– Male Wistar rats weighing 400-600 g were also anesthetized with pentobarbital. The jejunum was rapidly removed and a segment of about 16 cm was taken, washed with cold 9 g /L NaCl solution and opened longitudinally. The whole wall of the segment was mounted on modified Ussing chamber. Both mucosal (m) and serosal (s) sides of the chambers were attached to reservoirs containing 5 ml of the buffer solution described below and gassed with 95 % O_2, 5 % CO_2. Reservoirs were jacketed with a circulating water bath maintained at 37 °C. The control isoosmotic buffer solution consisted (in mmol/L) of 140 NaCl, 10 $KHCO_3$, 2.4 K_2HPO_4, 0.4 KH_2PO_4, 1.2 $CaCl_2$ and 1.2 $MgCl_2 \cdot H_2O$ at pH 7.4. In both mucosal and serosal media 20 mmol/L inert D-mannitol was added, a little amount of labelled ^{14}C-mannitol being added only to the mucosal solution. The mucosal to serosal flux of these labelled molecules was measured. The flux was also measured using a hypertonic medium (500 mosm/L in mucosal and serosal) by adding 200 mmol/L sorbitol to the control solution. Preliminary observations indicated that enough stable flux rates were achieved within 20 min after isotope addition. Thus, flux determinations were initiated after a minimum equilibration period of 20 min. The flux mucosa-to-serosa was calculated from

200 µl aliquots taken from the unlabelled side every 20 min along a 140 min period. Unidirectional flux calculations from the mucosal to serosal for the 140 min periods was calculated and expressed as previously described (13).

Chemical products were all of analytical grade. D-galactose and D-mannitol were supplied by Merck; 2-deoxy-D-glucose and D-sorbitol by Sigma; 1-(^{14}C)-2-deoxy-D-glucose (50.3 mCi/mmol), 1-(^{14}C)-D-mannitol (4.3 mCi/mmol) and 1-(^{14}C)-D-galactose (60 mCi/mmol) were from Du Pont.

Statistics.– The significance of differences between the means was assessed by the Student's *t* test and the single factor ANOVA test. Statistical analyses were performed by running the Stat View programs. Both matematical and statistical calculations were carried out on a Macintosh computer. Results are presented through mean ± SEM.

Results

Effects of hyperosmolarity on the passive permeability.– In *in vivo* experiments, the passive absorption of the non-transportable substrate 2-deoxy-D-glucose (10 mmol/L) was determined in the presence of high osmotic loads (400 or 500 mosm/L) in either the absence or presence of 25 mmol/L galactose during 5 or 10 min absorption periods.

Results with 400 mosm/L, 80 mmol/L NaCl perfusion solution are indicated in fig. 1. In experiment A the passive absorption is first measured with isoosmotical 80 mmol/L NaCl solution, secondly with the same solution plus 25 mmol/L galactose, thirdly with the 80 mmol/L NaCl hyperosmotical solution plus 25 mmol/L galactose and lastly again with isoosmotic Na^+-low solution plus galactose. As in previous results from this laboratory, a markedly increase in the passive absorption of the non-transportable substrate is observed when 25 mmol/L galactose was present in the isoosmotical perfusion solution. However, this stimulatory effect is not manifested when the Na^+-low, 400 mosm/L hyperosmotic solution is used. If this last solution is removed, and the

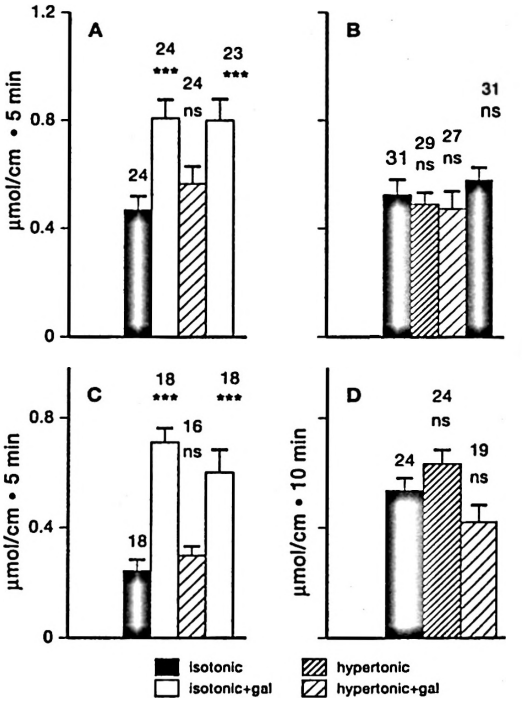

Fig 1. *Intestinal passive absorption of 10 mmol/L 2-deoxy-D-glucose under hypertonic stress.*
A-B) Isotonic solution: 300 mosm/L, 80 mmol/L NaCl; hypertonic solution: 400 mosm/L, 80 mmol/L NaCl. Galactose concentation, 25 mmol/L. C-D) Isotonic solution: 300 mosm/L, 140 mmol/L NaCl; hypertonic solution: 500 mosm/L, 140 mmol/L NaCl; Galactose concentration, 25 mmol/L. The consecutive experimental conditions were carried out with the perfusion solutions and the order indicated. In each condition three successive periods of 5 or 10 min duration were made. Number of determinations, on the top of the column. Statistical signification of the differences refers to the corresponding initial control values. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant.

intestine is washed with isotonic solution for 5 min and subsequently perfused again with isotonic solution containing galactose, the stimulation of 2-deoxyglucose absorption reappears. Experiment B shows that the basal passive absorption of 2-deoxyglucose is not significantly modified by perfusion with the hyperosmotical solutions. Very similar results were obtained with the 500 mosm/L hyperosmotical perfusion solution containing 140 mmol/L NaCl in 5 or 10 min (fig. 1, C and D).

In other experiments the temporal evolution of the passive absorption of 10 mmol/L 2-deoxy-D-glucose from perfusion solutions of 500 mosm/L was followed. After measuring the absorption with control isoosmotical solution, it was measured succesively along 60 min with the hyperosmotic solution and again with isotonic medium. Results (data not shown) indicate that passive absorption does not significantly change by continued exposition to luminal hypertonic solution.

Net changes in fluid volume under the different experimental conditions (table I) were determined by measuring (weigh) the initial and final volumes of the perfusion solution for each absorption period. Results indicated that with isotonic solutions a small liquid net flux from lumen to blood was taking place. This absorption flux was not significantly different when 25 mmol/L galactose was present. However, the final volume was higher than the initial one when hypertonic perfusion solutions were used indicating a net fluid secretion to the lumen. Significant differences in water secretion under high osmotic loads due to the presence of galactose were not observed either.

Subsequent *in vitro* studies using modified Ussing chambers with rat jejunum revealed that when mucosal and serosal sides were exposed to hypertonic solutions (500 mosm), the passive mucosal to serosal flux of mannitol was markedly higher than that measured in control condition (isotonic medium) (fig. 2 A). This increase appears as early as after 20 min of exposition to the high osmotic loads and remains almost constant throughout time. In sacs of everted jejunum (15 min incubation) the same hyperosmolarity (500 mosm/L) in either the mucosal or serosal or both sides also enhances (fig. 2 B) the passive transfer of 2-deoxyglucose from mucosal (20 mmol/L) to serosal (0 mmol/L) as well as the amount of substrate retained in the tissue. These *in vitro* findings suggest that high osmotic loads provoke increases in paracellular permeability.

Moreover, the total transport of 20 mmol/L galactose from mucosal to serosal by the jejunum sacs is not significantly changed with the hypertonic solutions, while the passive non mediated transfer measured in the presence of 0.5 mmol/L phloridzin, increased. The mediated transport could, therefore, be inhibited by hypertonicity.

Table I. *Net flux of water from lumen to blood during passive absorption of 10 mmol/L 2-deoxy-D-glucose by rat jejunum: Different conditions of luminal osmolarity and absence (-) or presence (+) of 25 mmol/L galactose.*
Positive flux values mean fluid absorption; negative, secretion to the lumen. Results (mean ± SEM) in µl.

Osmolarity (mosm/L)	25 mmol/L galactose	Time (min)	Net flux (µl)	Stat. signif.
300	–	5	+110 ± 40	
300	+	5	+163 ± 45	ns
400	–	5	–138 ± 57	***
400	+	5	–30 ± 50	*
500	–	5	–333 ± 55	***
500	+	5	–329 ± 56	***
180	–	5	+745 ± 142	***
180	+	5	+566 ± 89	***
160	–	10	+984 ± 135	***
160	+	10	+959 ± 111	***

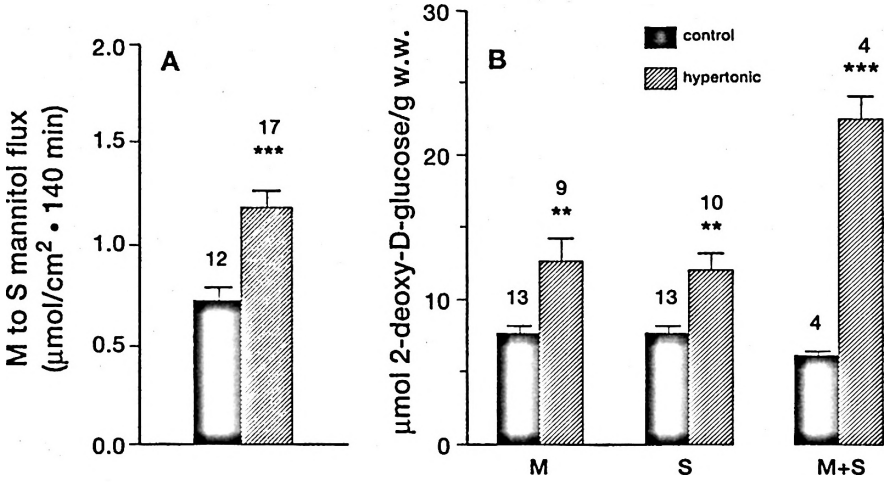

Fig. 2. *Effect of hypertonicity on passive intestinal absorption* in vitro.
A) Ussing chambers: mucosal to serosal mannitol flux; hypertonicity (+200 mmol/L sorbitol) in both sides. B) Everted sacs: µmol of 2-deoxy-D-glucose transferred from mucosal to serosal/g w. w. Hypertonicity (+200 mmol/L mannitol) in mucosal (M), serosal (S) or in both mediums (M + S). At the top of the column, number of data.

Effects of hypoosmolarity on passive permeability in vivo.– The experiments with hypoosmotic perfusion solutions were carried out similarly to those described for hyperosmotic solutions. The absorption was measured for 5 min or 10 min periods.

The stimulatory action of 25 mmol/L galactose on the passive absorption of 10 mmol/L 2-deoxy-D-glucose in 5 min periods is again observed when the perfusion solution is isoosmotic (fig. 3 A), but it is not when the galactose is solved in the 180 mosm/L (80 mmol/L NaCl) hypoosmotical solution. The stimulation reappears, however, when the perfusion, after 5 min jejunal washing with 25 mmol/L galactose containing isoosmotical solution, is made with this same solution. Moreover, the perfusion with the 180 mosm/L solution does not significantly modify the passive absorption of 2-deoxyglucose (fig. 3 B). The results obtained with a hypoosmotical solution (160 mosm/L, 70 mmol/L NaCl) and 10 min absorption periods were very similar (data not shown).

As both hypoosmotical solutions were low in Na^+ concentration and Na^+-galactose cotransport is dependent on Na^+ concentration, the lack of the stimulatory action of galactose could be ascribed to the hypoosmoticity or to the low Na^+ level. Another experiment was, therefore, made to compare the absorption of 10 mmol/L 2-deoxyglucose along 10 min periods when the perfusion solution is the isoosmotical control (300 mosm/L, 140 mmol/L NaCl), isoosmotical Na^+-low (300 mosm/L, 70 mmol/L NaCl) or isoosmotical Na^+-low containing 25 mmol/L D-galactose. Results obtained show that the passive absorption is not significantly changed when Na^+ concentration diminishes from 140 (0.228 ± 0.01) to 70 mmol/L (0.232 ± 0.02), but the increasing effect of galactose on passive absorption is not observed if Na^+ concentration is only 70 mmol/L (0.234 ± 0.02).

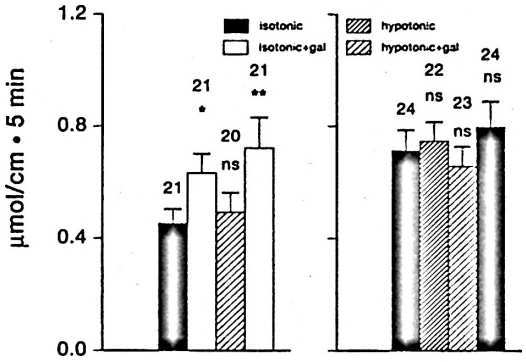

Fig 3. *Effect of luminal hypotonicity on intestinal passive absorption of 10 mmol/L 2-deoxy-D-glucose* in vivo.
The consecutive experimental conditions were carried out with the perfusion solutions and the order indicated. Hypoosmotic solution, 180 mosm/L; galactose concentration, 25 mmol/L. Experiments and results as in figure 1.

On perfusing the intestine with the hypoosmotical solutions either in the presence or absence of galactose, the net flux of water from lumen to blood increases and the final volume decreases in a very significantly higher proportion than those measured with isoosmotical solutions. Therefore, a clear osmotic flux to blood takes place, more easily observed with 10 min absorption periods. Significant differences in net flux were not shown by either the presence or absence of 25 mmol/L D-galactose (table I).

Discussion

Solutes may cross the intestinal epithelium by a transcellular pathway involving transfer through the apical and basolateral membranes, or by the paracellular pathway through the tight junctions and lateral spaces between enterocytes. Most transepithelial transfer of mannitol and 2-deoxyglucose, as the apical enterocyte membrane lacks in transporters for both substrates, would be made by a paracellular pathway and may be considered as suitable models for the measure of this passive absorption component of sugars and amino acids.

Although a direct quantification of the paracellular route in nutrient absorption under physiological conditions is still lacking, recent evidences suggest that the sugar and amino acid concentrations just at the apical membrane during digestion may reach high values, and that the tight junction permeability may markedly increase, two factors enhancing the use of the paracellular pathway, which becomes a significant part of the total nutrient absorption (18).

The *in vivo* passive absorption of 2-deoxyglucose, mannitol or D-galactose (mediated transport blocked by phloridzin) increases if 25 mmol/L galactose or other Na^+-cotransported substrates are present in the perfusion solution (20). This may be ascribed to tight junction structural changes (23) which decrease the transepithelial electrical resistance (11) as well as to solvent drag enhancing from mucosal to the subepithelial spaces (17).

The present results show that the stimulation of mannitol and 2-deoxyglucose passive paracellular transfer by the presence of Na^+-cotransported substrates such as D-galactose, as well as the basal passive transfer of those substrates, are influenced by osmolarity, a factor that has attracted particular atention during the last years, as a modifier of the tight junction structure and permeability (8, 11).

The *in vivo* perfusion experiments have completely confirmed the passive absorption stimulation of 2-deoxyglucose across rat jejunum by the presence of galactose at a 25 mmol/L concentration. However, if the perfusion solutions are hyperosmotic or hypoosmotic, that stimulation by galactose does not appear, a not easily explainable fact. As the stimulation has been ascribed to an activation of the Na^+-

substrate cotransporter (20), some of the used solutions being Na^+-low, changes in Na^+ level may interfere with that activation. Nevertheless, the results obtained with 500 mosm/L (140 mmol/L NaCl) hyperosmotic solution are very similar to those with 400 mosm/L (80 mmol/L NaCl). Besides, in the experiments carried out with an isotonic 300 mosm (80 mmol/L NaCl) solution, this low Na^+ level does not hinder the enhancing effect of galactose on 2-deoxyglucose absorption. With hypoosmotic solutions, the suppresion of the stimulatory action of galactose on passive absorption has been obtained both with 180 mosm/L (80 mmol/L Na^+) and with 160 mosm/L (70 mmol/L Na^+) solutions. Experiments with 300 mosm/L solutions show that the stimulation by galactose is observed when Na^+ concentration is 140 or 80 mmol/L, but not when it is 70 mmol/L. It may be concluded, therefore, that the absence of a galactose-increasing effect on passive absorption by luminal hyper or hypoosmolarity is not dependent on the Na^+ level when it is 80 mmol/L or higher. With 70 mmol/L Na^+ or less, however that effect may be ascribed to the low Na^+ level, since it is also observed under isotonicity.

If luminal hyperosmolarity provokes diminution of electrical resistance, opening of the tight junctions and enlargement of the intracellular spaces (11, 23), and the Na^+-cotransporter activation produces similar effects (9, 17), the addition of both actions might be not possible, the presence of galactose failing to stimulate passive absorption. Another explanation could be that the observed net water flux under mucosal hyperosmolarity is reverted from blood to lumen diminishing the solute transfer through a solvent drag, a fact that would balance the increase in paracellular permeability caused by Na^+-cotransporter activation. Finally, hyperosmolarity might impair Na^+-sugar cotransporter function: as enterocytes shrink, volume regulatory responses are elicited (2, 12, 14-16) involving cytoskeleton, ionic cotransporters and exchangers that may affect Na^+-sugar cotransporter, directly or indirectly through diminution of Na^+-gradients. In fact, the experiments with everted jejunum sacs seem to indicate inhibition of the mediated galactose transport under hyperosmolarity.

On the other hand, hypotonic perfusion solutions, even containing 80 mmol/L Na^+, also block the stimulatory action of galactose on 2-deoxyglucose passive absorption. Hypotonic stress activates regulatory volume decrease mechanisms (2, 24) involving increases in cytosolic Ca^{2+} (6, 25) and cAMP (19) as initial responses (1). Mutual interactions between Na^+-substrate cotransport, ionic channels and exchangers have also been reported in enterocytes (14). It is, therefore, possible that hypotonicity could in this way alter Na^+-galactose cotransporter.

Hyperosmolar solutions (500 mosm/L) markedly enhance the passive fluxes of mannitol or 2-deoxyglucose across the jejunum wall in Ussing chambers or by sacs of everted intestine. These results agree with previous observations *in vitro* (11, 23) reporting structural and functional changes in tight junctions by osmotic loads that increase paracellular permeability. In the experiments with Ussing chambers, the hypertonic solutions were always at both mucosal and serosal sides, whithout an osmotic gradient between the two compartments. With everted sacs, the hyperosmolar solutions was at either the mucosal, the serosal or both compartments and in spite of the different orientation and value of the osmotic gradient, an increase in passive transfer from mucosal to serosal side was in all situations observed.

Although previous *in vivo* results (3) suggested an enhancing in passive absorption when perfusion was made with a hypertonic solution, the more accurate *in vivo* experiments here presented do not support such observation. The in *vitro/in vivo* difference may be ascribed to various factors including the integrity of the vascular, nervous and humoral intestinal strategies in the most physiological *in vivo* conditions. Thus a net fluid secretion from blood to lumen takes place *in vivo* with hyperosmolarity at the lumen and a collapse of the intercellular spaces is produced (7) which may counteract the increase of solute absorption by the paracellular pathway. In the same way, luminal hypotonicity neither changes 2-deoxyglucose passive absorption, in spite of the observed increase in mucosal to blood net fluid flux. Perphaps cell swelling due to hypotonicity restricts the intercellular spaces and paracellular transfer.

In summary, the present results reveal that passive transfer of solutes like 2-deoxyglucose or mannitol, which is mostly done through the paracellular pathway, is modified by osmolarity, which might act more through the regulation of the tight junction structure, intercellular space dimensions and permeability than by changes in the intensity and direction of the solvent drag.

Acknowledgements

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M. PÉREZ, A. BARBER y F. PONZ. *Efecto de la osmolaridad sobre la permeabilidad paracelular en yeyuno de rata*. J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (2), 103-112, 1996.

Resultados previos *in vivo* demostraron un aumento significativo de la permeabilidad pasiva intestinal por la presencia de sustratos activamente transportables en la luz. En este trabajo se estudia *in vivo* e *in vitro* la influencia de los cambios de osmolaridad sobre la absorción pasiva de manitol o 2-deoxiglucosa tanto en condiciones control como en presencia de galactosa. *In vivo*, la perfusión luminal con soluciones de alta osmolaridad (400 ó 500 mosm/L) no afecta a la absorción pasiva de 2-deoxiglucosa 10 mmol/L, aunque se observa una secreción neta de líquido hacia la luz. Sin embargo, la hiperosmolaridad luminal anula el efecto estimulador debido a la presencia de galactosa 25 mmol/L sobre la absorción pasiva, estimulación que se manifiesta claramente en la misma asa intestinal con solución isoosmótica. Los resultados *in vitro*, tanto con sacos intestinales evertidos como con preparaciones de pared intestinal montadas en cámaras tipo Ussing, indican que la hiperosmolaridad (500 mosm/L) incrementa claramente el flujo neto pasivo mucosal-serosal de D-manitol o de 2-deoxiglucosa. Con soluciones de baja osmolaridad (180 ó 160 mosm/L, por disminución del NaCl) la absorción pasiva de manitol *in vivo* no se afecta pero la acción estimulante de la galactosa no aparece, aunque se observa un aumento de la absorción neta de líquido. La estimulación de la absorción pasiva por la presencia de galactosa en soluciones isoosmóticas es dependiente de los niveles de Na^+, requiriéndose al menos 80 mmoles Na^+/L. Estos resultados sugieren que la absorción pasiva paracelular a través del intestino delgado puede ser modificada por los cambios de la composición luminal.

Palabras clave: Epitelio intestinal, Uniones estrechas, Permeabilidad paracelular, Flujo líquido, Cargas osmóticas, Activación cotransportador.

References

1. Ackerman, M. J., Wickman, K. D. and Clapham, E. (1994): *J. Gen. Physiol.*, 103, 153-179.
2. Chen, J. G. and Kempson, S. A. (1995): *PEBM*, 210, 1-6.
3. Gato-Peciña, J. J. and Ponz, F. (1990): *Rev. esp. Fisiol.*, 46, 343-352.
4. Gumbiner, B. (1987): *Am. J. Physiol.*, 253, C749-C758.

5. Hollander, D. (1992): *Scand. J. Gastroenterol.*, 27, 721-726.
6. Leung, A. Y. H. and Wong, P. Y. D. (1993): *Pflügers Arch.*, 425, 77-81.
7. Loeschke, K. and Bentzel, C. J. (1994): *Am. J. Physiol.*, 266, G722-G730.
8. Madara, J. L. (1983): *J. Cell. Biol.*, 97, 125-136.
9. Madara, J. L. and Pappenheimer, J. R. (1987): *J. Membr. Biol.*,, 100, 149-164.
10. Madara, J. L. (1989): *J. Clin. Invest.*, 83, 1089-1094.
11. Marcial, M. A. and Madara, J. L. (1987): *J. Membr. Biol.*, 56, 424-433.
12. Matthews, J. B., Kevin, M. D., Tally, J. and Smith, J. A. (1995): *Am. J. Surg.*, 169, 50-56.
13. Naftalin, R. J. and Curran, J. (1974): *J. Membr. Biol.*, 16, 257-278.
14. O'Brien, J. A., Walters, R. J., Valverde, M. A. and Sepulveda, F. V. (1991): *Biochim. Biophys. Acta*, 1070, 501-504.
15. O'Brien, J. A., Walters, R. J., Valverde, M. A. and Sepulveda, F. V. (1993): *Pflügers Arch.*, 423, 67-73.
16. Orlov, S. N., Kolosova, I. A., Cragoe, E. J., Gurlo, T. G., Mongin, A. A., Aksentsev, S. L. and Konev, S. V. (1993): *Biochim. Biophys. Acta*, 1151, 186-192.
17. Pappenheimer, J. R. (1988): *J. Membr. Biol.*, 100, 137-148.
18. Pappenheimer, J. R. (1993): *Am. J. Physiol.*, 265, G409-G417.
19. Peña-Rasgado, C., Kimler, V. A., Mc Gruderr, K. D., Tie, J. and Rasgado-Flores, H. (1994): *Am. J. Physiol.*, 267, C1319-1328.
20. Perez, M., Barber, A. and Ponz, F. (1993): *Rev. esp. Fisiol.*, 49, 259-264.
21. Ponz, F., Ilundain, A. and Lluch, M. (1979): *Rev. esp. Fisiol.*, 35, 97-104.
22. Proulx, P. (1991): *Biochim. Biophys. Acta*, 1071, 255-271.
23. Rawlins, F. A., González, E., Pérez-González, M. and Whittembury, G. (1975): *Pflügers Arch.*, 353, 287-302.
24. Strange, K. (1994): *News Physiol. Sci.*, 26, 223-228.
25. Tilly, B. C., Edixhoven, M. J., Van der Berghe, N., Bot, A. G. M. and de Jonge, H. R. (1994): *Am. J. Physiol.*, 267, C1271-C1278.
26. Wilson, T. H. and Wiseman, G. (1954): *J. Physiol.*, 123, 116-12.