REVISTA ESPAÑOLA DE FISIOLOGIA, 44 (2), 147-150, 1988

The Effect of $BaCl_2$ on Intestinal Sugar Transport in the Rat *in vitro*

A. I. Alcalde and A. Ilundáin*

Departamento Biomedicina
Facultad de Veterinaria
50013 Zaragoza (Spain)

(Received on February 1, 1988)

A. I. ALCALDE and A. ILUNDAIN. *The Effect of $BaCl_2$ on Intestinal Sugar Transport in the Rat* in vitro. Rev. esp. Fisiol., 44 (2), 147-150, 1988.

The effect of $BaCl_2$ on galactose transport across isolated rat small intestine has been investigated. The addition of 5 mM $BaCl_2$ or theophylline (3 mM) to the bathing solutions increased cell water free sugar accumulation and decreased mucosal to serosal sugar fluxes. However the effects of $BaCl_2$ were smaller than those induced by theophylline. Removal of Ca^{2+} from the bathing solutions did not modify the response to $BaCl_2$, though the response to theophylline was partially reduced. In the presence of 0.1 mM trifluoperazine, both theophylline and $BaCl_2$ were without effect on sugar transport. These findings are discussed in terms of an effect of Ba^{2+} on intestinal smooth muscle tone.

Key words: Sugar transport, Intestine, $BaCl_2$

Over the last decade it has been demonstrated that intracellular Ca^{2+} plays a major role in regulating a number of important cellular functions. We have previously reported (9) that calcium appears to mediate, via calmodulin, the theophylline-dependent decrease in serosal sugar permeability in intact rat ileum. The results also suggested that both internal and external sources of calcium were involved in the response to theophylline.

Several agents have been described to either induce or prevent the release of Ca^{2+} from intracellular stores, so that they could prove a useful tool in determining the role played by these stores in the control of intestinal functions. $BaCl_2$ has been reported to release Ca^{2+} from intracellular stores in intestinal smooth muscle (1) and it has been suggested (7) that $BaCl_2$ induces intestinal secretion by mobilizing intracellular calcium. Ba^{2+} has also been reported to increase smooth muscle tone (1).

The present study was designed to investigate the actions of $BaCl_2$ on D-galactose transport in intact rat small intestine.

* To whom all correspondence should be addressed: Departamento de Fisiología Animal. Facultad de Farmacia. 41012 Sevilla (Spain).

Materials and Methods

Animals, incubation solutions. — Male albino Wistar rats weighing 150-200 g were anaesthetized with ether overdose. A segment of distal small intestine was removed and rinsed free of intestinal contents with ice-cold Ringer's solution. The tissue was then stripped of its serosal and external muscle layers using the method of POWELL *et al.* (15). The Ringer's solution contained, in mM: 140 NaCl, 10 $KHCO_3$, 0.4 KH_2PO_4, 2.4 K_2HPO_4, 1.2 $CaCl_2$ and 1.2 $MgCl_2$, and was continuously bubbled with 95 % O_2/5 % CO_2. In experiments where Ca^{2+}-free conditions were required, Ca^{2+} was omitted from the serosal solution and 0.1 mM EGTA was added to remove interstitial Ca^{2+}.

Sugar uptake measurements. — Pieces of distal small intestine, weighing about 50 mg were incubated at 37° C in Ringer's solution containing 0.5 mM D-galactose labelled with ^{14}C for 30 min. At the end of the experiments the tissues were washed with gentle shaking in ice-cold Ringer's solution and blotted carefully on both sides to remove excess moisture. The tissue was weighed wet and extracted by shaking for 15 h in 1 ml 0.1 N HNO_3. Samples were taken from the bathing solutions and from the extracts of the tissues for radioactivity counting.

All the modifiers were added to the incubation solution at the beginning of the incubation period.

The extracellular space was estimated using ^3H-PEG 4,000 as previously reported (9). None of the modifiers caused a significant effect either on the extracellular space, or in tissue water fraction, or on cell water fraction.

The total amount of sugar sequestered withing 1 g of wet tissue was corrected for the phosphorilated galactose and extracellular space as previously reported (9).

Transepithelial flux measurements. — The stripped mucosa was mounted as a flat sheet in Ussing-type chambers. The bathing solutions on the mucosal and serosal surfaces of the tissue were maintained at 37° C using a circulating water bath. Both solutions contained 0.5 mM galactose. Mucosal to serosal sugar fluxes were measured by placing the ^{14}C-labelled galactose in the mucosal side. Samples were removed from the non radioactively labelled side at 10 min intervals for 30 min after a 20 min preincubation period. One sample only was taken for counting from the radioactively labelled side. Samples were counted using a liquid scintillation counter.

Theophylline (Sigma) and trifluoperazine Smith, Kline and French Lab. were added to both bathing solutions at the start of the incubation period. $BaCl_2$ was only added to the serosal solution.

Statistics. — Results are expressed as mean ± S.E. Statistical significance was evaluated by the two-tailed Student's t-test.

Results and Discussion

The results show that, as previously reported (9), theophylline increased tissue sugar accumulation (table I) and decreased mucosal to serosal galactose fluxes (table II). The action of $BaCl_2$ on galac-

Table I. *Effect of $BaCl_2$ (5 mM) and theophylline (3 mM) on tissue galactose (0.5 mM) accumulation.* TFP: trifluoperazine (0.1 mM). Values are the means ± S.E. of 20 independent determinations.

	μmol D-galactose · ml^{-1} cell water		
	Control	Theophylline	TFP
No addition	1.12±0.05	2.50±0.08*	1.14±0.04
$BaCl_2$	1.91±0.06*	2.31±0.06*	0.97±0.06

* $p < 0.001$ test compared with control without modifiers.

Table II. *Effect of $BaCl_2$ (5 mM) and theophylline (3 mM) on mucosal to serosal galactose (0.5 mM) fluxes.*
TFP: trifluoperazine (0.1 mM). Values presented are the means ± S.E. of ten independent observations.

	Jms (μmol D-galactose · cm^{-2} h^{-1})		
	Control	Theophylline	TFP
No addition	0.15±0.01	0.07±0.01*	0.16±0.01
$BaCl_2$	0.10±0.007*	0.06±0.01*	0.12±0.01
0 mM Ca^{2+} + $BaCl_2$	0.10±0.005*	0.10±0.005*	0.13±0.01

* $p < 0.001$ test compared with control without modifiers.

tose transport was similar to that caused by theophylline, though the effects of $BaCl_2$ on both tissue sugar accumulation and transepithelial sugar fluxes were smaller than those induced by theophylline. Furthermore the response to theophylline was not significantly modified by the simultaneous addition of $BaCl_2$. These results are in keeping with the suggestion that $BaCl_2$ and theophylline affect the same system, i.e. serosal sugar exit, and that the serosal sugar permeability is maximally inhibited by theophylline.

The calmodulin antagonist trifluoperazine (TFP) abolished the changes in sugar transport produced by $BaCl_2$ (table I and II), which suggest that the action of $BaCl_2$ may be mediated by calmodulin. Ba^{2+}, as does Ca^{2+}, could activate calmodulin directly, but Ba^{2+} has been shown (12) to be ineffective in promoting the binding of trifluoperazine to purified calmodulin. Although the possibility that TFP may have a non-specific action cannot be ruled out, these findings suggest that $BaCl_2$ might act by rising intracellular Ca^2, which would lead to activation of Ca-calmodulin complex.

As opposed to theophylline, the response to $BaCl_2$ was not modified by removal of serosal Ca^2 (table II). Under these experimental conditions theophylline and Ba^{2+} inhibited sugar fluxes to the same extent.

Together all these results may indicate that Ba^{2+} acts by releasing Ca^{2+} from the intracellular stores, as has been shown in intestinal smooth muscle (1). HARDCASTLE *et al.* (7) have reported that $BaCl_2$ stimulates intestinal secretion in the rat by mobilizing intracellular calcium.

However, it is worth pointing out that the preparation used in the present study consists of intestinal epithelial cells and muscularis mucosa. Several studies (2, 3, 6, 11, 14) have indicated that the subepithelial intersticial forces may play an important role in intestinal transport. An increase in smooth muscle tone leading to decreasing permeability of the non-epithelial barriers could explain both, decreased mucosal to serosal flux and higher tissue sugar accumulation. Both theophylline (4, 17) and Ba^{2+} (1) cause smooth muscle contraction.

Therefore the effects of Ba^{2+} on sugar transport in intact intestine could result from its action on smooth muscle tone. Supporting this view are the results obtained using isolated enterocytes. Thus, BROWN and SEPÚLVEDA (5) reported that Ba^{2+} reduced by 40 % the ability of isolated rabbit enterocytes to accumulate α-methyl-glucoside. We have also observed the same effect in isolated chicken enterocytes (unpublished results).

Although the same experiments were not carried out in isolated rat enterocytes, the fact that Ba^{2+} reduces sugar uptake into enterocytes of two different animal species to the same extent, suggests that the same response would be observed in rat enterocytes.

It could also be thought that the increased sugar accumulation and the reduced mucosal to serosal sugar flux may be due to cell swelling and consequent collapse of the intercellular spaces. Thus,

Ba^{2+} acting as a K^+ channel blocker (5, 13, 16) could inhibit volume regulatory events that prevent cell swelling during sugar transport (10). Cell swelling could lead to an apparent increased sugar accumulation. On the other hand, collapse of the intracellular spaces has been reported to reduce sugar absorption (8). However, the Ba^{2+} effects on cell volume will be also present in isolated enterocytes and, as mentioned above, sugar accumulation was reduced by Ba^{2+}. Furthermore, neither extracellular volume nor the intracellular volume were significantly modified by the presence of Ba^{2+}. It is possible however that changes in these parameters did occur but were beyond detection.

In conclusion, comparing the effects of Ba^{2+} on sugar accumulation in isolated enterocytes with those observed in intact intestine, it appears that the current findings result from an effect of Ba^{2+} on smooth muscle tone. These findings also suggest that it may not be always right to consider that the enterocytes are the only ones responsible for the response of the intact intestine to modifiers that can also affect subepithelial tissues.

Acknowledgements

We thank Smith, Kline and French Laboratories for the gift of trifluoperazine.

Resumen

Se estudia el efecto del Cl_2Ba sobre el transporte de galactosa en intestino delgado de rata *in vitro*. La presencia de 5 mM Cl_2Ba ó 3 mM teofilina en el medio de incubación aumenta la acumulación de galactosa en el tejido, mientras que el flujo del azúcar desde el lado mucosal al serosal disminuye. La eliminación en el medio de incubación del Ca^{2+} no modifica la respuesta del tejido al Cl_2Ba, mientras que la debida a la teofilina se reduce ligeramente. La trifluoperazina (0.1 mM) anula el efecto de la teofilina o del Cl_2Ba sobre el transporte del azúcar. Todos estos resultados se discuten en relación con un efecto del Ba^{2+} sobre el tono del músculo liso.

Palabras clave: Transporte de azúcares, Intestino, Cl_2Ba.

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