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Stimulation of Brush Border Enzyme Activity Along the Rat Small Intestine by Misoprostol

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The effect of Misoprostol (0.3 mg/kg b.w., orally for four weeks) on the brush border membrane enzyme activity, is studied in growing rats. Misoprostol enhanced stomach and intestine relative weights as well as the mucosal weight of the duodenum and proximal jejunum. In treated rats, disaccharidases, alkaline phosphatase and aminopeptidase enzyme activity were measured in brush border purified fraction throughout the small intestine. Sucrase, maltase, aminopeptidase and alkaline phosphatase specific activities were significantly increased along the small intestine. In the proximal jejunum, sucrase (62 %; $p < 0.001$) and maltase (42 %; $p < 0.01$) activities were significantly greater. Sucrase activity was also significantly ($p < 0.001$) increased by about 103 % in the distal jejunum. There was also a significant ($p < 0.05$) increment of 32 % in the duodenal and ileal alkaline phosphatase activity after treatment. Similarly, aminopeptidase activity was significantly ($p < 0.05$) higher in duodenum (67 %) and jejunum (24 %). In conclusion, Misoprostol appreciably increased the ability of the small intestine to perform its digestive functions although further studies will be necessary to examine the cellular and molecular mechanism(s) which may be responsible for these effects.

Key words: Misoprostol, Small intestine, Brush border enzymes, DNA.

PGEs are short-lived derivatives of fatty acids with a wide range of biological actions including a key role in the control of gastrointestinal mucosa morphology and function. They can protect the gastrointestinal tract from the adverse effects of many noxious agents (22) in addition to being potent inhibitors of acid secretion.

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The study and use of PGs have been limited by their short life span, but stable long-acting analogues are now available. One such analogue is Misoprostol, a methyl ester of PGE1, which has been used as an oral antiulcer agent, especially when associated with non-steroidal anti-inflammatory agents.

The effect of Misoprostol on the gastrointestinal epithelium has been studied in the stomach (7, 19), whereas few studies have been performed in the small intestine. GOODLAD *et al.* (6) found that Misoprostol, administered at pharmacological doses, produces a significant increment in crypt area and in the number of mitoses per crypt throughout the dog small intestine. On the other hand, in ulcer patients a 4 week Misoprostol treatment has been reported to increase alkaline phosphatase and leucyl β-naphtilamidase activities toward normal values (21).

Our aim was to study the effect of Misoprostol on intestinal function by assessing the response of brush border membrane enzyme throughout the rat small intestine.

Materials and Methods

Animals.— Male Wistar rats *(Rattus norvegicus)* initially weighing 70 g (n = 28) were used (Local breeder at the University of Pamplona, Spain). They were housed in a room at 21 °C with alternating 12-hour periods of light and darkness and were fed a standard rat chow and had free access to water.

Misoprostol treatment.— Animals were distributed in two experimental groups. Treated rats received oral Misoprostol (0.3 mg/kg , b. w.) every day for four weeks and control rats (C) received saline (0.15 M NaCl) following the same schedule. On the 29th treatment day, after 24 hours of starvation, the animals were decapitated between 9-11 a. m.

The abdomen was opened and the stomach, liver and small intestine were removed and weighed. The entire small intestine was washed in cold saline and separated in four parts: duodenum (from the pyloric ring to the ligament of Treizt), proximal and distal jejunum (the first 15 cm segment and the second 15 cm segment distal from the ligament of Treizt, respectively) and ileum (the last 20 cm before the ileocecal valve). Each part of the small intestine was weighed and measured and was then cut longitudinally. The mucosa was scraped off with a glass slide and frozen separately (–30 °C). Specimens of each intestinal segment were taken for protein, DNA and RNA determinations, as it will be described later.

Enzyme assays.— The intestinal mucosa was homogenized (3 min, 300 rpm) in a homogenizer and the brush border membrane was isolated and purified using $CaCl_2$ precipitation (10, 17). Preliminary assays revealed that sucrase specific activity was 20 times higher in the brush border membrane fraction (P_2) than in the crude homogenate. Sucrase (EC 3.2.1. 48), maltase (EC 3.2.1. 20) and lactase (EC 3.2.1. 23) activities were measured by the method of DAHLQVIST *et al.* (3). Glucose 1-phosphate (Sigma), 0.056 mmol/L in 0.1 mol/L amine-methyl-propanol at pH = 10.0, was used as substrate for the determination of alkaline phosphatase (EC 3.1.3) according to the method of BERGMEYER *et al.* (1). Aminopeptidase N (EC 3.4.11. 2) was determined according to MAROUX *et al.* (13) using L-leucine-*p*-nitroanilide as substrate. Enzyme activities were expressed as specific activities in the purified brush border membranes (U/g protein). One unit of activity equals 1 μmol substrate hydrolyzed per minute at 37 °C.

Other assays.— Protein content was assayed according to the method of LOWRY *et al.* (12). DNA and RNA were extracted by the method of MUNRO and FLECK (15)

and DNA was assayed according to the method of BURTON (2) as modified by GILES and MYERS (5).

Statistical analysis.— All the results are presented as the mean ± SEM. Statistical comparisons were made by the Student's *t* test. Differences were taken as statistically significant at $p<0.05$.

Results and Discussion

All animals were checked daily for any adverse effects; none were observed. During Misoprostol-treatment the weight gain was 196±6 in control rats and 199±6 in treated rats. As shown in table I, Misoprostol significantly increased stomach (11 %, $p<0.01$) and small intestine (6 %; $p<0.05$) relative weights, expressed as a percentage of the total body weight. The increment in the stomach and small intestine weights is consistent with the literature (11).

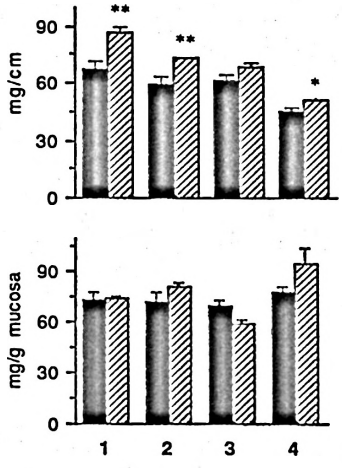

Fig.1. *Mucosal weight (mg/cm of bowel) and protein content (mg/g of mucosa) along the small intestine.* Values are mean ± SEM. 1: Duodenum. 2: Proximal jejunum. 3: Distal jejunum. 4: Ileum. Solid bars indicate control animals and hatched bars animals treated with Misoprostol. * $p<0.05$; ** $p<0.01$ as compared to control rats.

As it can be seen in figure 1, mucosal mass (mg per cm of bowel) was significantly ($p<0.01$) higher in the duodenum (30 %) and proximal jejunum (20 %) of treated rats as compared to control rats

However mucosal protein, DNA, and RNA contents were scarcely modified by the Misoprostol treatment in all four segments of the small intestine (figs. 1 and 2). Misoprostol enhanced significantly ($p<0.05$) duodenal DNA content (27 %) and in the distal jejunum RNA levels were higher (21 %) than in the control rats.

Table I. *Body weight (g) and relative weight (expressed as percentage of body weight) of gastrointestinal organs from Control and Misoprostol rats.* Results are expressed as media ± SEM; n = 14 per group.

Weight	Control	Misoprostol
Initial (g)	71±2.00	68±2.00
Final (g)	268±7.00	267±6.00
Liver (%)	3.42±0.04	3.41±0.05
Stomach (%)	0.65±0.02	0.72±0.02**
Small intestine (%)	3.31±0.07	3.51±0.05*

*p<0.05, **p<0.01.

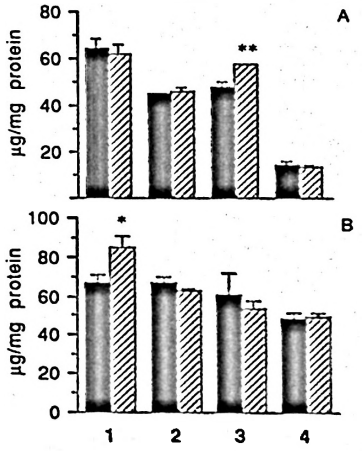

Fig. 2. *Mucosal RNA (A) and DNA (B) values along the small intestine.* Results (media ± SEM) are expressed as µg/mg of mucosal protein. Legend as in figure 1.

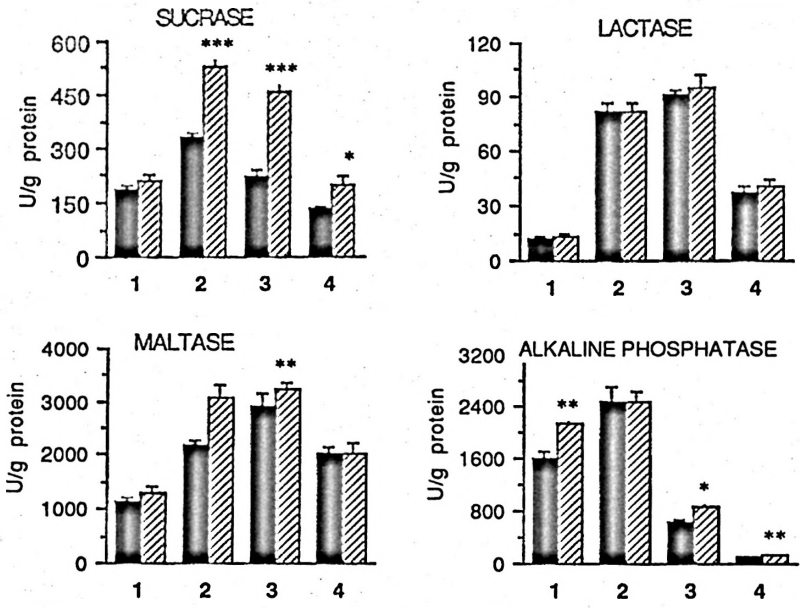

Fig. 3. *Comparison of enzyme specific activities in purified brush border membranes along the small intestine.* Values (media ± SEM) are expressed as μmoles of substrate hydrolyzed/min (U)/g of protein. Legend as in figure 1. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ as compared to control rats.

The specific activities of brush border enzyme throughout the small intestine from control and treated rats are shown in figures 3 and 4. The specific enzyme activity is a useful measure of intestinal maturity and function. Enzyme activity has been measured in purified brush border membranes which did not exhibit changes in their protein content between control and treated rats in any of the four segments of the small intestine.

Our results show for the first time that Misoprostol treatment increases sucrase, maltase, aminopeptidase and alkaline phosphatase activities of growing rats irrespective of the intestinal segment or the enzyme location in the villi membrane: sucrase, maltase, aminopeptidase are located in the external surface of the villi membrane whereas alkaline phosphatase is buried deeper.

Regional disaccharidase activity differs throughout the intestinal tract, being highest in villus regions and the proximal small intestine. In the present study the jejunoileal gradient of the disaccharidase activity was seen in all animals, and it showed peak levels in the jejunum and low values in the ileum.

Misoprostol produces an increase in sucrase and maltase activities whose magnitude is greater in the jejunum with a marked gradient for these enzymes. Jejunal sucrase activity was significantly increased ($p<0.001$) by Misoprostol treatment. The increment was about 62 % in the proximal part and 103 % in the distal jejunum of treated rats. Similarly, maltase activity was significantly ($p<0.01$) raised by 42 % in the proximal jejunum of treated rats. Ileal sucrase activity was also enhanced by Misoprostol treatment (49 %; $p<0.05$). Specific activities of sucrase-isomaltase are also modulated by developmental, hormonal, differentiation and nutritional factors (9).

In the duodenum disaccharidase activities were not modified by Misoprostol

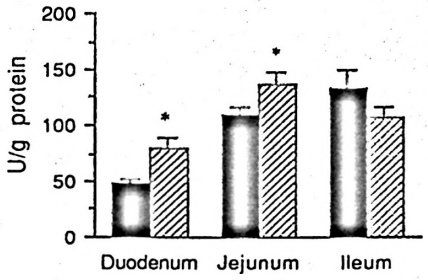

Fig. 4. *Aminopeptidase N specific actitity from duodenum, jejunum and ileum.*
Values (media ± SEM) are expressed as µmoles of substrate hydrolyzed/min (U)/ g protein. Solid bars indicate control animals and hatched bars indicate animals treated with Misoprostol. * $p<0.05$ as compared to control rats.

treatment. As it can be seen in figure 3, lactase activity was not modified by Misoprostol in either the jejunum or ileum. For this enzyme, the lowest values of activity were found in the duodenum.

The gradient of alkaline phosphatase fell steeply from duodenum to ileum (14). In control rats duodenal alkaline phosphatase values were 1604±84 U/g protein and fell to 93±6 U/g protein in the ileum. Alkaline phosphatase activity was significantly greater in the duodenum (32 %; $p<0.05$) and the ileum (32 %; $p<0.01$) when compared with the control group.

In contrast, aminopeptidase activity had low values in the duodenum and rose progressively from duodenum to the ileum. Aminopeptidase activity was significantly ($p<0.05$) increased in the duodenum (67 %) and jejunum (24 %) from treated rats.

The stimulation of brush border enzyme activity can be explained by the mucosal hyperplasia due to PGs administration (6, 18, 20). In addition, after PGs-treatment there is an increase in mucosa glycoprotein production (4, 8, 16) that may protect disaccharidases from their degradation by pancreatic enzymes.

In conclusion, Misoprostol increases brush border enzyme activities along the rat small intestine although further studies will be necessary to clarify the mechanism(s) involved.

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A. MARTÍ y M.P. FERNÁNDEZ-OTERO. *Estimulación por el misoprostol de la actividad enzimática del borde en cepillo en intestino de rata.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (2), 75-80, 1994.

Se estudia el efecto del misoprostol (0,3 mg/kg/día durante cuatro semanas) sobre la actividad enzimática del borde en cepillo de rata, en periodo de crecimiento. En las ratas tratadas se observa un incremento significativo ($p < 0,05$) de los pesos relativos del estómago e intestino y de la mucosa del duodeno y del yeyuno proximal, así como de la actividad específica de las disacaridasas, fosfatasa alcalina y aminopeptidasa. En el yeyuno proximal la actividad sacarasa y maltasa aumentan significativamente (62 % y 42 %, respectivamente), este aumento para la actividad sacarasa llega hasta un 103 % ($P<0,001$) en el yeyuno distal. Hay también un incremento significativo del 32 % ($P<0,05$) de la actividad fosfatasa alcalina en el duodeno e ileon. La aminopeptidasa es significativamente más alta en duodeno (67 %) y yeyuno (24 %). Se concluye que el misoprostol incrementa la capacidad del intestino delgado en relación con sus funciones digestivas, aunque son necesarios más estudios para examinar los mecanismos celular y molecular que pueden ser responsables de estos efectos.

Palabras clave: Misoprostol, Intestino delgado, Enzimas del borde en cepillo, DNA.

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