BBA 76018

DETERGENT ACTION OF SODIUM TAUROCHOLATE ON RAT GASTRIC MUCOSA

ANDREW J. THOMAS, DAVID L. NAHRWOLD AND RICHARD C. ROSE

Departments of Physiology and Surgery, College of Medicine, The Pennsylvania State University, Hershey, Pa. (U.S.A.)

(Received March 20th, 1972)

SUMMARY

Rats were prepared with a vagotomized whole stomach pouch. The pouch was exposed to a solution of HCl and Na_2SO_4 (control) or HCl, Na_2SO_4 and sodium taurocholate (treatment) for 30 min. Transmural net fluxes of H^+, Na^+ and K^+ were determined and the amount of phospholipid extracted from the mucosa was measured. Exposure of the mucosa to sodium taurocholate was associated with increased transmural ionic diffusion and increased recovery of phospholipid. It is possible that sodium taurocholate disrupts the gastric mucosal barrier through its detergent action on cell membranes.

INTRODUCTION

Normal gastric mucosa allows only a low rate of H^+ diffusion from the gastric lumen toward the serosa. Several investigators[1-3] have shown that pretreatment of human or canine gastric mucosa with bile or solutions of bile salts disrupts this physiologic barrier to back-diffusion of H^+, but the mechanism by which this phenomenon occurs is not clear. Bile salt solutions have detergent properties which enable them to solubilize lipids. Theoretically, solubilization of the phospholipid component of cell membranes could result in cellular damage and disruption of the gastric mucosal barrier.

The present study was done to determine the effect of sodium taurocholate on ionic fluxes across rat gastric mucosa, a study not previously reported. In addition, we sought to quantitate the detergent action of bile salt by measuring the amount of phospholipid extracted by sodium taurocholate.

METHODS

Sprague–Dawley rats (150 to 370 g) were deprived of food, but not water, for 48 h, and were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally. The trachea was intubated with polyethylene tubing (0.055 inch internal diameter), and the viscera were exposed through a midline incision. An intubated pouch of the entire stomach was prepared by inserting a polyethylene tube (0.055 inch internal diameter) 1 cm into the proximal stomach through an incision in the distal esophagus,

and another polyethylene tube (0.148 inch internal diameter) 1 cm into the distal stomach through an incision in the proximal duodenum. The tubes were secured by ligature at the esophagogastric junction and the pylorus, respectively. Solutions were introduced through the proximal tube and aspirated through the distal tube. Bilateral subdiaphragmatic truncal vagotomy was performed to eliminate gastric acid secretion[4].

The stomach of six rats was rinsed with Solution 1 (100 mM HCl, 27 mM Na_2SO_4, 20 mM mannitol) until it was judged to be clean. 5 ml of Solution 1 were then placed in the stomach and aspirated after 30 min (Period 1). After four 5-ml washes with Solution 1, 5 ml of Solution 2 (40 mM sodium taurocholate, 100 mM HCl, 7 mM Na_2SO_4) were placed in the stomach and aspirated after 30 min (Period 2). This was followed by four 5-ml washes with Solution 1. 5 ml of Solution 1 were then placed in the stomach and aspirated after 30 min (Period 3). In parallel control studies on six rats, Solution 1 was used in all three periods.

The method of Hunt and Knox[5] was used to determine the residual volume of each stomach during Period 1 by using phenol red (40 mg/l) as a dilution marker. The residual volume was 0.47 $\pm$ 0.07 ml.

Samples from each period were centrifuged and aliquots taken of the clear supernatant for phospholipid and electrolyte measurements. Total phospholipid was determined by the method of Patton and Thomas[6] with adjustment for smaller volumes. Briefly, lipid was extracted from samples using chloroform–methanol (2:1, v/v). The phospholipids were isolated from the neutral and glycolipids using column chromatography. Purity of the phospholipid was documented by thin layer chromatography on representative samples. Quantification of phospholipid was performed by analysis for lipid phosphorus.

Ionic fluxes (μequiv/30 min) were determined for each period by the difference between net ion recovered and net ion instilled. Net ion instilled and net ion recovered were calculated from the measured initial and recovered solutions corrected for initial and final residual volumes. Positive values are used to indicate a net flux of ion into the lumen and negative values to indicate a net flux of ion from the lumen.

Na^+ and K^+ concentrations were measured with a flame photometer, and H^+ concentration was determined by electrometric titration to pH 7.0 against 0.2 M NaOH.

RESULTS

When an isosmotic acid solution (Solution 1) was used in all three periods there was net flux of H^+ out of the lumen and net flux of Na^+ and K^+ into the lumen (Table I). None of the differences in flux values of individual ions between periods were statistically significant. However, when an isosmotic sodium taurocholate solution (Solution 2) was used during the second period there was a marked increase in net flux of H^+ out of the lumen and a marked increase in net flux of Na^+ and K^+ into the lumen. These changes tended to persist, but were less striking, during the subsequent period.

Small amounts of phospholipid were found in the gastric contents after exposure to the acid solution used in control experiments (Table II). Differences between phospholipid values for all control periods were not significant. Total phospholipid was markedly increased after exposure of the mucosa to sodium taurocholate.

TABLE I

NET IONIC FLUX ACROSS GASTRIC MUCOSA

In control experiments Solution 1 was used in all three periods. In test experiments Solution 1 was used in Periods 1 and 3, and Solution 2 in Period 2. Values indicate mean ± S.E. in μequiv/30 min. Positive values indicate net flux into the lumen.

Experiment	*Ion*	*Period 1*	*Period 2*	*Period 3*
Control ($N = 6$)	H^+	−48.0 ± 22.5	−60.8 ± 39.1	−25.5 ± 46.0
	Na^+	44.9 ± 17.2	19.8 ± 9.1	67.4 ± 19.4
	K^+	5.1 ± 1.6	6.6 ± 2.2	4.4 ± 1.3
Taurocholate ($N = 6$)	H^+	−43.0 ± 10.7	−201.3 ± 34.0*	−116.3 ± 28.3+
	Na^+	40.3 ± 8.9	139.1 ± 29.3**	68.0 ± 40.2
	K^+	3.1 ± 0.5	12.3 ± 1.3***	17.1 ± 2.1++

* $P < 0.025$ compared with Period 2 of control experiment and $P < 0.005$ compared with Period 1 of test experiment.

** $P < 0.005$ compared with Period 2 of control experiment and $P < 0.01$ compared with Period 1 of test experiment.

*** $P < 0.05$ compared with Period 2 of control experiment and $P < 0.001$ compared with Period 1 of test experiment.

\+ $P < 0.05$ compared with Period 1 of test experiment.

++ $P < 0.001$ compared with Period 1 of test experiment.

TABLE II

PHOSPHOLIPID RECOVERED FROM STOMACH

In control experiments Solution 1 was used in all three periods. In test experiments Solution 1 was used in Periods 1 and 3, and Solution 2 in Period 2. Values indicate mean ± S.E. in μg/30 min.

Experiment	*Period 1*	*Period 2*	*Period 3*
Control ($N = 6$)	5.6 ± 3.7	11.1 ± 7.4	8.5 ± 4.9
Taurocholate ($N = 6$)	9.8 ± 2.5	293.8 ± 84.7*	54.3 ± 15.0

* $P < 0.05$ compared with Period 2 of control experiment and $P < 0.01$ compared with Period 1 of test experiment.

DISCUSSION

The high rate of gastric acid secretion known to occur in the pylorus-ligated rat is essentially eliminated by vagotomy[4]. We used the vagotomized rat preparation because secretion of gastric acid could mask ion flux changes resulting from alteration of the mucosal barrier. The vagus nerve apparantly has no effect on maintainance of the permeability characteristics of the rat gastric mucosa[4].

Our experiments were designed to provide substantial concentration gradients of Na^+ and K^+ from plasma to gastric lumen and a gradient of H^+ from lumen to plasma under both control and experimental conditions. Thus, an increase in ionic conductance, resulting from disruption of the mucosal barrier, would be evidenced by larger diffusional net fluxes of Na^+ and K^+ into the lumen and a larger net flux of H^+ out of the lumen.

Our control studies confirm the work of Overholt *et al.*[4] who found a significant back diffusion of H^+ in the vagotomized rat stomach. Our experiments clearly show

that sodium taurocholate severely disrupts the gastric mucosal barrier in the vagotomized rat. Others[1] have shown that the mucosal barrier is disrupted in dogs by sodium taurocholate in both neutral and acidic solutions. Black *et al.*[7] found that bile damages the gastric mucosal barrier in dogs. This effect was more pronounced with high bile concentrations and at low pH.

The histologic studies of Grant *et al.*[8] and Smith[9] have shown that bile exerts a cytolytic effect on cat gastric mucosa. Conjugation of bile acids with taurine or glycine converts them into acid-resistant detergents[10]. Our data show that treatment of rat gastric mucosa with sodium taurocholate is associated with increased ionic flux across the mucosa and a simultaneous increase in the dissolution of phospholipid from the mucosa. It is possible that the detergent action of taurocholate alters the mucosal barrier either by disruption of the lipoprotein content of the plasma membrane or by loosening the tight junctions between epithelial cells[1].

ACKNOWLEDGEMENTS

This work was partially supported by N. I. H. Grant AM 15244-01. The authors wish to thank Mr George Felder, III for technical assistance.

REFERENCES

1 H. W. Davenport, *Gastroenterology*, 54 (1968) 175.
2 K. J. Ivey, L. Den Besten and J. A. Clifton, *Gastroenterology*, 59 (1970) 683.
3 J. L. Werther, H. D. Janowitz, W. P. Dyck, M. L. Chapman and J. Rudick, *Gastroenterology*, 59 (1970) 691.
4 B. F. Overholt, D. A. Brodie and B. J. Chase, *Gastroenterology*, 56 (1969) 651.
5 J. N. Hunt and M. T. Knox, *J. Physiol. London*, 163 (1962) 34.
6 S. Patton and A. J. Thomas, *J. Lipid Res.*, 12 (1971) 331.
7 R. B. Black, D. Hole and J. Rhodes, *Gastroenterology*, 61 (1971) 178.
8 R. Grant, M. I. Grossman, K. J. Wang and A. C. Ivy, *J. Cell. Comp. Physiol.*, 37 (1951) 137.
9 G. M. Smith, *J. Med. Res.*, 30 (1914) 147.
10 D. M. Small, *Am. Chem. Soc. Monograph*, 84 (1968) 31.