Cimetidine and the gastric mucosal barrier

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SUMMARY The histamine H_2 -receptor antagonist, cimetidine, when given topically or parenterally, did not affect net luminal Na^+ gain or net luminal H^+ loss in vagally-denervated pouches of antrectomised dogs. Cimetidine did not affect disruption of the mucosal barrier by 5 mmol/l or 20 mmol/l sodium taurocholate.

The histamine H_2 -receptor antagonists are potent inhibitors of gastric secretion and are likely to prove useful in the treatment of peptic ulcer (Black *et al.*, 1972; Milton-Thompson *et al.*, 1974; Blackwood *et al.*, 1976). Previous studies in our laboratory have shown that, while the H_2 -receptor antagonist metiamide does not protect the canine gastric mucosal barrier against permeability changes induced by sodium taurocholate solution, the compound does not in itself increase mucosal permeability to hydrogen or sodium ion (O'Brien and Carter, 1975). Metiamide has now been superseded by the apparently non-toxic compound cimetidine (Brimblecombe *et al.*, 1975; Burland *et al.*, 1975), and it is important to define the effect of this new compound on the mucosal barrier before its widespread clinical use. The present studies were therefore undertaken to define the effect of cimetidine on hydrogen and sodium movement across the gastric mucosa in an experimental model, and to determine the effect of cimetidine on permeability changes induced by the barrier breaker, sodium taurocholate.

Methods

Three male mongrel dogs weighing 12, 15, and 17 kg were prepared by antrectomy and construction of a vagally denervated (Heidenhain) pouch drained to the exterior by a vitallium cannula. No experiments were performed in the month after surgery, during which the animals were trained to the stand.

The animals were deprived of food, but not water, for 18 hours before the start of each experiment. All experiments consisted of six 30-minute periods. Twenty millilitres of solution containing a known

amount of Na^+ and H^+ ion were instilled into the gastric pouch at the beginning of each period, mixed thoroughly by repeated withdrawal and instillation using a 20 ml syringe attached to the cannula, and a 5 ml sample (initial sample) removed to measure Na^+ and H^+ ion concentration. The remaining 15 ml were retained in the pouch for 30 minutes, and after further thorough mixing a further 5 ml aliquot (final sample) was removed to determine Na^+ and H^+ concentration. The remaining fluid was discarded and the next period commenced.

A standard *basal solution* was instilled during periods 1, 2, 4, 5, and 6 during all tests, while period 3 was used to instil solutions containing test substances. Control tests were performed by instilling basal solution in all six periods. Basal solution consisted of 80 mmol/l HCl made isotonic with NaCl.

The test solutions instilled during period 3 were (1) taurocholate solution, (2) cimetidine solution, or (3) a combined solution of cimetidine in taurocholate solution. The taurocholate solution consisted of basal solution in which either 5 mmol/l or 20 mmol/l sodium taurocholate (Koch Light) replaced equivalent amounts of NaCl.

The cimetidine solution consisted of 200 mg cimetidine (N' -cyano-N-methyl- N' -[2-[(5-methylimidazol-4-yl)methylthio]ethyl]guanidine) dissolved in 20 ml basal solution. The combined solution consisted of 200 mg cimetidine dissolved in either 5 mmol/l or 20 mmol/l taurocholate solution.

In a further series of experiments, the effect of parenteral cimetidine on mucosal permeability was assessed by giving a bolus intravenous injection of 200 mg of the compound at the beginning of period 2, and instilling basal solution in all six periods. The effect of parenteral cimetidine on permeability changes induced by 5 mmol/l taurocholate solution was assessed by injecting 200 mg of the compound

before period 2, and instilling 5 mmol/l taurocholate solution during period 3.

To minimise error caused by incomplete recovery of fluid from the pouch, polyethylene glycol labelled with C^{14} (New England Nuclear) was added to all solutions as a volume marker, and unlabelled polyethylene glycol (1 g per litre) was also added to prevent loss of the labelled material by surface adherence.

The initial and final samples from each period were assayed for Na^+ ion concentration by flame photometry (IL 343, Digital Flame Photometer, Instrumentation Lab. Inc.), and for H^+ ion concentration using an automatic titrator (Radiometer, Copenhagen) and glass electrode. The amount of 100 mmol/l NaOH solution required to bring the sample to pH 7.0 was determined and an aliquot of the derived neutral solution was then added to liquid scintillant (NE 260, Nuclear Chicago) and C^{14} activity determined by scintillation counting. Knowing the initial volume instilled at the start of each period, the amount of 100 mmol/l NaOH solution needed to neutralise the initial and final sample from each period, and the change in C^{14} activity over each period, the difference between the initial and final mass of Na^+ and H^+ could be calculated as: difference in mass =

$$\begin{aligned} & (\text{final concentration} \times \text{final volume}) \\ & - (\text{initial concentration} \times \text{initial volume}). \end{aligned}$$

Two tests were carried out in each of the three dogs at each stage of the experiments, and the mean $\pm$ SEM ($\mu\text{mol}/30 \text{ min}$) for the change in mass of each ion calculated from the six results available for each 30 minute period.

The statistical significance of the difference between changes in ion mass has been assessed by comparing periods 2 and 3 within each set of experiments, and by comparing period 3 after exposure to test solutions with period 3 in control tests using basal solution. The analysis of the six pairs of results available was performed using a t test for paired data.

Results

CONTROL TESTS

Instillation of basal solution during all six 30-minute periods produced a small but constant gain in Na^+ by the pouch fluid, and a comparable small but constant loss of H^+ , once the initial period was completed (Fig. 1). There was no statistically significant difference between periods 2 and 3 when gain of Na^+ or loss of H^+ ion was assessed (Table 1).

CIMETIDINE SOLUTION

Instillation of cimetidine solution during period 3

did not produce any statistically significant alteration when the pattern of ion mass changes was compared with that observed in control tests (Fig. 1; Table 1).

Fig. 1 Effect of basal solution and cimetidine solution on net Na^+ gain and net H^+ loss. Each point represents the mean $\pm$ SEM from six experiments.

Table 1 Net Na^+ gain and H^+ loss under basal conditions and after administration of topical or parenteral cimetidine

	Basal solution alone	Topical cimetidine	P	Parenteral cimetidine	P
Net Na^+ gain					
Period 2	+ 140 $\pm$ 26	+ 183 $\pm$ 19	NS	+ 175 $\pm$ 37	NS
Period 3	+ 146 $\pm$ 14	+ 148 $\pm$ 26	NS	+ 171 $\pm$ 61	NS
P	NS	< 0.05		NS	
Net H^+ loss					
Period 2	- 83 $\pm$ 28	- 65 $\pm$ 14	NS	- 94 $\pm$ 37	NS
Period 3	- 57 $\pm$ 20	- 37 $\pm$ 19	NS	- 73 $\pm$ 17	NS
P	NS	NS		NS	

Net ion gain or loss expressed in $\mu\text{mol}/30 \text{ min}$; each figure is the mean $\pm$ SEM of six values.

TAUROCHOLATE SOLUTION

Instillation of either 5 mmol/l or 20 mmol/l sodium taurocholate solution during period 3 caused a statistically significant increase in Na^+ net gain and H^+ net loss when compared with the preceding period 2 (Figs. 2 and 3; Table 2). These changes were greater after exposure to the higher concentration of taurocholate.

COMBINED SOLUTION

The addition of 200 mg cimetidine to either concentration of taurocholate solution did not reduce

Fig. 2 Effect of topical cimetidine on net Na^+ gain and net H^+ loss induced by 5 mmol/l taurocholate solution. Each point represents the mean $\pm$ SEM from six experiments.

the net Na^+ gain or net H^+ loss induced by taurocholate alone (Figs 2 and 3). In the case of 5 mmol/l taurocholate solution, the addition of cimetidine actually increased net H^+ loss to a statistically significant extent. The mean net loss of H^+ ion during period 3 was $-289 \mu\text{mol}/30 \text{ min}$ after exposure to the combined solution, as opposed to $-235 \mu\text{mol}/30 \text{ min}$ after exposure to taurocholate alone (Table 2).

PARENTERAL CIMETIDINE

Injection of 200 mg cimetidine before period 2 did not produce any statistically significant alteration in the pattern of ion movement when compared with control tests (Fig. 4; Table 1).

Injection of cimetidine did not prevent the changes

Fig. 3 Effect of cimetidine on net Na^+ gain and net H^+ loss induced by 20 mmol/l taurocholate solution. Each point represents the mean $\pm$ SEM from six experiments.

in permeability induced by instillation of 5 mmol/l taurocholate during period 3 (Fig. 5); indeed, net H^+ loss was significantly increased when compared with period 3 in tests where 5 mmol/l taurocholate was instilled without previous injection of cimetidine.

Discussion

Damage to the gastric mucosal barrier resulting in increased permeability to H^+ ion may be important in the aetiology of gastritis and gastric ulcer (Rhodes,

Table 2 Net Na^+ gain and H^+ loss after exposure to taurocholate solutions with and without administration of cimetidine ($\mu\text{mol}/30 \text{ min}$; mean $\pm$ SEM of six values)

	20 mmol/l taurocholate alone	20 mmol/l taurocholate + topical cimetidine	P	5 mmol/l taurocholate alone	5 mmol/l taurocholate + topical cimetidine	P	5 mmol/l taurocholate + parenteral cimetidine	P
Net Na^+ gain								
Period 2	+ 126	+ 122	NS	+ 149	+ 141	NS	+ 120	NS
	$\pm$ 34	$\pm$ 40		$\pm$ 9	$\pm$ 11		$\pm$ 37	
Period 3	+ 427	+ 494	NS	$\pm$ 331	$\pm$ 340	NS	$\pm$ 406	NS
	$\pm$ 73	$\pm$ 45		$\pm$ 30	$\pm$ 25		$\pm$ 47	
P	< 0.001	< 0.001		< 0.001	< 0.001		< 0.001	
Net H^+ loss								
Period 2	- 63	- 88	NS	- 59	- 78	NS	- 55	NS
	$\pm$ 30	$\pm$ 20		$\pm$ 26	$\pm$ 23		$\pm$ 13	
Period 3	- 647	- 591	NS	- 235	- 289	< 0.05	- 335	< 0.0
	$\pm$ 29	$\pm$ 90		$\pm$ 20	$\pm$ 27		$\pm$ 37	
P	< 0.001	> 0.001		< 0.001	< 0.001		< 0.001

Fig. 4 Effect of parenteral cimetidine on net Na^+ gain and net H^+ loss. Each point represents the mean $\pm$ SEM from six experiments.

Fig. 5 Effect of parenteral cimetidine on net Na^+ gain and net H^+ loss induced by 5 mmol/l taurocholate solution. Each point represents the mean $\pm$ SEM from six experiments.

1972). The barrier can be damaged by a number of agents (Davenport, 1964, 1966, 1967; Ivey *et al.*, 1970) and release of mucosal histamine is considered to be of central importance in the pathophysiology of barrier disruption (Johnson, 1966, 1968).

Output of Na^+ across an intact mucosa is usually less than 300 $\mu\text{mol}/30$ min in gastric pouches of the type used in the present study (Davenport and Barr, 1973). After exposure to 20 mmol/l taurocholate we have observed net Na^+ gains in excess of 400 $\mu\text{mol}/$

30 min, but this increase in mucosal permeability was not reduced by concurrent cimetidine instillation (Table 2). Exposure to 5 mmol/l taurocholate produced net Na^+ gains just exceeding 300 $\mu\text{mol}/30$ min, suggesting that this was a threshold concentration of taurocholate for barrier disruption in the animals used. However, even when given in conjunction with this lower concentration of taurocholate, cimetidine failed to prevent increases in net Na^+ gain. Previous intravenous injection of cimetidine also failed to prevent increases in net Na^+ flux induced by 5 mmol/l taurocholate (Table 2).

Changes in mass of H^+ ion are generally accepted as less reliable indices of barrier disruption. After severe mucosal damage, histamine release may stimulate H^+ secretion by the pouch mucosa, thus masking increased H^+ back-diffusion from the pouch lumen (Davenport, 1970). There is no evidence from the present study that cimetidine prevented the increased net loss of H^+ induced by taurocholate instillation. At first sight, the small but statistically significant increase in net H^+ loss, noted when cimetidine was given in association with 5 mmol/l taurocholate (Table 2), could reflect inhibition of pouch acid secretion by cimetidine. However, the absence of a similar pattern after exposure to a higher concentration of taurocholate suggests that these differences are caused by experimental variation rather than by a biologically significant mechanism involving histamine H_2 -receptor blockade.

The failure of H_2 -receptor antagonists to protect against barrier disruption by taurocholate is not unexpected. If histamine has central importance in the pathophysiology of disruption, it seems probable that its deleterious effects will be mediated by H_1 as well as H_2 receptors in the underlying tissues. Rees and his associates (1976) have recently produced support for this thesis by demonstrating that, in an animal model similar to that used in the present study, barrier disruption by taurocholate can be prevented by a combination of mepyramine (H_1 -receptor antagonist) and metiamide (H_2 -receptor antagonist), whereas administration of either agent alone did not prevent taurocholate-induced increases in ion flux.

Although we have been unable to detect any protective action of cimetidine on the mucosal barrier challenged by taurocholate, our results in no way detract from the potential value of this compound in the treatment of conditions such as gastritis and gastric ulceration. Cimetidine showed no signs of increasing mucosal permeability when given in the absence of taurocholate (Table 1), and, as a potent inhibitor of gastric secretion, the compound could prove valuable by reducing the amount of secreted H^+ ion available to exploit a mucosal barrier damaged by

agents such as bile salts. The encouraging preliminary results reported from H_2 -receptor antagonist therapy in patients with stress-induced upper gastrointestinal haemorrhage (MacDonald *et al.*, 1976) and in prevention of haemorrhagic gastritis in patients with liver disease (Bailey *et al.*, 1976), lend support to this suggestion.

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