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Protective Effect of Cimetidine on Tannic Acid-induced Gastric Damage in Rats

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Abstract—The effect of cimetidine on the acidified tannic acid-induced back diffusion of gastric acid and mucosal damage was investigated in the vagotomized rat. After intragastric irrigation for 1 to 3 h, tannic acid (20–500 mg kg^{-1}) produced a dose-related increase in gastric volume and the loss of luminal H^+. The change of mucosal permeability to the electrolyte, either the loss of H^+ or the gain of Na^+, K^+, and Ca^{2+}, induced by tannic acid was significantly diminished by the combination with intragastric cimetidine. However, intravenous injection of cimetidine did not protect this damage and back diffusion of acid neutralized intragastric cimetidine did not reduce the back diffusion of acid and Na^+, K^+, and Ca^{2+} output provoked by acid solution. Thus, the neutralizing action of cimetidine seems responsible.

Tannic acid has been used as an antidote for the oral treatment of alkaloid intoxication and/or heavy metal poisoning. Tannic acid was found to cause hepatotoxocity and colonic mucosal necrosis when used as an adjuvant of barium enema in the roentgen diagnosis of colonic disease (Harris et al 1966). However, the effect of tannic acid on the stomach and the back diffusion of gastric acid remains obscure. Cimetidine is known to reduce the gastric secretion through the blockade of histamine H_2-receptors in both man (Henn et al 1975; Adland & Berstad 1978) and experimental animals (Hirschowitz 1979; Hung et al 1979). The protection of gastric mucosa by cimetidine against a variety of agents, such as aspirin, indomethacin and ethanol or taurocholic acid-induced damage is well documented (Robert et al 1979; Puurunen 1980; Utley et al 1985; Liss et al 1986; Bauer et al 1986). This study is an attempt to understand the effect of cimetidine on tannic acid-induced damage of the stomach and acid back diffusion.

Materials and Methods

Animals

Male Sprague-Dawley rats, 200–250 g, were fasted but allowed free access to water for 24 h before the experiments.

Surgical procedures

Under light ether anaesthesia, the stomach was surgically exposed for the ligation of pylorus and lower oesophagus. In order to prevent spontaneous gastric secretion, bilateral subdiaphragmatic vagotomy was conducted. Care was taken to avoid injury of blood vessels. A polypropylene tube (1·0 mm i.d. × 20 mm) was inserted through an incision which was made in the forestomach. The tube was secured with a ligature.

Experimental protocol for the back diffusion of gastric acid

The stomach was rinsed meticulously with double distilled water (37°C). Seven mL of the acid solution containing 100 mM HCl and 54 mM NaCl was injected with a syringe into the lumen. The luminal content was well mixed with the same syringe by repeated aspiration and injection for 30 s, and 3 mL of the fluid was taken as the initial sample. The incision of the forestomach was tightly closed. The abdominal wound was also sutured.

After 1 or 3 h, the animal was killed with an overdose of ether. The gastric sample (final sample) was collected and centrifuged at 3000 rev min^{-1} for 20 min. The initial and final samples were analysed for titratable acidity and Na^+, K^+, and Ca^{2+} concentrations. As soon as the final sample was collected, the stomach was filled with 1% formalin for 10 min. The mucosa was exposed by opening the stomach along the greater curvature. The length (mm) and the width (mm) of the ulcer on the gastric mucosa were measured by a planimeter (1 × 1 mm) under a dissecting microscope (× 0·7-× 3·0; A. O. Scientific Instrument 569). The ulcer area was calculated as described by Kauffman & Grossman (1978), i.e. the ulcer area = length × width × $\pi/4$. The total ulcer area (mm^2) of each stomach was recorded. The mucosal damage was determined by a person unaware of the schedules. Histological studies of the stomach were conducted by the method of Hollander et al (1985).

Drugs

The solutions of tannic acid (20–500 mg kg^{-1}), or tannic acid (100 mg kg^{-1}) + cimetidine (50–300 mg kg^{-1}) were prepared by mixing each substance with acid solution (100 mM HCl + 54 mM NaCl). Neutralized cimetidine was obtained by titrating cimetidine solution with 0·3 M HCl to pH 7·0 before adding to the acid solution or acidified tannic acid solution. Each solution was freshly prepared before use. Cimetidine, tannic acid, and $NaHCO_3$ were purchased from Sigma Chemicals (St. Louis, USA).

Quantitation of gastric samples

The acidity of the initial and final sample was measured by titrating a 1 mL sample of gastric contents with 0·1 M NaOH to pH 7·0 on an autoburette titrator (Radiometer, Copenhagen, Denmark). The concentrations of Na^+, K^+, and Ca^{2+} were measured using a flame photometer (Eppendorf, FCM 6341, Germany). The net flux of ions through gastric mucosa

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was calculated as the difference between the product of the final volume and concentration, and the product of the initial volume and concentration. Negative values indicate that the net flux was from the lumen to the mucosa. Positive values indicate that the net flux was from the mucosa to the lumen. The osmolarity of each solution was tested by using an advanced Digimatic Osmometer (Model 3D2).

Statistical methods

Data were expressed as mean ± s.e.m. and were analysed for statistical significance by the Student's *t*-test for unpaired comparison. $P < 0.05$ was considered significant.

Results

When the stomach was irrigated with acidified tannic acid solution, gastric inflammation, oedema, white protein precipitation and mucosal necrosis were observed. The mucosa became fragile and eroded. In most cases, exfoliation of the mucosal layer was found (Fig. 1, upper). The severity of the mucosal necrosis was dependent on the concentration of tannic acid used. Furthermore, from the observation of gastric contents, the initial sample appeared a transparent light yellow colour while the final sample showed white turbidity accompanied by exfoliated mucosal protein precipitation in the centrifuge tubes. Histological study also indicated that tannic acid produced gastric oedema and mucosal cell damage (Fig. 2, upper).

Cimetidine (50–300 mg kg^{-1}) dissolved in tannic acid (100

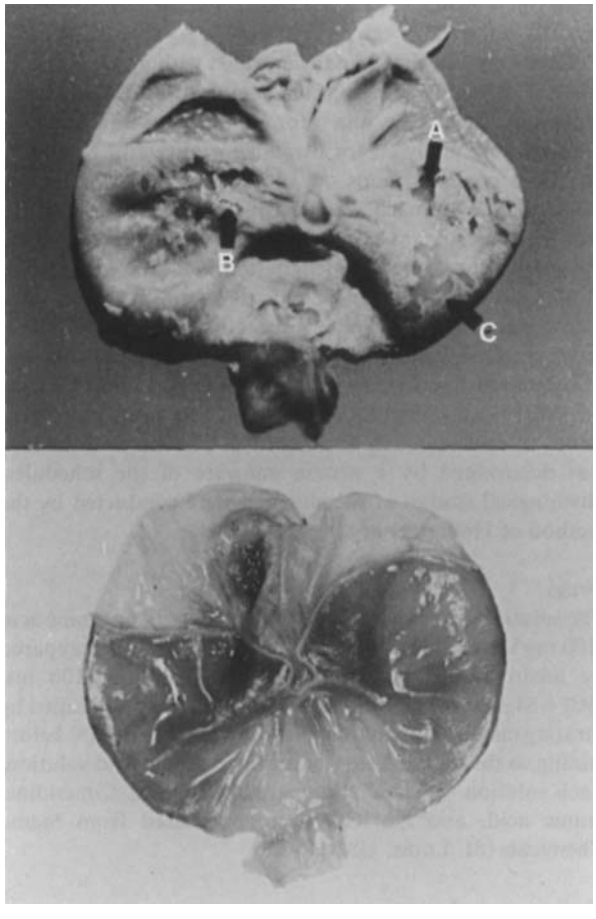

FIG. 1. Upper: Gastric mucosal damage caused by 3 h irrigation of the acidified tannic acid (tannic acid 100 mg kg^{-1} dissolved in 100 mM HCl + 54 mM NaCl) solution in the rat stomach. Note that the epigastric layer is exfoliated (A); the mucin is coagulated (B); and the mucin layer is desquamated (C) by acidified tannic acid. Lower: cimetidine (300 mg kg^{-1}) protects gastric mucosa against acidified tannic acid (100 mg kg^{-1})-induced gastric damage. The mucin precipitation and epigastric exfoliation are markedly reduced.

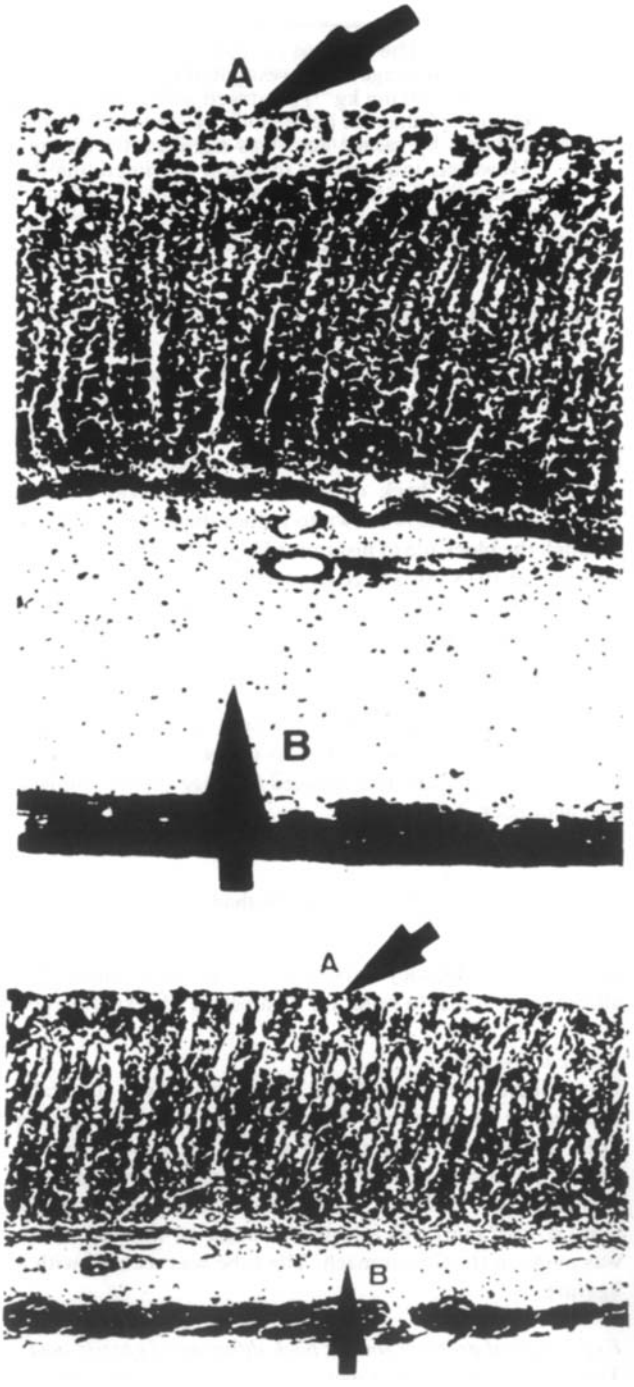

FIG. 2. Upper: histological study of the acidified tannic acid (100 mg kg^{-1})-induced gastric mucosal damage. The mucin and the epigastric layer are extensively damaged (A), and gastric oedema (B) is also observed. Lower: cimetidine (300 mg kg^{-1}) protects the mucosa against acidified tannic acid (100 mg kg^{-1})-induced damage. Gastric oedema is also inhibited.

Table 1. Gastric mucosal necrosis induced by acidified tannic acid and protective effects of cimetidine or $NaHCO_3$ or neutralized cimetidine.

Treatment	Dose (mg kg^{-1})	Ulcer area (mm^2)
Acid solution		1·2±0·6
acidified tannic acid	20	17·2±3·2[a]
	100	42·8±5·8[a]
	500	64·0±9·2[a]
Acidified tannic acid 100 mg kg^{-1} plus		
cimetidine	50	44·0±7·8
	100	34·2±6·6[b]
	300	9·4±2·4[b]
$NaHCO_3$	52	21·4±3·2[b]
neutralized cimetidine	300	27·0±2·3[b]

[a] $P<0·05$ vs tannic acid (100 mg kg^{-1}); [b] $P<0·001$ vs acid solution. Each test used 10 rats.

mg kg^{-1}) solution, produced a dose-related inhibition in the mucosal necrosis provoked by tannic acid solution (Table 1). The morphological evidence that cimetidine (300 mg kg^{-1}) protected gastric mucosa against tannic acid-induced damage is shown in Fig. 1 (lower); the exfoliation of gastric mucosa was markedly prevented. Also, the gastric oedema and epithelial cell damage was markedly improved by cimetidine (Fig. 2, lower).

The mucosal permeability to electrolytes influenced by tannic acid is indicated in Fig. 3. After 1 or 3 h of intragastric irrigation with acid solution, a reduction in luminal acidity and an increase of Na^+, K^+, and Ca^{2+} fluxes were obtained. Tannic acid enhanced this back diffusion of acid and gain of Na^+, K^+, and Ca^{2+} in the stomach in a dose-dependent manner from 20 to 500 mg kg^{-1}. Thus, the decrease in acidity and the increases in Na^+, K^+, and Ca^{2+} concentrations were observed in the final sample.

Table 2 shows the effect of cimetidine on the back diffusion of acid induced by tannic acid (100 mg kg^{-1}), regarding the change in gastric volume, ionic concentrations and ionic fluxes. Below the dose of 50 mg kg^{-1}, intragastric irrigation of cimetidine did not have significant influence on the tannic acid-induced back diffusion of acid (data not shown).

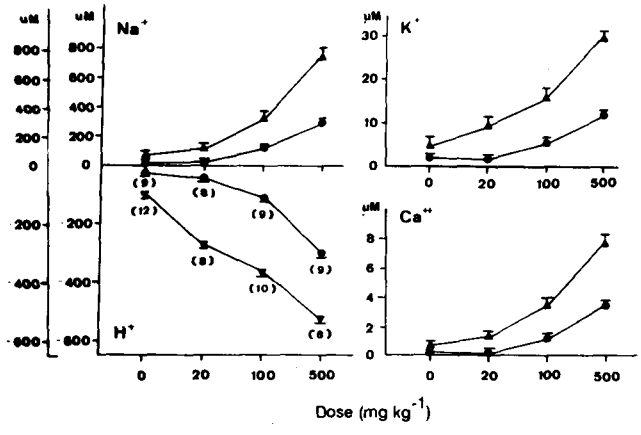

FIG. 3. Graded doses of acidified tannic acid-induced changes in the luminal electrolytes. Tannic acid causes a dose-dependent enhancement in the luminal H^+ loss and an increase in Na^+, K^+ gain. The stomach was irrigated with each test solution for 1 (O– –O) or 3 h (▲– –▲).

However, at doses higher than 50 mg kg^{-1}, it produced a dose-dependent (50–300 mg kg^{-1}) reduction in tannic acid-provoked gastric volume increase, H^+ loss, and gain of Na^+, K^+, and Ca^{2+} in the lumen. The H^+ concentration in the initial sample was decreased while that in the final sample was increased by cimetidine; both decrease and increase were dose-dependent. In addition, $NaHCO_3$ (52 mg kg^{-1}) diminished the acidified tannic acid-induced changes of luminal electrolytes in a manner equal to 300 mg kg^{-1} of cimetidine. When neutralized cimetidine (300 mg kg^{-1}) was concomitantly administered with tannic acid into the stomach, the back diffusion of acid induced by tannic acid was also markedly inhibited. However, the inhibitory effect was less pronounced compared with that of cimetidine at the same dose. Fig. 4 shows that the luminal H^+ loss and Na^+, K^+, and Ca^{2+} output induced by the lower doses of tannic acid (20 mg kg^{-1}) was not affected by intravenous administration of cimetidine at the dose of 50 mg kg^{-1}; on the contrary, significant ($P<0·01$) enhancement was found. Fig. 5 shows an acid concentration-dependent increase in the luminal H^+ loss and Na^+, K^+, and Ca^{2+} gain when various acid solutions (100–300 mM HCl + adequate amount of NaCl for isotonicity) were applied to the stomach. Cimetidine (300 mg kg^{-1}), but not the same dose of neutralized cimetidine, significantly ($P<0·001$) reduced the back diffusion of acid and Na^+, K^+, and Ca^{2+} output provoked by acid solution as mentioned above. Moreover, none of the doses of tannic acid and/or cimetidine used in the present study produced any toxicity, such as tremor or respiratory inhibition, in the experimental animals.

Discussion

The back diffusion of gastric acid is one of the important factors that cause exacerbation of peptic ulcer. In the normal gastric mucosa, back diffusion of acid is prevented by gastric mucosal barriers. Davenport (1969) reported that once the gastric mucosal barriers were disrupted by noxious agents, such as aspirin and ethanol, the luminal acidity decreased while Na^+ concentration increased. The net flux of Na^+ from the mucosa to lumen is a sensitive index for the gastric mucosal barriers. In the present study, acidified tannic acid caused a substantial increase in the luminal H^+ loss and Na^+, K^+, and Ca^{2+} gain and gastric volume. These results indicate that gastric mucosal barriers were seriously damaged by tannic acid. The extent of mucosal damage and back diffusion of acid were dependent on the time and concentration of tannic acid in contact with the gastric mucosa. The enhancement in the gastric volume, K^+ and Ca^{2+} fluxes also explain why mucosal cells were killed by tannic acid. Morphological and histological observations showed that tannic acid produced gastric oedema, mucus precipitation and mucosal necrosis.

These results are similar to the observations made by Rambo et al (1966) in the rat colonic mucosa. Since tannic acid is an astringent and protein coagulant, it damages gastric mucosal cells probably through a direct corrosive effect and protein denaturation. In particular, it reacts with mucin which plays a very important role in the cytoprotection of the gastric mucosa. The results are not in agreement with those of Satoru (1976) who reported an increase in the

Table 2. Effects of cimetidine, $NaHCO_3$ and neutralized cimetidine on acidified tannic acid (100 mg kg^{-1})-induced changes in gastric volumes, ionic concentration and ionic fluxes in the rat gastric lumen.

	Tannic acid (100 mg kg^{-1})					
Treatment	Control	Cimetidine			$NaHCO_3$	Neutralized cimetidine
Dose (mg kg^{-1})	100	50	100	300	52	300
Gastric content						
Initial volume (mL)	4·0±0·1	4·0±0·1	4·0±0·1	4·0±0·1	4·0±0·1	4·0±0·1
Final volume	50·0±0·1	4·6±0·1*	4·4±0·1*	4·1±0·1*	4·8±0·1*	3·9±0·4*
Initial $[H^+]$ (mM)	110·4±0·8	106·4±1·6*	97·7±0·9*	78·7±1·4*	78·7±0·2*	107·4±3·0
Final $[H^+]$ (mM)	18·6±1·7	20·4±1·3	24·5±1·5*	38·5±1·7*	20·6±2·7	42·8±1·3*
Net flux (μM/3 h)	−349·3±7·9	−332·8±8·9	−283·2±8·0*	−159·0±4·0*	−215·2±13·6*	240·5±7·7*
Initial $[Na^+]$ (mM)	58·1±0·2	60·7±1·7	61·0±1·1*	61·4±1·6*	86·0±3·2*	55·7±0·3*
Final $[Na^+]$ (mM)	103·5±1·9	111·0±2·4*	98·9±3·1	87·5±2·9*	108·6±2·0*	83·6±1·6*
Net flux (μM/3 h)	282·5±16·8	269·0±22·1	193·8±17·5*	109·5±11·1*	177·3±12·1*	138·8±12·7*
Initial $[K^+]$ (mM)	3·0±0·1	0·1±0·1*	0·2±0·1*	0·2±0·1*	0·3±0·1	0·5±0·2*
Final $[K^+]$ (mM)	2·9±0·1	3·4±0·3*	2·9±0·3	1·5±0·1*	3·7±0·1*	1·8±0·1*
Net flux (μM/3 h)	13·8±0·4	15·0±1·1	12·4±0·3*	5·3±0·4*	11·7±0·5*	6·0±0·2*
Initial $[Ca^{2+}]$ (mM)	0·1±0·1	0·1±0·1	0·1±0·1	0·1±0·1	0·1±0·1	0·2±0·1
Final $[Ca^{2+}]$ (mM)	0·8±0·4	0·7±0·3	0·5±0·3	0·4±0·1*	0·6±0·4	0·6±0·3
Net flux (μM/3 h)	3·3±0·2	2·8±0·2*	2·0±0·2*	1·2±0·1*	2·2±0·2*	1·5±0·2*

The stomach was irrigated with each drug solution for 3 h. Each test used 10 rats. *$P<0·05$ vs control, using Student's t-test.

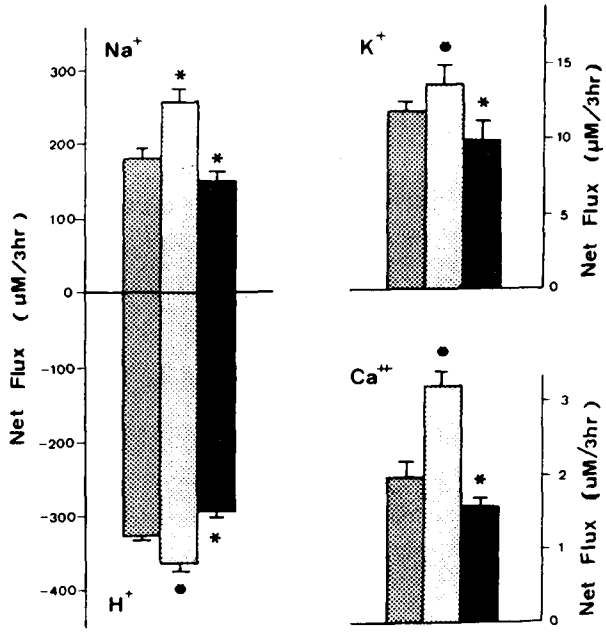

FIG. 4. Effect of intravenous injection (i.v.) or intragastric irrigation (i.g.) of cimetidine on acidified tannic acid-induced changes in the luminal electrolyte fluxes. Each test used 8 rats. *$P<0·01$, **$P<0·001$. The key, reading from left to right; large black dots on white, control (tannic acid): 20 mg kg^{-1}. Small black dots on white, cimetidine 50 mg kg^{-1} (i.v.). White dots on black, cimetidine 50 mg kg^{-1} (i.g.).

gastric volume as well as a reduction in acidity and pepsin activity of gastric juice by tannic acid; the difference might be due to the damage of mucosal cells and the occurrence of back diffusion of gastric acid during the intragastric administration of tannic acid. Tannic acid is toxic to living cells; death from centrolobular hepatic necrosis following multiple tannic acid enema has been reported by McAlister et al (1963) and Lucke et al (1963).

The present study found that intragastric combination of cimetidine markedly reduced tannic acid-induced back diffusion of acid and mucosal necrosis. This effect of cimetidine may be through three possible mechanisms: (i) cimetidine has a direct cytoprotective effect on the gastric mucosa, (ii) cimetidine can reduce the back diffusion through gastric acid inhibition or neutralizing action, (iii) via chemical interaction, cimetidine may reduce the corrosive effect of tannic acid. Because intravenous administration of cimetidine failed to inhibit acidified tannic acid-induced back diffusion of acid and mucosal necrosis, a direct cytoprotective effect on the gastric mucosa can be ruled out. In the clinic, cimetidine is effective in preventing and curing gastric lesions in patients suffering from gastroduodenal ulcers (Barakat et al 1988; Bardhan et al 1988; Howden et al 1988). The inhibition of gastric secretion may play an important role in cimetidine-induced prevention and healing of gastric ulcers. In the present study, we also observed that cimetidine given by intragastric irrigation with tannic acid or acid solution reduced the acidity of the initial samples (Table 2). Furthermore, intragastric administration of cimetidine (300 mg kg^{-1}), but not of neutralized cimetidine (300 mg kg^{-1}), was significant in diminishing the back diffusion of acid induced by acid solution of various concentrations (100–300 mM HCl). These results indicate that the effect of cimetidine was produced possibly through the neutralizing action. The inhibitory effects of cimetidine (300 mg kg^{-1}) on tannic acid solution-induced back diffusion of acid and mucosal necrosis were more pronounced than that of $NaHCO_3$ or neutralized cimetidine. Moreover, neutralized cimetidine was effective in diminishing tannic acid-induced, but not acid solution-induced, back diffusion of acid and mucosal necrosis. The mechanism of this effect is still not clear. One possible explanation is that cimetidine reduced the corrosive effects of tannic acid via chemical interaction.

Our results suggest that tannic acid breaks down the gastric mucosal barrier and can be used as a good ulcer-inducer in the animal models. Cimetidine can reduce the

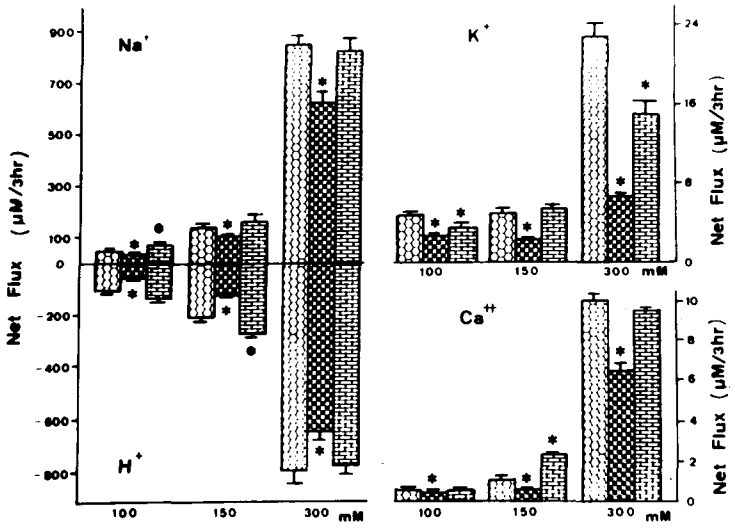

FIG. 5. Effect of cimetidine (300 mg kg^{-1}) or neutralized cimetidine on acid solution (100–300 mM HCl + adequate amount of NaCl)-induced changes in the luminal electrolyte fluxes. Each test used 10 rats. * $P < 0.01$. The key, reading from right; honeycomb, acid solution; chequer, cimetidine 300 mg kg^{-1}; bricks, neutralized cimetidine 300 mg kg^{-1} (pH 7·0).

tannic acid-induced back diffusion of gastric acid and mucosal necrosis, via chemical interactions, in rat stomach.

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