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The influence of chronic or acute nicotine pretreatment on ethanol-induced gastric ulceration in the rat

S. H. Wong, C. W. Ogle, C. H. Cho*, *Department of Pharmacology, Faculty of Medicine, University of Hong Kong, 5 Sassoon Road, Hong Kong*

The effects in rats of chronic or acute nicotine pretreatment were studied on three gastric parameters: ethanol-induced ulceration, gastric wall mucus content and gastric acid secretion, under basal or histamine-stimulated conditions. Oral administration of ethanol (40%, 10 ml kg^{-1}) depleted gastric wall mucus and produced ulceration in the gastric glandular mucosa. Ten-day nicotine pretreatment (15 or 25 μg ml^{-1} drinking water) worsened the adverse effects of ethanol on mucosal ulceration and mucus content, potentiated the gastric secretory action of histamine, but did not affect basal acid secretion. Single oral doses of nicotine (2 or 4 mg kg^{-1}, given 1 h beforehand) prevented ulceration and mucus depletion in ethanol-treated animals; however, they did not influence either basal or histamine-stimulated gastric acid output. It is concluded that chronic nicotine administration aggravates ethanol ulceration, possibly by decreasing gastric wall mucus content and sensitizing the stomach to the acid secretory action of histamine. On the other hand, an acute oral dose of nicotine preserves the mucus content and prevents ethanol-induced ulcer formation.

Chronic nicotine pretreatment has been found to worsen ethanol-induced ulceration in the gastric glandular mucosa of rats (Ogle et al 1985). The association between peptic ulcers and cigarette smoking has been shown in man (Friedman et al 1974), but the mechanism for the aggravation of ethanol-induced ulceration by chronic nicotine administration in rats is yet to be defined.

It is known that gastric wall mucus plays an important role in the defensive mechanism of the stomach against acid-pepsin digestion (Bickel & Kauffman 1981; Pfeiffer 1981; Williams & Turnberg 1980), and that excessive gastric histamine leakage appears to contribute largely to ethanol ulceration (Cho et al 1983; Dinoso et al 1976). The present study examines the influence of chronic or acute nicotine pretreatment on gastric ulceration and mucus content, both in the absence and presence of ethanol, and on the acid-secretory action of histamine in rat stomachs.

* Correspondence.

Methods

Male Sprague-Dawley rats (180–210 g) were reared on a standard laboratory diet (Ralston Purina Co.) and given tap water to drink. They were kept in a temperature (22 ± 1 °C)- and humidity (65–70%)-controlled room where the experiments were conducted.

In the chronic nicotine experiments, rats drank either ordinary tap water or nicotine bitartrate (BDH), 5 or 25 μg ml^{-1} of tap water, for 10 days (each rat drank 31 ± 1·8 ml per day, i.e. 155 ± 9 or 775 ± 45 μg nicotine per day); the weight of the alkaloid is expressed as its salt. Food, but not drinking fluid, was removed on the 9th day. On the 10th day, rats were given either distilled water or 40% v/v ethanol (BDH) in distilled water, in a volume of 10 ml kg^{-1}, orally via a stainless steel gastric tube. All animals were killed by a sharp blow on the head 5 h later. Stomachs were removed, opened along

the greater curvature and examined for ulcers or for mucus lining the glandular mucosae. Ulcer size was measured using a grid (each grid square was 1 mm^2) placed on the glandular mucosal surface; measurements were carried out by an observer who was unaware of the treatment regimen. In the case of petechiae, five such lesions were recorded as the equivalent of 1 mm^2. The sum of the ulcer areas in each group of rats was divided by the number of animals and expressed as the mean ulcer index (Ogle et al 1985). The amount of mucus on the glandular mucosa was determined by the alcian blue method (Corne et al 1974). The glandular portions of the stomachs were removed, weighed and immediately transferred to 10 ml of 0·1% w/v buffered alcian blue (Sigma) solution (pH 5·8). Tissues were stained for 2 h in alcian blue solution at room temperature (23 °C); excess dye was removed by two successive rinses in 10 ml of 0·25 M sucrose (Sigma) solution. Dye complexed with gastric wall mucus was extracted with 10 ml of 0·5 M magnesium chloride (BDH) solution which was intermittently shaken for 1 min at 30 min intervals for 2 h. Four millilitres of the blue extract solution were then vigorously shaken with an equal volume of diethyl ether (BDH). The amount of alcian blue in the aqueous layer was measured spectrophotometrically at a wavelength of 540 nm. The quantity of alcian blue extracted per g wet glandular tissue was calculated from standard curves which obeyed the Beer-Lambert law at the dye concentrations used.

Studies on gastric acid secretion were carried out in separate groups of animals which had also been pretreated with nicotine for 10 days and starved on the 9th day for 24 h before use. On the day of experimentation, the rats were anaesthetized with sodium pentobarbitone (Abbott) (50 mg kg^{-1}, i.p.); their tracheae and left jugular veins were then cannulated before a midline laparotomy was performed. A gastric chamber was prepared, as described by Mersereau & Hinchey (1973). The glandular mucosa in the chamber was initially washed with three changes of 0·9% w/v NaCl solution (saline). Luminal solution, consisting of 1·5 ml saline, was then instilled into the gastric chamber; this solution was replaced after 15 min and the procedure repeated at 15 min intervals until a total of five sequential collections had been made over the 75 min observation period. Rats were given an intravenous bolus injection of histamine diphosphate (Sigma) 500 μg kg^{-1} (weight expressed as the salt), or saline 1 ml kg^{-1}, immediately after the first collection of luminal solution. The acid content in the gastric chamber luminal samples was measured using an autotitration system (Radiometer model TTT 80), and expressed as μequiv. HCl produced in 15 min by 100 mm^2 of gastric mucosal area forming the base of the chamber.

The influence of a single oral dose of nicotine bitartrate on ethanol-induced ulceration and gastric wall mucus content, and on basal or histamine-provoked gastric acid output, was also evaluated. Rats were starved for 24 h and subsequently pretreated with either saline 10 ml kg^{-1} or nicotine bitartrate 2 or 4 mg kg^{-1} via an intragastric tube 1 h before receiving ethanol or before starting the stomach chamber experiment. The severity of gastric ulceration and the stomach wall mucus content were measured 5 h after ethanol administration. Acid secretion was measured over a 75 min period.

The data were analysed for statistical significance of difference by the two-tailed Student *t*-test.

Results

Table 1 shows the results of chronic nicotine pretreatment on ethanol-induced gastric lesion formation and gastric wall mucus content. Nicotine administration for 10 days dose-dependently increased the ulcer index but depressed the mucus content; statistically significant changes of both parameters were seen with the higher dose of nicotine (Table 1A). The lesions appeared as petechiae with occasional small haemorrhagic ulcers. Administration of a single oral dose of ethanol (10 ml kg^{-1} of a 40% solution, i.e. about 3·2 g kg^{-1}) provoked severe haemorrhagic ulceration which was accompanied by decreased mucus content of the mucosal wall (Table 1B). These effects were markedly potentiated by chronic nicotine pretreatment. Chronic administration with the alkaloid did not influence basal gastric acid output in the stomach chamber preparation (Fig. 1), but the higher dose of the drug noticeably enhanced (an increase of 511% during the 60 min period after histamine injection) histamine-induced acid secretion (Fig. 1).

Table 1. Effects of chronic pretreatment with nicotine (5 or 25 μg ml^{-1} drinking water given for 10 days) on ethanol-induced gastric glandular ulceration and mucus level changes (rats killed 5 h after ethanol).

Pretreatment	No. of rats	Ulcer index (mm^2)	Mucus content (μg alcian blue g^{-1} wet wt)
A. Rats given distilled water (10 ml kg^{-1}) p.o.			
Tap water	10	0·02 ± 0·02	318·23 ± 21·08
Nicotine 5 μg ml^{-1}	9	0·27 ± 0·17	260·95 ± 17·55
Nicotine 25 μg ml^{-1}	10	0·47 ± 0·11**	241·67 ± 15·84*
B. Rats given 40% ethanol (10 ml kg^{-1}) p.o.			
Tap water	10	27·57 ± 3·09†	277·18 ± 22·59
Nicotine 5 μg ml^{-1}	9	61·89 ± 10·63†	218·97 ± 18·69†
Nicotine 25 μg ml^{-1}	10	74·67 ± 15·01*†	198·26 ± 19·38*†

Values indicate means ± s.e.m.
*$P < 0·05$, **$P < 0·01$ when compared with its own control pretreated with tap water.
†$P < 0·01$ when compared with the corresponding group given distilled water intragastrically in A.

Acute oral administration of nicotine did not increase the ulcer index, however, the bigger dose significantly elevated the stomach wall mucus content (Table 2A). The severe ulceration and decrease in gastric wall mucus content produced by ethanol were dose-dependently reduced by acute nicotine pretreatment (Table 2B). The same doses of nicotine did not influence basal gastric

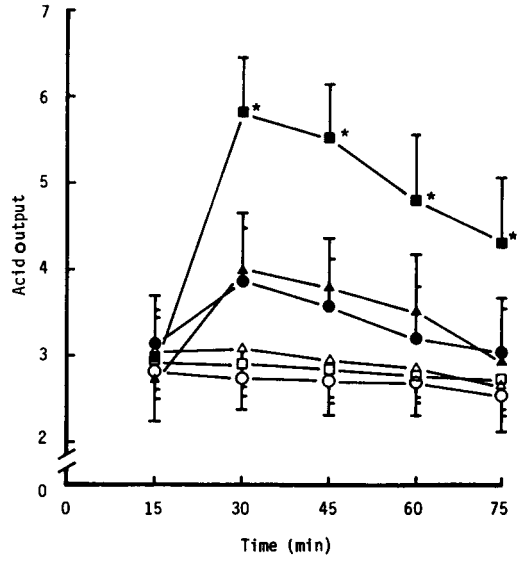

FIG. 1. Effects of 10-day nicotine pretreatment (○, ● = drinking water; △, ▲ = nicotine 5 μg ml^{-1}; □, ■ = nicotine 25 μg ml^{-1} drinking water) on basal (saline 1 ml kg^{-1}, open symbols) and histamine-evoked (histamine 500 μg kg^{-1}, closed symbols) gastric acid output. Either saline or histamine was given as a single i.v. bolus injection at 15 min. Values show means ± s.e.m. *$P < 0.05$ when compared with the corresponding tap water-drinking group. Acid output = μequiv. 15 min^{-1} 100 mm^{-2} of gastric mucosa; n = ■ 16, ● 12, ▲ 11, □ 7, △ 7, ○ 8.

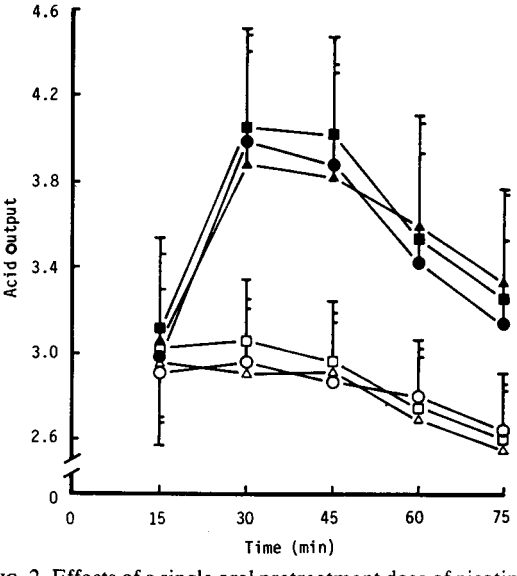

FIG. 2. Effects of a single oral pretreatment dose of nicotine (○, ● = saline 10 ml kg^{-1}; △, ▲ = nicotine 2 mg kg^{-1}; □, ■ = nicotine 4 mg kg^{-1}) on basal (saline 1 ml kg^{-1}, open symbols) and histamine-evoked (histamine 500 μg kg^{-1}, closed symbols) gastric acid output. Either saline or histamine was given as a single i.v. bolus injection at 15 min. Values show means ± s.e.m. Acid output = μequiv. 15 min^{-1} 100 mm^{-2} of gastric mucosa; n = ▲ 11, ■ 10, ● 8, ○ 10, □ 10, △ 8.

Table 2. Effects of a single oral dose of nicotine (2 or 4 mg kg^{-1}, given 1 h beforehand) on ethanol-induced gastric glandular ulceration and mucus level changes (rats killed 5 h after ethanol).

Pretreatment (p.o.)	No. of rats	Ulcer index (mm^2)	Mucus content (μg alcian blue g^{-1} wet wt)
A. Rats given distilled water (10 ml kg^{-1}) p.o.			
Saline 10 ml kg^{-1}	8	0·03 ± 0·03	324·35 ± 38·80
Nicotine 2 mg kg^{-1}	8	0·02 ± 0·02	397·33 ± 44·31
Nicotine 4 mg kg^{-1}	8	0·01 ± 0·01	441·25 ± 36·26*
B. Rats given 40% ethanol (10 ml kg^{-1}) p.o.			
Saline 10 ml kg^{-1}	9	34·05 ± 8·06†	261·53 ± 23·69
Nicotine 2 mg kg^{-1}	10	16·63 ± 4·04†	343·02 ± 64·90
Nicotine 4 mg kg^{-1}	11	14·22 ± 4·11*†	378·50 ± 49·21*

Values indicate means ± s.e.m.
*$P < 0.05$, when compared with its own control pretreated with saline.
†$P < 0.01$ when compared with the corresponding group given distilled water intragastrically in A.

acid output or histamine-provoked acid secretion in the gastric chamber preparation (Fig. 2).

Discussion

This study not only confirms the potentiating effect of chronic nicotine treatment on ethanol-induced gastric ulcers (Ogle et al 1985) but also shows that pretreatment with an acute oral dose of the alkaloid has an opposite action on ethanol-induced ulceration (Table 2). Although the mechanisms of ethanol-evoked ulcer formation are not clear, gastric mucosal mucus (Cho et al 1983; Koo et al 1986) and histamine (Cho et al 1983; Dinoso et al 1976) have been found to play important roles in its pathogenesis. Ethanol has been shown to deplete gastric mucosal mucus possibly by mobilizing the mucopolysaccharide into the lumen (Cho et al 1983; Koo et al 1986); this effect could indeed greatly weaken the mucosal barrier to acid-pepsin digestion (Bickel & Kauffman 1981; Pfeiffer 1981; Williams & Turnberg 1980). Ethanol also increases gastric histamine secretion from the histamine storage cells into the gastric lumen and blood vessels (Cho et al 1983; Dinoso et al 1976), thus producing stomach wall microvascular dilatation and permeability changes (Cho & Ogle 1978; Guth & Hall 1966). Therefore, it is possible that chronic or acute nicotine pretreatment could have produced their aggravating or protecting effects on ethanol ulceration, respectively, through an influence on gastric mucosal mucus and histamine.

Administration of cholinergic drugs has been shown to increase mucus secretion from the gastric glandular mucosa (Takagi & Okabe 1970; Williams & Turnberg 1980). Thus, the elevation of gastric wall mucus content by acute nicotine administration (Table 2) is probably the result of activation of the nicotinic receptors in the stomach parasympathetic ganglia. However, continued exposure to the alkaloid might depress the secretory function of the mucus cells possibly by desensitization of the ganglia in the stomach (Cho et al 1985b), to account for the lower gastric mucus content seen in rats chronically treated with nicotine.

Chronic nicotine treatment in drinking water for 10 days (155 ± 9 or 775 ± 45 μg of nicotine per day) not only depressed the mucus content but also increased ulcer formation in the gastric glandular mucosa. The inter-relationship between these actions remains to be defined, but may be related to the mucosal wall mucus profile. The observation that chronic nicotine pretreatment led to greater mucus depletion and ulceration by ethanol would suggest that the nicotine-ethanol-induced ulcers could indeed be, at least partially, causally related to mucus depletion. The finding that acute nicotine treatment significantly increased the glandular mucus level and prevented ethanol-evoked ulceration could indicate the importance of mucus in antagonizing these alcohol-induced lesions.

It is unclear as to how chronic nicotine pretreatment potentiates the effect of histamine on gastric acid secretion; acute nicotine administration did not have this potentiating action. As chronic nicotine administration with similar doses significantly increases urinary histamine excretion in rats (Svensson & Wetterqvist 1968; Sixt et al 1973), it is, therefore, conceivable that such an action on histamine metabolism may sensitize the histamine receptors and influence gastric responses to exogenous histamine. This hypothesis needs more study. The observed increased stomach sensitivity to histamine most likely contributed to the aggravation of alcohol-induced ulcer formation.

Nicotine is known to be a ganglion stimulator; excessive or prolonged stimulation of the gastrointestinal parasympathetic nervous system has been shown to produce gastric ulceration (Cho et al 1976; Cho et al 1985a). This action could also account for the ulcerogenic effect of chronic nicotine treatment and the worsening of ethanol-evoked ulceration.

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