gastric secretion observed in rats by Tachè et al (1980) as opposed to the inhibitory effects found in dogs in the present study. Thus, in accordance with the results of Gascoigne et al (1980) concerning the inhibitory effects of TRH on insulin-stimulated acid and pepsin secretion in cats, the inhibitory action of TRH on gastric secretion in dogs might be the result of an impairment of cholinergic transmission. Further studies are in progress to establish whether the inhibitory effects of TRH on canine gastric secretion are centrally and/or peripherally mediated.

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# Lysosomal enzyme release and ethanol-induced gastric lesions in rats

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Leakage of lysosomal enzymes into cells and the surrounding extracellular space has been implicated in the pathogenesis of gastric ulceration caused by acute stress (Ferguson et al 1972), 5-hydroxytryptamine (5-HT) (Ferguson et al 1973) and vitamin A (Watanabe et al 1981). It is possible that gastric lesions commonly seen in experimental animals after intragastric administration of ethanol are accompanied by labilization of the lysosomal membrane (Dinoso et al 1976; Puurunen et al 1980; Aures et al 1981), and the resulting enzyme release could further exacerbate the mucosal damage. Carbenoxolone and prednisolone have been reported to be lysosomal membrane stabilizers in-vitro (Weissman 1969; Symons & Parke 1980), and were recently shown to provide protection against ethanol-induced gastric injury in rats (Derelanko & Long 1981, 1982). Moreover, prostaglandin $E_1$, which is capable of stabilizing gastric lysosomes in-vivo and in-vitro (Ferguson et al 1973), is highly active in inhibiting erosive damage on the gastric mucosa due to ethanol and other noxious agents, a property defined as 'cytoprotection' (Robert et al 1979). With pretreatment of $PGE_1$, 5-HT-induced gastric ulceration in the rat was reduced while the accompanying release of the lysosomal enzyme cathepsin D from the gastric mucosa was partially prevented (Ferguson et al 1973).

Lewis et al (1971) demonstrated that phenylbutazone, which is ulcerogenic in man and animals, enhanced release of acid phosphatase from rat isolated stomachs. In the present study, by using a modified protocol we monitored changes in the release of acid phosphatase from the stomach after intragastric ethanol challenge and ascertained whether prevention of ethanol-induced gastric lesions by various known cytoprotective agents could be linked to inhibition of lysosomal enzyme release. The role of lysosomal enzymes in the reported protective effect through previous exposure to low concentrations of ethanol against subsequent insult with high concentrations of ethanol was also investigated (Robert et al 1978; Code 1981).

*Methods*

Male Charles River CD rats, 180 to 200 g, were fasted overnight with free access to water before experiments. Before the intragastric administration of 1 ml absolute ethanol, individual animals were treated with one of the following: vehicle (0·4% aqueous methylcellulose) 0·5 ml per rat by mouth or s.c.; prostaglandin $E_2$ (Sigma), 0.1 mg $kg^{-1}$ by mouth; SC-29333 [(±)-15-deoxy-16α,β-hydroxy-16-methyl prostaglandin $E_1$ methylester] (Searle, Chicago, Ill., U.S.A.), 30 μg $kg^{-1}$ s.c.; prednisolone (Schering, U.S.A.), 30 mg $kg^{-1}$ by mouth; epidermal growth factor (EGF; Collaborative Research, Waltham, Mass., U.S.A.), 30 μg $kg^{-1}$ s.c.; carbenoxolone (Biogastron, Biorex, London, U.K.), 100 mg $kg^{-1}$ by mouth; 25% ethanol, 1 ml per rat by mouth. All were administered 30 min before absolute ethanol except EGF which preceded ethanol by 10 min. There was always a separate control group receiving only the vehicle.

Ten minutes after absolute ethanol, the animals were killed by a blow to the head. The stomachs were rapidly removed and placed on ice after brief rinsing in 0·9% NaCl (saline). The individual stomachs were opened

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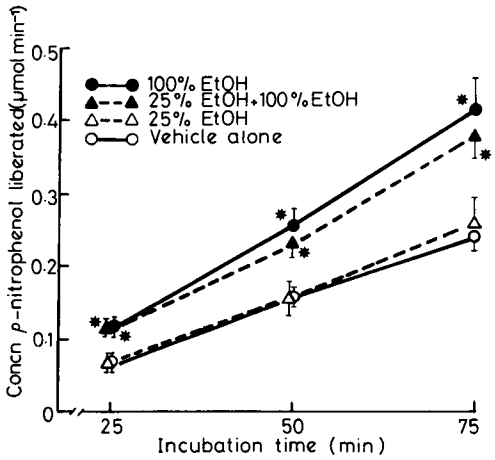


FIG. 1. Effect of previous exposure to 25% ethanol on acid phosphatase release from the rat stomach following intragastric challenge of absolute ethanol. Each of the 4 groups consisted of 8 rats. The enzyme activity is expressed by μmol of *p*-nitrophenol liberated $min^{-1}$ per stomach. There were no significant ($P > 0.05$) differences between '100% ethanol' and '25% ethanol + 100% ethanol' groups.

and placed in 22 ml of fully oxygenated Ringer-Tyrode solution at pH 6·0 (composition in mM: NaCl, 112; KCl, 1·9; $MgSO_4.7H_2O$, 1·0; $NaH_2PO_4.H_2O$, 29; glucose, 5·6; $NaHCO_3$. 4·8; $CaCl_2$, 1·8) and containing 15 mM *p*-nitrophenyl phosphate (Sigma) in stoppered flasks. These were gently shaken in a Dubnoff Metabolic Shaking Incubator set at 37 °C and 1 ml samples of the medium were taken at 25, 50 and 75 min after the incubation was started. The samples were spinned down in a cold centrifuge for 3 min and 0·5 ml aliquots of the supernatant transferred to test tubes containing 5 ml of 0·1 M NaOH. The absorbance of the final solutions was then determined with a Coleman spectrophotometer at 420 nm. For each assay, *p*-nitrophenol (PNP, Calbiochem) was used to construct the caliberation curves with minimal variations. Acid phosphatase activity was expressed by the rate of PNP liberated (μmol $min^{-1}$ per stomach). Since the stomach weight in the rats used (body wt: 180–200 g) was closely similar [wet wt(g): 1·314 (0·0027 s.d., N = 21; dry wt(g): 0·245 (0·018 s.d.), N = 21], the enzyme activity was not factored by the stomach weight. At the end of each 75 min incubation, the stomachs were examined by an investigator who did not know which treatment each animal received. The length of each lesion was measured in mm and summed up for each individual stomach.

All values represent mean ± s.e. Significant differences ($P < 0.05$) were determined by Duncan's multiple range test.

*Results*

*Effect of ethanol on lysosomal enzyme release from, and lesion formation in, the stomach.* As shown in Table 1 and Fig. 1, there was a spontaneous release of acid phosphatase activity increasing linearly with time in the control stomachs treated with vehicle alone. The stomachs exhibited markedly enhanced release of the enzyme activity 10 min following ethanol challenge, as indicated by the accelerated rate of liberation of *p*-nitrophenol from *p*-nitrophenyl phosphate. Severe haemorrhogic lesions were detected in the ethanol-treated stomachs but none in the control receiving vehicle alone.

Table 1. The effects of intragastric administration of absolute ethanol on acid phosphatase release from stomachs and on gastric lesions in rats. The enzyme activity is expressed by the amount of *p*-nitrophenol (PNP) in μmol liberated from *p*-nitrophenyl phosphate per minute per stomach. Three groups were treated with $PGE_2$, SC-29333 and prednisolone respectively 30 min before ethanol and one group treated with EGF 10 min before ethanol. The rats were killed 10 minutes after ethanol and the stomachs removed for incubation. Lesions were examined at the end.

| Treatment (no. in group) | μmol of PNP liberated $min^{-1}$ at different times 25 min | 50 min | 75 min | Gastric lesions mm/rat |
|---|---|---|---|---|
| Vehicle alone (32) | 0·08 ± 0·01 | 0·17 ± 0·01 | 0·25 ± 0·01 | 0 |
| Ethanol alone (32) | 0·15 ± 0·01* | 0·33 ± 0·01* | 0·47 ± 0·01* | 75 ± 7 |
| $PGE_2$ 0·1 mg $kg^{-1}$ oral + ethanol (8) | 0·16 ± 0·03* | 0·31 ± 0·04* | 0·46 ± 0·05* | 4 ± 2† |
| SC-29333 30 μg $kg^{-1}$ s.c. + ethanol (8) | 0·13 ± 0·01* | 0·31 ± 0·03* | 0·43 ± 0·04* | 3 ± 1† |
| Predinsolone 30 mg $kg^{-1}$ oral + ethanol (8) | 0·14 ± 0·01* | 0·28 ± 0·04* | 0·43 ± 0·05* | 30 ± 14† |
| EGF 30 μg $kg^{-1}$ s.c. + ethanol (8) | 0·15 ± 0·01* | 0·34 ± 0·03* | 0·52 ± 0·04* | 9 ± 2† |

* $P < 0.05$, vs 'vehicle alone'.
† $P < 0.05$, vs 'ethanol alone'.

*Effects of various cytoprotective agents on ethanol-induced lysosomal enzyme release and gastric lesions.* Since the results with vehicle or ethanol did not vary much from day to day, the vehicle control and ethanol control data were put together and presented in Table 1. However, the daily ethanol control values accompanying the carbenoxolone-treated group were somewhat lower than usual and hence will be presented separately (vide infra).

None of the cytoprotective agents was able to suppress the elevation in the rate of enzyme release caused by ethanol. Nevertheless, each respective drug significantly ($P < 0.05$) reduced the length of the macroscopic gastric lesions produced by ethanol. Carbenoxolone 100 mg $kg^{-1}$ by mouth significantly ($P < 0.05$) inhibited ethanol-induced mucosal damage, the treated stomachs showing a mean lesion length of 0·2 ± 0·2 mm per rat (n = 8) in comparison with 100 ± 16 mm per rat in the ethanol control ($P < 0.05$, n = 8). Again there were no significant ($P > 0.05$) differences in enzyme release between the two groups at corresponding time intervals (carbenoxolone: 0·11 ± 0·01, 0·24 ± 0·02 and 0·34 ± 0·03 μmol

PNP $min^{-1}$ vs ethanol control: 0·11 ± 0·01, 0·26 ± 0·01 and 0·37 ± 0·03 μmol PNP $min^{-1}$).

*Effects of previous exposure to 25% ethanol.* Exposure of the stomachs to 25% ethanol 30 min before absolute ethanol resulted in significantly ($P < 0·05$) reduced gastric lesions (1·6 ± 0·8 mm per rat, vs ethanol control, 26 ± 6·6 mm per rat), but this was not accompanied by any significant ($P > 0·05$) diminution in enzyme release (Fig. 1). 25% Ethanol alone did not provoke lesion formation or any acceleration of enzyme release.

*Discussion*

We have confirmed that $PGE_2$ (Robert et al 1979), SC-29333 (Colton et al 1979), prednisolone (Derelanko & Long 1982), EGF (Konturek et al 1981) and carbenoxolone (Derelanko & Long 1981) have cytoprotective activity in animals and protect the stomach from injury caused by ethanol.

We have demonstrated that as early as 10 min after the rats were challenged with absolute ethanol intragastrically, incubation of the excised stomachs showed marked enhancement in the release of acid phosphatase. Since all cytoprotective compounds achieved substantial reduction in gastric lesions without appreciably abating the accelerated enzyme release due to ethanol, it then appears that cytoprotection is mediated through mechanisms other than lysosomal stabilization.

The strongly disruptive action of absolute ethanol on the cellular membrane components including lysosomal membranes probably exceeded the membrane stabilizing effects of the cytoprotective agents, leading to an increase in enzyme release. Consequently if elevations in lysosomal enzyme release signify cellular injury, it could be inferred from the present data that the preventive effect of the cytoprotective compounds against ethanol-induced gastric damage, while effective, may not be complete. A recent study showed that 16,16-dimethyl $PGE_2$ prevented macroscopic lesions in rat stomachs challenged with ethanol, but cytological disruption in these specimens was still observed with electron microscopy (Lacy & Ito 1982).

Exposure to a damaging agent can produce an increase in the resistance of the gastric mucosa to subsequent exposures to the same substance at higher concentrations (Code 1981). Our morphological findings using 25% ethanol before absolute ethanol are in agreement with those of Robert et al (1978). Nevertheless, the reduction in lesions by previous exposure to 25% ethanol was not accompanied by a concomitant suppression in the release of degradative enzymes from the lysosomes.

The exact mechanism by which prostaglandins and other agents exert cytoprotective effects against noxious stimuli on the gastric mucosa remains unclear (Miller & Jacobson 1979). One possibility relates to increased production of gastric mucus which acts as a barrier protecting epithelial cells, as shown in man and animals using prostaglandins (Bolton et al 1978; Domschke et al 1978; Mahoney & Waterbury 1981) and carbenoxolone (Gheorghiu et al 1975; Bickel & Kauffman 1981). From the present data we can only conclude that the cytoprotective action of prostaglandins and other agents is not mediated through prevention of lysosomal enzyme release from the gastric mucosal cells.

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