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# Ethanol-stimulated acid secretion in the isolated whole stomach of the rat

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The application of ethanol to the serosal surface of the isolated whole rat stomach stimulated a high rate of acid secretion, with a maximum response being obtained to 4% v/v ethanol. This acid response to ethanol was not inhibited by high concentrations (1 mM) of atropine or cimetidine, indicating that the response was probably not mediated by either histamine, gastrin or acetylcholine. These results using the secretory antagonists were confirmed by experiments in an isolated gastric mucosa preparation. The acid response to ethanol was significantly, but not completely inhibited by 10 mM thiocyanate, indicating the existence of an acid component of non-parietal cell origin in the mucosal bathing solution. However, analysis of the mucosal solution failed to reveal the presence of an acidic metabolite of ethanol.

It is well established that the parenteral administration of ethanol in the dog causes gastric acid secretion, but some controversy exists about the mechanism of this stimulation. Kondo & Magee (1977) have reported that in the conscious dog ethanol-stimulated acid secretion is mediated by the release of antral gastrin, although other workers (Woodward et al 1957; Irvine et al 1960) have found that antrectomy does not abolish this response. In addition, the failure of vagotomy to affect the acid response to ethanol (Woodward et al 1957; Irvine et al 1960) does not support the view that ethanol is acting centrally via the vagus nerves (Hirschowitz et al 1956). The possibility that histamine is involved in this acid response in the dog has also been considered (Woodward et al 1957), although there is no evidence to substantiate this idea (Irvine et al 1960; Daves et al 1965).

In the present study the stimulation of acid secretion by ethanol in the isolated rat stomach preparation is reported, and the mechanism of this stimulant action is investigated.

## MATERIALS AND METHODS

*Isolated stomach preparation.* Gastric acid secretion in the isolated stomach of the immature rat (35–45g) was measured by the method described by Bunce & Parsons (1976). In brief, the rats were anaesthetized with pentobarbitone, the stomach exteriorized and the oesophagus ligated. An incision was made in the rumen of the stomach, and the contents washed out with warm Krebs-Henseleit solution. A second incision was made at the pyloric sphincter and polythene cannulae were inserted and tied into the stomach via these incisions. The stomach was rapidly dissected out and placed in Krebs-Henseleit solution at 37°C. The lumen of the stomach was perfused at a rate of 1 ml $min^{-1}$ with a modified Krebs-Henseleit solution from which the buffers ($NaHCO_3$ and $KH_2PO_4$) were omitted.

*Isolated gastric mucosa preparation.* Gastric acid secretion by an isolated rat gastric mucosa preparation was measured essentially according to the method described by Main & Pearce (1978). Male rats of the Wistar strain, approximately 120g, were anaesthetized with pentobarbitone (60 mg $kg^{-1}$ i.p.). The abdomen was opened, the stomach exteriorized, and the rumen of the stomach dissected away. The lumen of the stomach was rinsed with warm Krebs-Henseleit solution. The serosal muscle layer of the fundic region of the stomach was then separated from the gastric mucosa using the "blistering" technique described by Forte et al (1975). The muscle coat was dissected away, and the remaining sheet of mucosa was tied over the end of a Perspex chamber (1·13$cm^2$ area). The serosal surface of the gastric mucosa was bathed with Krebs-Henseleit solution, and the mucosal surface was superfused with unbuffered Krebs-Henseleit solution at a rate of 1 ml $min^{-1}$ using a technique similar to that described for the isolated whole stomach preparation.

For both preparations the hydrogen ion activity of the effluent mucosal perfusate was continuously recorded as previously described (Bunce & Parsons

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1976), and the rate of acid secretion expressed as nmol $min^{-1}$.

After setting up the tissue preparations the basal $H^+$ output was allowed to stabilize before the effect of ethanol was investigated. All drugs were added to the solution bathing the serosal surface of the whole stomach and gastric mucosa preparations. The acid response to a single dose of ethanol was calculated as the amount of acid secreted at peak response above the preceeding basal level. On adding a secretory antagonist to the serosal bathing solution, the tissue preparations were equilibrated in these solutions for at least 1 h before a dose of ethanol was given.

*Materials.* Ethanol (analar quality, James Burrough Ltd), histamine acid phosphate and atropine sulphate (BDH Ltd), sodium thiocyanate (Koch-Light Laboratories Ltd), pentobarbitone (Sagatal, May & Baker Ltd), cimetidine was synthesized in our own laboratories.

*Analysis of results.* Results are expressed as mean ± standard error of the mean. The difference between two means was examined statistically using Student's *t*-test for unpaired data. A *P*-value of less than 0·05 was considered to be significant.

## RESULTS

### *Isolated stomach preparation*

*Stimulation of acid secretion.* Ethanol stimulated the secretion of acid and sequential dose-response curves were constructed in seven stomach preparations by the addition of graded doses of ethanol in the concentration range 0·5 to 6% v/v. The results are shown in Fig. 1. Ethanol at a concentration of 0·5% v/v was threshold for the stimulation of acid secretion. The acid responses were dose-related in the concentration range 1 to 4% v/v, with a maximum acid response of 120·1 (± 32·1, n = 7) nmol $min^{-1}$ to 4% v/v ethanol. A concentration of ethanol of 6% v/v was supramaximal for acid secretion. A concentration of ethanol of 4% v/v was therefore used in subsequent studies.

A prerequisite for the subsequent antagonist studies was to determine whether there was any fade of response to repeated doses of ethanol. For this purpose five repeated doses of ethanol at 4% v/v were given over a mean period of 4·6h (± 0·13, n = 5), and the results are shown in Fig. 2. During this time there was a 46% reduction in the acid response to ethanol.

*The effects of cimetidine and atropine.* The inherent fade of the acid response to ethanol would complicate the interpretation of results in experiments where acid responses were measured both in the absence and the presence of an antagonist in the same stomach preparation. For this reason single doses of 4% v/v ethanol were applied to separate stomach preparations either under control conditions or in the presence of an antagonist. The results are shown in Fig. 3. Under these conditions neither the $H_2$-receptor antagonist, cimetidine (1mM), nor atropine (1mM) produced a significant inhibition of the acid response to ethanol.

*The effect of thiocyanate.* In these experiments one dose of 4% v/v ethanol was given under control conditions, the stomach was then equilibrated with

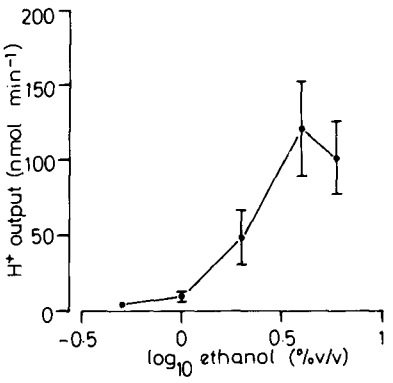


FIG. 1. Sequential dose-response curve to ethanol. Each point is the mean of 7 observations. Means and standard errors of the mean are shown.

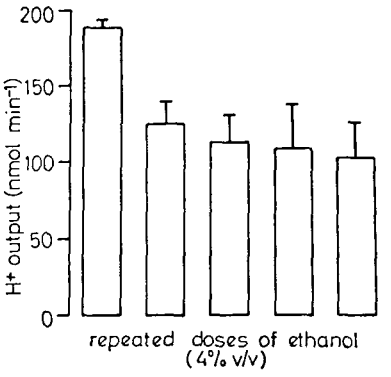


FIG. 2. Tachyphylaxis of the acid secretory response to five repeated doses of ethanol at 4% v/v given over a mean period of 4·6 ± 0·13 h (s.e.m.). Each column is the mean of five observations. Means and standard errors of the mean are shown.

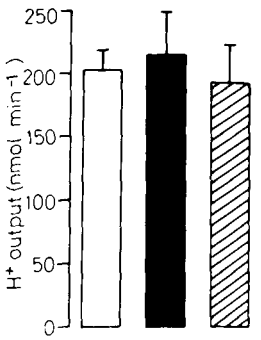


FIG. 3. The effect of atropine (1 mM) and cimetidine (1 mM) on the acid response to single doses of ethanol (4% v/v). Open column: ethanol alone (n = 8); closed column: ethanol plus cimetidine (n = 6); hatched column: ethanol plus atropine (n = 6). Means and standard errors of the mean are shown.

10mM thiocyanate and a second dose of ethanol (4% v/v) applied. The stomach was finally equilibrated in "normal" serosal solution, and a third dose of ethanol (4% v/v) given. The results are shown in Fig. 4. Thiocyanate (10mM) produced a significant inhibition of the acid response to ethanol ($P < 0.01$), and this was readily reversed on bathing the stomachs in a "normal" serosal solution.

*Isolated gastric mucosa preparation*

A small number of experiments were carried out on the isolated mucosa preparation to confirm the observations in the whole stomach. Ethanol was a relatively poor secretagogue in the isolated mucosa preparation and the acid responses showed some considerable variability. However it was found that 6% v/v ethanol stimulated a mean acid output of 14·9 ± 6·7 (n = 4) nmol $min^{-1}$ in mucosa preparations which were responsive to 0·1mM histamine (63·2 ± 23·9 nmol $min^{-1}$, n = 4) and that, as with the isolated whole stomach, this preparation still responded to ethanol in the presence of high concentrations (1mM) of both atropine and cimetidine.

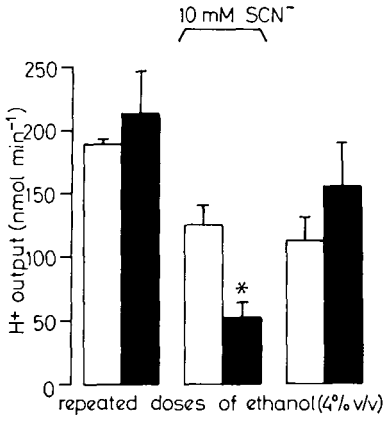


FIG. 4. The effect of thiocyanate (10 mM) on the acid response to ethanol (4% v/v). The open columns represent the control acid secretion (n = 5). The closed columns represent the test procedure as indicated by the horizontal bar (n = 5). *$P < 0.01$. Means and standard errors of the mean are shown.

## DISCUSSION

The first point to note in the present study is that ethanol stimulated a high rate of acid secretion in the isolated whole stomach preparation; a maximum acid response of 120 nmol $min^{-1}$ in response to ethanol compared with approximately 50–70 nmol $min^{-1}$ for histamine, gastrin and acetylcholine (Bunce & Parsons 1976; Bunce et al 1976). Also a gradual tachyphylaxis to ethanol was occurring during the construction of the sequential dose-response curve (Fig. 1), and the single application of 4% v/v ethanol to each stomach preparation (Fig. 3) shows that the true maximum acid response is in the region of 200 nmol $min^{-1}$.

Little information is available about the effect of serosally applied ethanol on acid secretion in isolated stomach preparations for direct comparison with the present work. However, Durbin et al (1973) have reported that a high concentration (16% v/v) of ethanol applied to the serosal surface of the isolated frog gastric mucosa inhibits acid secretion. The effect of applying ethanol topically to the mucosal surface of the rat stomach has also been investigated, and under these conditions acid secretion is inhibited both in vivo (Puurunen & Karppanen 1975) and in vitro (De Saint-Blanquat & Derache 1966). However, in the latter experiments the presence of ethanol on the mucosal surface of the stomach may not be affecting parietal cell activity, but rather damaging the mucosa and thus increasing the back-diffusion of hydrogen ions (Deregnaucourt 1979). The stimulation of acid secretion by the application of ethanol to the serosal surface of the stomach in the present experiments shows that this response is not mediated exclusively by a central mechanism involving either a neural (Hirschowitz et al 1956) or a humoral (Weise et al 1961) pathway. Also the failure of atropine and cimetidine to inhibit the acid response to ethanol shows that none of the secretagogues generally regarded as being of physiological significance, viz. histamine, acetylcholine and gastrin (the latter being susceptible to high concentrations of these antagonists) were involved.

A further possibility is that ethanol is stimulating acid secretion by increasing the intracellular level of cAMP in the parietal cell. Determination of nucleotide levels in the gastric mucosa has been restricted to studies in which ethanol has been applied topically to the gastric mucosa; a procedure which did not increase the cAMP content of this tissue (Tague & Shanbour 1974; Puurunen & Karppanen 1975). Thus, the effect of serosally applied ethanol on gastric mucosal cAMP levels requires investigation since it has been previously reported that, like ethanol, the acid response to dibutyryl cAMP in vitro is resistant to both atropine and $H_2$-receptor antagonists (Bunce et al 1976; Watanabe et al 1977).

Thiocyanate (8·5–10·0 mM) completely, or almost completely, inhibits secretagogue-induced acid secretion in isolated mammalian gastric mucosa preparations (Holton & Spencer 1976; Watanabe et al 1977; Main & Pearce, 1978). Thus the inhibition of ethanol-induced gastric acid secretion by thiocyanate in the present study does suggest that ethanol was stimulating parietal cell activity. However, part of the acid response to ethanol was resistant to thiocyanate indicating the existence of a non-HCl component. One possibility was that some of the ethanol was being oxidized in the stomach wall to acetic acid which was then diffusing into the mucosal bathing solution. However, acid secretion was also stimulated in both the isolated stomach and mucosa preparations by t-butanol (2% v/v), an alcohol which is resistant to oxidation. This result suggests, firstly, that alcohols may stimulate acid secretion by a non-specific effect on the parietal cell, and, secondly, that the oxidation of ethanol, if it occurs, does not explain the present results. Indeed, analysis of the mucosal solution collected during ethanol stimulation using a nuclear magnetic resonance spectrometer, failed to reveal either acetic acid or dissociated acetate ions. Application of this technique did show the presence of ethanol in the unbuffered mucosal solution, although this does not account for the observed decrease in pH since control experiments showed that the direct application of ethanol (in the concentration range 0·5 to 4% v/v) to this medium caused a slight increase in recorded pH.

At the present time it is difficult to explain the observation that the isolated mucosa preparation, which responded normally to histamine, gave only poor acid responses to ethanol. The stimulation of acid secretion in the whole stomach may indicate that ethanol was releasing a substance with secretagogue activity from the serosal layer of the stomach although the resistance of this response to both atropine and cimetidine certainly does not help to reveal its identity.

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