ACTION OF ETHANOL AND SOME ALCOHOLIC BEVERAGES ON
GASTRIC ACID SECRETION IN ANAESTHETIZED RATS

STEPHAN TEESSSEN, GLORIA GONZÁLEZ-CALERO, ANNELEN KORN
and MANFRED V. SINGER*

Department of Medicine IV (Gastroenterology), University Hospital of Heidelberg at Mannheim, Theodor-Kutzer-Ufer, 68135
Mannheim, Germany

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Abstract — The action of intragastric ethanol in various concentrations equivalent to those found in alcoholic beverages (1.5–40% v/v), ethanol 96% (v/v) and of some commonly ingested alcoholic beverages produced by alcoholic fermentation (beer, wine, champagne, armagnac and rum) or by fermentation plus distillation (e.g. whisky, cognac, calvados, armagnac and rum) on gastric acid output (GAO) was studied in anaesthetized Wistar rats. Ethanol concentrations of 1.5%, 4% and 10% v/v did not significantly alter basal GAO, whereas all higher concentrations of ethanol (20%, 40% and 96% v/v) significantly (P < 0.05) and dose-dependently decreased the GAO. All alcoholic beverages produced by fermentation significantly increased GAO by 30–35% of maximal acid output (MAO; 48 µg/kg pentagastrin intramuscularly), whereas alcoholic beverages produced by fermentation plus distillation significantly decreased GAO as compared to control (isotonic glucose and distilled water). Glucose solutions to which yeast was added, resulting in fermentation, were as potent stimuli of GAO as beer. Lyophilized fermented glucose at different concentrations (dilution of 1:20 to 1:1) dose-dependently stimulated GAO: the highest dilution (1:20) had no effect, the 1:5 dilution significantly increased gastric acid secretion similarly to that of beer and fermented glucose. The highest concentration of lyophilized fermented glucose (1:1) was as potent as the MAO after pentagastrin (90% of MAO). In conclusion, the present investigation shows for the first time that, in rats: (1) ethanol in a concentration >10% v/v inhibits GAO; (2) lower ethanol concentrations (<10% v/v) do not alter GAO; (3) alcoholic beverages produced by fermentation but not those produced by alcoholic fermentation plus distillation are powerful stimulants of GAO; (4) the stimulatory non-alcoholic ingredients of these alcoholic beverages are most likely produced during the process of fermentation of carbohydrates and removed during the following process of distillation.

INTRODUCTION

In humans, the non-stimulatory action of intragastric ethanol and the powerful stimulatory effect of some alcoholic beverages on gastric acid output (GAO) and release of gastrin are well established (McArthur et al., 1982; Singer et al., 1987). In non-alcoholic volunteers, intragastric instillation of 500 ml of 1.4% and 4.0% (v/v) pure ethanol has a small stimulatory effect on GAO with a response of ~23% of the pentagastrin-stimulated incremental GAO [i.e. maximal acid output (MAO)]. Higher concentrations of pure ethanol (up to 40% v/v) have either no effect or a mild inhibitory one (McArthur et al., 1982; Singer et al., 1983a,b; Lenz et al., 1983; Peterson et al., 1986; Singer et al., 1987; Chittenden et al., 1898; see also the review by Singer and Leffmann, 1898; Singer et al., 1991; Chari et al., 1993a,b). By contrast some of the commonly ingested alcoholic beverages are potent stimuli of GAO (Singer et al., 1987). Oral or intragastric instillation of beer causes a stimulation of ~95% of that produced by pentagastrin (i.e. the MAO). Red and white wine increase GAO up to 61% of MAO. Beverages with a high alcohol content, such as whisky and cognac (40% v/v), do not stimulate GAO and release of gastrin (Singer et al., 1987).

The search for the stimulatory substances in beer has shown that the powerful stimulants of GAO are produced during the process of alcoholic fermentation and that they are thermostable and anionic polar substances with a molecular weight of <700 Da (Teysen et al., 1991, 1992, 1993, 1994).
In animals, however, many reports exist about the acute effects of ethanol on the mechanisms of injury on the gastric mucosa, but the acute effect of oral or intragastric ethanol on gastric acid secretion is still unclear and that of alcoholic beverages unknown. In the earlier literature it was reported that ingestion of alcohol stimulates GAO and gastrin in the dog (Elvin, 1969; Woodward et al., 1972; Treffot et al., 1975). Intravenous infusion of ethanol stimulates GAO both in man (Hirschowitz et al., 1956; Köbel et al., 1986) and dog (Kondo and Magee, 1977; Sarles et al., 1977). Earlier studies in rats (Puurunen and Karppanen, 1975; Puurunen, 1978) showed that intragastric application of ethanol had an inhibitory effect on GAO. Most of these earlier studies were uncontrolled and not quantitative for GAO (for a review see Chari et al., 1993a).

The purpose of the present investigation in normal rats was threefold: (1) to determine in detail and systematically the effect of various concentrations of pure ethanol equivalent to those found in alcoholic beverages on GAO; (2) to investigate the action of some alcoholic beverages with a high ethanol content produced by fermentation plus subsequent distillation, such as aperitifs and spirits on the GAO, and to compare their effects with those of alcoholic beverages produced by fermentation only, such as beer, champagne, sherry and other aperitifs; (3) to compare such effects with those of a maximal exogenous stimulus.

**MATERIALS AND METHODS**

**Animals**

Male Wistar rats (Charles River Wiga GmbH, Sulzfeld, Germany) weighing 250–300 g were used (n = 6 in each test group). They were maintained under controlled housing conditions of light (12 h light:12 h dark), relative humidity (65%) and temperature (22–25°C). Feeding was routinely performed with a standardized food (Altromin®, Altromin International, Lage, Germany) ad libitum. Each subject was investigated only once. The sequence of tests was randomized. The research protocol was approved by the University Hospital’s ethics committee.

**Animal preparation**

The rats were anaesthetized with pentobarbital (Nembutal®, Sanofi GmbH, Hannover, Germany; 6 mg/100 g body wt, i.m.). Body temperature was maintained at 38°C by an electric heating pad controlled by a rectal thermistor probe. Spontaneous breathing was facilitated by a cannula inserted into the trachea. When necessary, supplementary doses of pentobarbital were administered during the operation procedure and the subsequent experiment. In no instance did any of the rats show any signs of pain during the operation and the experiment.

The abdomen was opened through a ventral laparotomy and the pylorus was ligated. A soft catheter (diameter 1.5 mm) was inserted into the stomach through an incision in the distal oesophagus and held in place by ligature.

**Plan of experiments**

This is summarized in Fig. 1. The rats were starved for 18 h before each test, but had free access to water. Each experiment was started at 09.00. Before instillation of the first solution through the intraoesophageal implanted intragastric soft catheter at the beginning of the experiment, the stomach was rinsed with water (1 ml) and emptied by aspiration. GAO in response to the various liquid test meals was determined at 30-min intervals. During each experiment, six liquid meals (volume 1 ml each, pH 5.5) were instilled into the stomach at 30-min intervals. After each 30-min period, the remaining gastric contents were emptied and the volume was measured. Thereafter, the next meal was instilled. The first two meals of each study consisted of isotonic glucose (5.76% w/v; C1 and C2 in Fig. 1), which has been shown to inhibit gastric emptying without altering the acid-secretory response (Maxwell et al., 1984). To investigate the kinetics of

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Fig. 1. Schematic representation of the experimental procedure.

One ml volumes of the test solutions were given. C1 to C4 = controls = 1.0 ml of 5.76% (w/v) glucose each. For a detailed description of the protocol, see text.
Table 1. Alcoholic beverages produced by alcoholic fermentation or distillation

<table>
<thead>
<tr>
<th>Alcoholic beverage</th>
<th>Ethanol content (% v/v)</th>
<th>Volume given (ml)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcoholic fermentation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer (Eichbaum Pilsener)</td>
<td>4.9</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Alcoholic free beer (Clausthaler)</td>
<td>–</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>White wine (Zeltinger Riesling, dry)</td>
<td>10.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Champagne (Pommery Drapeau sec)</td>
<td>12.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Martini Bianco (sweet)</td>
<td>15.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Harvey's Bristol Fino Sherry (dry)</td>
<td>16.5</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Fermented glucose (11.5% wt/vol)</td>
<td>7.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td><strong>Distillation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scotch whisky (Ballentines)</td>
<td>43.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Cognac (Rémy Martin)</td>
<td>40.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Calvados Hors d’Age</td>
<td>40.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Armagnac Clés des Ducs</td>
<td>40.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Bacardi Superior Gold Rum</td>
<td>37.5</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Pernod Fils</td>
<td>40.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Cointreau</td>
<td>40.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Campari</td>
<td>25.0</td>
<td>1.0</td>
<td>5.5</td>
</tr>
</tbody>
</table>

GAO in response to the liquid test meals after ending perfusion, the actual test period was followed by two control periods (C3 and C4 in Fig. 1) in which isotonic glucose was administered for 30 min each (data not shown).

The acid concentrations of the six reaspirates of the 30-min intervals were determined by automatic titration (TTT 81 Radiometer Copenhagen, Denmark) of aliquots (1.0 ml each) of the perfusate to pH 7.0 with 0.01 M NaOH, and the GAO was calculated.

**Test meals**

Before instillation of the liquid test meals (2 x 1.0 ml each), the pH of the solution was adjusted to 5.5 by addition of HCl or NaOH in the appropriate amount.

The following test meals (1 ml each) were given intragastrically:

(A) 1.0 ml of pure ethanol at concentrations of 1.5%, 4%, 10%, 20%, 40%, and 96% (v/v).

(B) The following alcoholic beverages were tested (Table 1): German beer (Eichbaum Pilsener), German white wine (Zeltinger Riesling, dry), French champagne (Pommery Drapeau sec), Armagnac Clés Des Ducs, Rum (Bacardi superior Gold), Cointreau, Campari bitter, Calvados Hors D’Age, Pernod Fils, Sherry (Harveys Bristol Fino, dry), Scotch whisky (Ballentines), Cognac (Rémy Martin), and Martini Bianco (sweet).

(C) For a non-alcoholic beverage, an alcohol-free beer (Clausthaler, Binding Brauerei, Frankfurt, Germany) was tested (Table 1).

(D) Three different solutions of glucose were tested: (1) 11.5% w/v; (2) 11.5% w/v (fermented). Fermentation of glucose was used to mimic the effect of alcoholic fermentation. The 11.5% w/v glucose was used for fermentation because it is equal to the concentration of barley extract in finished wort (preproduct of beer) (for more details see Singer et al., 1991). (3) Lyopholized fermented glucose (11.5% w/v) at different concentrations (dilution of 1:20 to 1:1).

(E) Distilled water was given as an isovolumetric control and 5.76% glucose (w/v) was given as an isotonic control solution (Chari et al., 1993a).

**Pentagastrin-stimulated secretion**

To determine the maximal acid output (MAO), a pentagastrin test at different concentrations (6–60 µg/kg, i.m.) was performed. Pentagastrin is the pentapeptide N-tert-butyl-oxy carbonyl-β-alanyl-l-tryptophyl-l-methionyl-l-aspartyl-l-phenylalaninamide. Pentagastrin (6–60 µg/kg, i.m.; Gastrodiagnost®, E. Merk, Darmstadt, Germany) was dissolved in 0.15 M NaCl. Control experiments were performed with the solvent alone. During the 60-min basal period, two meals of isotonic glucose solution (1.0 ml each) were administered intragastrically.
Fig. 2. One-hour incremental gastric acid output (mmol x 10⁻³/h).
Output was in response to different doses of pentagastrin given i.m. Results are means ± SEM of six rats. *P < 0.05 compared with both distilled water and isotonic glucose (5.76% w/v) control solution (see Figs 3-6).

RESULTS

Pentagastrin-stimulated secretion
Apart from the lowest dose (6 μg/kg) of pentagastrin, all higher doses (12–60 μg/kg) dose-dependently increased the GAO above basal and above control (water and isotonic glucose) values. As shown in Fig. 2, the maximal GAO was observed in response to the 48 μg/kg dose of pentagastrin.

The mean basal GAO in response to 1.0 ml isotonic glucose solution (5.8% w/v) was -0.4 ± 0.4 mmol x 10⁻³/h. The mean MAO was 23.5 ± 3.1 mmol x 10⁻³/h, and the mean incremental MAO was 23.1 ± 2.8 mmol x 10⁻³/h (mean ± SEM, n = 6). Intragastric instillation of 1 ml distilled water did not significantly stimulate GAO above basal levels (Fig. 3).

Ethanol
Intragastric instillation of 1.5%, 4% and 10% v/v ethanol did not significantly alter the GAO, whereas all higher concentrations (20%, 40% and

Calculations and statistics
All statistical analyses were performed on incremental response, i.e. on observed gastric acid response minus response to intragastric isotonic glucose. To calculate the 1-h incremental GAO, the value obtained during the second 30-min control period of intragastric glucose administration was multiplied by 2, and this value was subtracted from the observed 1-h gastric acid response to a given stimulus. This 1-h incremental GAO in response to the various test substances was calculated for each experiment and for each subject, and these individual values were used for statistical analysis. MAO was calculated by adding the two highest 30-min outputs produced by intramuscular injection of pentagastrin. To compare the gastric acid response to pentagastrin with that to the different test meals, the incremental MAO, i.e. observed MAO in response to pentagastrin minus GAO during intragastric instillation of isotonic glucose, was calculated.

The differences between the various treatments were evaluated using Wilcoxon's ranked-pair test. P values of <0.05 were considered significant.

Data are reported as means ± SEM unless stated otherwise.
Alcoholic beverages produced by fermentation

All alcoholic beverages produced by fermentation significantly stimulated GAO, compared to control (Fig. 4). Beer, alcohol-free beer, and wine were the most potent stimulants of GAO, causing 32–35% of incremental MAO. The 1-h incremental response to Martini Bianco, champagne and sherry, was 20–25% of incremental MAO. Lyophilized fermented glucose at different concentrations (dilution of 1:20 to 1:1) dose-dependently stimulated GAO (Figs 5 and 6): the 1:5 dilution significantly increased the gastric acid secretion similarly to that of beer and fermented glucose; the highest dilution (1:20) had no effect. The highest concentration of lyophilized fermented glucose (dilution of 1:1) was as potent as the MAO after pentagastrin (90% of MAO).
Alcoholic beverages produced by distillation

All alcoholic beverages produced by fermentation plus distillation significantly decreased GAO as compared with controls (Fig. 4).

DISCUSSION

Our finding that ethanol had an inhibitory effect on GAO confirms earlier studies in rats (Puurunen and Karppanen, 1975; Puurunen, 1978). Puurunen (1978) has shown in rats that ethanol at concentrations of 10% (v/v) or lower decreases the content of hydrogen ions by inhibiting the secretion of acid, not by causing a back-diffusion into the gastric mucosa. Based on the finding that 10.5% ethanol and some other hyperosmotic solutions decreased GAO in rats, others (Sernka and Jackson 1975, 1976) suggested that the inhibition of gastric acid secretion by ethanol is a non-specific hyperosmotic effect. However, in the investigations of Puurunen (1978), hypotonic (0.8%) ethanol caused a transient decrease in the spontaneous output of acid. Isotonic (1.7%) or higher concentrations of ethanol induced a constant inhibition of GAO. At a concentration of 10%, ethanol strongly inhibited GAO. In the presence of 20% ethanol in the gastric lumen, a significant disappearance of added hydrogen ions was observed, whereas 10% ethanol did not cause a loss of acid.

In the present study, ethanol concentrations <20% tended to decrease and higher ethanol concentrations significantly decreased gastric acid secretion in rats. This finding indicates that the decrease of GAO by ≤10% of ethanol is due to the inhibition of the secretory process. Higher concentrations may additionally decrease the gastric acid output also by inducing a back-diffusion of hydrogen ions into the gastric mucosa.

Corresponding studies in dogs have led to controversial results. Thus in some studies an inhibitory effect was observed (Davenport, 1967), whereas other studies have shown a stimulatory effect of ethanol on GAO (Davenport, 1969;
Elvin, 1969; Woodward et al., 1972; Treffot et al., 1975; Eysselein et al., 1984). In humans, however, the effect of ethanol on GAO has been investigated in much more detail. In earlier studies (Singer et al., 1987), we have shown that the action of pure ethanol is related to its concentration; concentrations of 1.4% and 4.0% (v/v) were moderate stimulants, whereas those of 5-40% exerted no effect, or rather an inhibitory effect. One possibility for the discrepancy of the results between humans and rats could be a species difference or differences in methods used for the measurement of GAO. In rats, we measured GAO by titration of aspirates, whereas in humans we measured it by intragastric titration (Fordtran and Walsh, 1973), which permits immediate measurement of GAO after the beverage (e.g. with a higher ethanol concentration) has entered the stomach, perhaps before backdiffusion of hydrogen ions into the gastric mucosa.

Reports on the mechanism by which ethanol decreases the content of hydrogen ions in the gastric lumen are controversial. Davenport (1967) attributed the ethanol-induced decrease of hydrogen ions to a back-diffusion into the gastric mucosa. He observed that ethanol >8% was capable of damaging the gastric mucosal barrier, and that topical ethanol <8% applied to the acid secretion mucosa of the dog directly stimulated secretion. However, other studies have challenged this view (Shanbour et al., 1973; Kuo et al., 1974; Sernka et al., 1974) and claimed that ethanol inhibits the active secretion of acid by the parietal cells due to the hyperosmolarity of the alcohol solution (Sernka and Jackson, 1975) or to the lowering of cyclic AMP and ATP (Puurunen and Karppanen, 1975; Puurunen et al., 1977) and/or increasing the synthesis of prostaglandins, which exert an inhibitory effect on gastric secretion (Puurunen, 1978).

The present study demonstrates that beer, alcohol-free beer, white wine and champagne are potent stimulants of GAO (30-35% of MAO) in rats. This is an extension of our studies in humans (Singer et al., 1987, 1991). In addition, we observed that Martini Bianco and sherry, which are based on wine, are almost as potent as beer and wine themselves (30% of MAO). All these beverages are produced by alcoholic fermentation only. That alcoholic fermentation is the most important event for producing the stimulatory substances in beer is underlined by our earlier experiments with fermented and non-fermented glucose in humans (Singer et al., 1991) and by the present study in rats. Only fermented glucose in a concentration found in barley extract in finished wort (the preproduct of beer before starting of alcoholic fermentation by yeast) caused an increase in GAO similar to that seen after beer. This finding underlines our suggestions in humans (Singer et al., 1991) that the alcoholic fermentation of carbohydrates is responsible for the generation of the stimulatory substances in rats.

Because alcoholic beverages contain a considerable amount of volatile organic substances produced during the fermentation process of carbohydrates, the action of lyophilized fermented glucose at different concentrations was also tested. Lyophilized fermented glucose dose-dependently stimulated the GAO: the highest dilution (1:20) had no effect, whereas the 1:5 dilution stimulated gastric acid secretion similarly to beer and fermented glucose, and the highest concentration of lyophilized fermented glucose (1:1 dilution) tested was as potent as the MAO after pentagastrin (90% of MAO). That the action of the powerful stimulants of fermented lyophilized glucose on gastric acid secretion is related to its concentration in the gastric lumen is surprising. It differs from our observation in humans (Singer et al., 1991). However, it could be an explanation of why we needed an eight-fold higher dose of pentagastrin to stimulate maximally the gastric acid secretion and why the gastric acid response to beer, wine, champagne, sherry and Martini could not exceed one-third of the gastric acid response to the same beverages given to humans.

Alcoholic beverages produced by fermentation plus distillation, such as aperitifs and spirits, had no stimulatory action on GAO. Earlier, we have shown (Singer et al., 1987) that intra-gastric instillation of pure ethanol in concentrations of 5-40% (v/v) did not stimulate GAO in humans. In the present study, it is shown that pure ethanol in concentrations of 1.5-10% (v/v) also did not stimulate, and in concentrations of 20-96% (v/v) inhibited, GAO. Based on these results and since we have shown that distilled beverages behave as an ethanol solution of an equivalent ethanol concentration, it could be theoretically concluded that the presence of ethanol is responsible for the non-stimulatory effect of alcoholic beverages.
produced by distillation on GAO in rats. We favour the hypothesis that, during the distillation process non-alcoholic substances, which have been generated during alcoholic fermentation and which stimulate gastric acid output, are removed. The fact that sherry, beer and wine are strong stimulants of GAO and that their distillates no longer stimulate GAO in humans although the remaining parts (supernatants) of distilled sherry, beer and wine do (partly published in Teyssen et al., 1995) is strong evidence in support of this hypothesis. In addition to the postulated loss in the stimulatory substances during distillation, it is possible that ethanol in concentrations found in distilled beverages will induce and/or support the inhibitory effect, since it is well known that ethanol dose-dependently damages the gastric mucosal barrier at concentrations >10% (v/v) (Davenport, 1967; Takeuchi et al., 1988) resulting in marked changes of net hydrogen ion loss and net sodium ion gain (Stern et al., 1984). The back-diffusion of hydrogen ions could hide a possible stimulatory action of some stimulatory substances inasmuch as the correct determination of secreted hydrogen ions is not possible.

We have previously investigated the stimulatory substances in beer (Singer et al., 1991; Teyssen et al., 1991, 1992, 1993, 1994). None of the known stimulants of GAO present in beer either alone or in combination could be implicated (Singer et al., 1991). Among the various preproducts of beer tested, only those produced after the addition of yeast — that is, after the onset of fermentation — had any capacity to stimulate acid secretion. Fermented glucose was the most potent stimulant (Singer et al., 1991). To identify this metabolic product of yeast, fermentation of glucose extracts obtained from fermented glucose by different extraction methods (e.g. ethylacetate extraction and eluate of anion exchange resin) were tested for their ability to stimulate acid secretion or gastrin release (Teyssen et al., 1991, 1992, 1993, 1994). Preliminary results suggest that the powerful stimulants of GAO are thermostable and anionic polar substances with a molecular weight <700 Da. We speculate that these substances are removed during distillation.

In conclusion, the present investigation shows for the first time that in anaesthetized rats: (1) ethanol in a concentration >10% v/v inhibits GAO; (2) ethanol concentrations <10% v/v do not alter GAO; (3) alcoholic beverages produced by fermentation but not those produced by alcoholic fermentation plus distillation are powerful stimulants of GAO; (4) the stimulatory non-alcoholic constituents of those alcoholic beverages which stimulate GAO are most likely produced during the process of fermentation of carbohydrates and removed during the following process of distillation. Both the inhibitory and, predominantly, the stimulatory, mechanisms of alcoholic beverages on GAO and release of gastrin remain to be further elucidated.

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