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Effects of Antral Ethanol on Gastric Acid Secretion, Potential Difference, and Serum Gastrin (39857)

GEORGE W. BO-LINN AND LINDA L. SHANBOUR[1]

Baylor College of Medicine, and the Department of Physiology, The University of Texas Medical School at Houston, Houston, Texas 77025

Introduction. The actions of ethanol on the stomach have long been of interest to scientists and physicians. It has been assumed that oral or parenteral administration of ethanol stimulates gastric acid secretion in both man and dog (1, 2). Recently, however, there have been reports suggesting that ethanol may not be a stimulant of gastric acid output (3, 4). Cooke (3), studying human volunteers, detected no increase in acid output following ethanol meals in concentrations ranging from 0.8 to 5.6 g%. Rehm and Hokin (5) first observed an inhibitory effect of ethanol on acid secretion using a dog chambered gastric segment. Supporting this finding are studies by Shanbour *et al.* (6), Sernka *et al.* (7), and Kuo *et al.* (8) in which the direct action of ethanol was inhibition of ion transport in the gastric mucosa.

Ethanol presumably stimulates acid secretion in fundic pouches of dogs during irrigation of the antrum via gastrin release (9, 10). Becker *et al.* (11) reported an increase in antral venous serum gastrin concentration to approximately one-third of that observed with 1.0% acetylcholine chloride (12) when 10% ethanol perfused an isolated antral pouch. However, neither the acid secretion from the fundic pouch nor peripheral venous serum gastrin concentration was reported. In the same study (11), 50% ethanol oral ingestion was necessary in human subjects to elicit a slight increase in peripheral venous serum gastrin concentration. Cooke and Turtle (13) determined that in man physiological stimuli released gastrin within 10 min of the start of a test meal but that 16% ethanol did not significantly change the measured serum gastrin levels.

The present studies were designed to permit direct measurement of gastric acid output from a fundic pouch and, simultaneously, the transmural potential difference of the pouch. Using these two parameters discrimination can be made as to whether ethanol perfusing a separate antral pouch causes stimulation, inhibition, or no effect on fundic acid secretion.

Materials and Methods. Mongrel dogs of either sex weighing 14-21 kg were fasted for 24 hr prior to study. All surgical procedures were performed under chloralose-ethyl carbamate (1:10, 0.7 g/kg) anesthesia, which does not affect gastric active transport. A femoral artery was cannulated and connected to a Sanborn pressure transducer for monitoring the blood pressure; the femoral veins were catheterized for infusion of supplemental anesthetic and histamine acid phosphate, and for obtaining peripheral venous samples for gastrin assay. A midline laporatomy was performed and the viscera were exposed. A ligature was placed tightly around the pyloric sphincter to prevent reflux of solutions and two clamps were placed across the antrofundic junction. A cannula was inserted into the antrum via a stab wound in the anterior wall of the antral pouch, thus permitting instillation of solutions directly onto the mucosal surface (Fig. 1A). The antral pouch was then washed with normal saline (0.9% NaCl). A wedge from the greater curvature of the fundus was clamped symmetrically with respect to the gastroepiploic vasculature that branches from the splenic artery. The fundic segment was cut free, exteriorized, and mounted in a Lucite chamber. The chamber was fixed in a suitable position with the blood supply maintained intact for the duration of each experiment. The exposed gastric mucosa, at

[1] Address reprint requests to Dr. Linda L. Shanbour, Department of Physiology, The University of Texas Medical School at Houston, P. O. Box 20708, Texas Medical Center, Houston, Tex. 77025.

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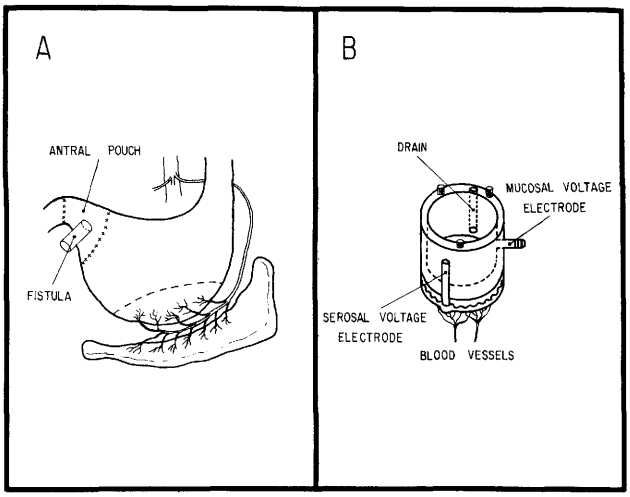

FIG. 1. (A) Antral pouch and fistula preparation (ligatures indicated by X's). Fundic wedge that was mounted in chamber is indicated by dashed line. (B) Chamber assembly for fundic wedge.

least 9.6 cm^2, was rinsed, then bathed with saline for $^1/_2$ hr following the completion of all surgical procedures to reach a steady state before the 30-min control period began. This procedure was followed for all experiments.

The complete chamber assembly is shown in Fig. 1B. The mucosal and serosal voltage electrodes detected the spontaneous transmural potential difference (PD) via saline columns and agar bridges connected to calomel electrodes (Radiometer K 410). The PD was recorded by a voltage-clamp system (14) to the nearest 0.5 mV immediately before each gastric juice sample was taken from the mucosal chamber. The gastric juice within the mucosal chamber was aspirated as completely as possible via the outlet drain at either 15- or 30-min intervals. The volume of each collection was recorded to the nearest 0.1 ml. The concentration of acid was determined by titration of the entire gastric juice sample to an endpoint of pH 7.0 with 0.1 *N* NaOH using a glass electrode and an automatic titrator (Radiometer-Autoburette, Copenhagen, Denmark). Immediately following each collection, 10.0 ml of fresh saline was instilled into the mucosal chamber. The pH of the pouch remained above 5.0 in all experiments except for acidification studies. The serum gastrin was measured according to the radioimmunoassay method of Yalow and Berson (15) using antibody characterized by Dockray and Walsh (16). Human synthetic gastrin I (Imperial Chemical Industries, Ltd.) was used as the standard.

Seven groups of experiments were performed, each group containing four to six dogs. Group 1 consisted of antral pouches continually perfused with saline (0.9%). In Group 2 the antral pouch was perfused with a solution of ethanol (20% w/v) and TES buffer (pH 7.4; composition in millimoles: NaCl 133.0, KCl 5.0, $MgSO_4$ 1.0, $CaCl_2$ 1.0, Na_2HPO_4 1.0, glucose 25.0, and *N*-tris hydroxymethylmethyl - 2 - aminoethanesul - fonic acid 1.0; Calbiochem). Group 3 consisted of a 180-min period during which a submaximal level of histamine (1.2 μg/kg-min) was infused intravenously at a constant flow rate of 0.42 ml/min. The antral pouch was rinsed and remained continually bathed in saline during the experiment. Group 4 was identical to Group 3 except that ethanol and TES buffer (20% w/v) perfused the antral pouch during the last 120 min. Group 5 was conducted to test the responsiveness of the preparation to exogenous gastrin. Pentagastrin (6 μg/kg-hr) was infused intravenously for 120 min while saline perfused the antrum. Group 6 was designed to determine whether acetylcholine chloride (Ach-Cl) would induce endogenous antral gastrin release and thus stimulate fundic acid secretion. Ach-Cl (0.5% w/v) in TES buffer was continually perfused in the antral pouch for 120 min. In these six groups of experiments,

acid output and PD were measured every 30 min. Group 7 was designed to correlate acid output and potential difference with serum gastrin determinations using a postulated gastrin receptor agent, glycine. The antral pouch was perfused with TES buffer for 30 min, followed successively with 10 and 20% ethanol in TES and glycine (pH 7.4) for 30, 30, and 60 min, respectively. During all experiments, the antral pouch was perfused at a rate which would prevent distension. Acid output was measured at 15-min intervals. Potential difference and serum gastrin were determined at 5- and then at 15-min intervals for each perfusion period.

Differences between the control period and test situations for acid output, PD, and serum gastrin levels of the same experimental group were evaluated by the one-tailed Student's paired *t* test (17). Differences between identical periods in different groups were evaluated by the onetailed Student's unpaired *t* test (17). The differences were considered significant if the *P* value was less than 0.05.

Results. The blood pressure remained stable for the duration of the experiment in all dogs used in these studies. Figure 2 illustrates the effects of various conditions on acid output from the fundic pouch. Saline and ethanol perfusion of the antral pouch produced essentially no effect during resting conditions (nonstimulated), although the ethanol group (3.6 ± 0.9 to 4.5 ± 1.3 μEq) was slightly higher than the saline group (2.6 ± 0.5 to 1.9 ± 0.3 μEq) during the course of the experiments. Histamine iv infusion increased acid output from 2.7 ± 0.4 to 107.0 ± 30.8 μEq after 60 min and to 139.6 ± 34.2 μEq after 180 min. In the histamine-plus-ethanol group, histamine preinfusion increased acid output from 5.3 ± 3.0 to 120.2 ± 25.0 μEq. During ethanol perfusion of the antrum, acid output further increased to 210.6 ± 63.4 μEq at 120 min. There were no significant differences during the ethanol perfusion as compared to preinfusion values (0 time) or at comparable time periods when compared with the histamine-only group. Pilot studies with lower (0.6 μg/kg-min) and higher (2.4 μg/kg-min) doses of histamine produced similar results. The dose of histamine selected (1.2 μg/kg-min) has been demonstrated to permit significant potentiation of acid secretion in response to dibutyryl cyclic AMP infusion (18). The preparation was proven responsive to exogenous pentagastrin as demonstrated by the increase in acid output from 7.9 ± 2.2 to 54.4 ± 7.7 μEq. Ach-Cl increased acid output from 5.6 ± 1.4 to 38.4 ± 20.2 μEq during the 120 min.

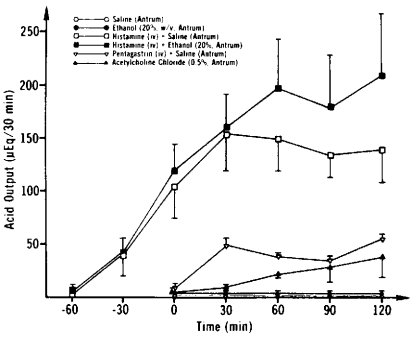

FIG. 2. Acid output (μEq/30 min) with time for the following groups: (1) ○——○, saline perfusion of the antrum for 120 min; (2) ●——●, ethanol perfusion of the antrum for 120 min; (3) □——□, histamine iv infusion (1.2 μg/kg-min) for 180 min and saline antral perfusion for 120 min; (4) ■——■, histamine iv infusion (1.2 μg/kg-min) for 180 min and ethanol (20%) antral perfusion for 120 min; (5) ▽——▽, pentagastrin iv infusion (6 μg/kg-hr) for 120 min and saline antral perfusion for 120 min; and (6) ▲——▲, acetylcholine chloride (0.5%) perfusion of the antrum for 120 min. Means ± SEM for six experiments in Groups 1-4 and four experiments in groups 5 and 6.

Figure 3 illustrates the corresponding PD values for the six groups. Values were essentially unchanged in the saline and ethanol groups. The higher values in the ethanol group reflect slightly higher initial levels. Pilot studies with 5 and 10% ethanol also failed to show changes in PD or acid secretion. Histamine infusion produced the characteristic decrease in PD due to initiation of acid secretion; 62 ± 2 to 39 ± 2 mV in Group 3 and 75 ± 1 to 40 ± 1 mV in Group 4 during the preperfusion period. Ethanol perfusion in Group 4 did not significantly alter the PD during the 120 min and the values were essentially the same as for comparable time periods in Group 3. Pentagastrin also decreased the PD which corresponded to onset of acid secretion, 68 ± 6 to 37 ± 6 mV at 120 min. Ach-Cl produced a slight decrease in PD from a control value of 67 ± 4 to 57 ± 8 mV during the 120 min.

Figure 4 illustrates changes in PD, acid

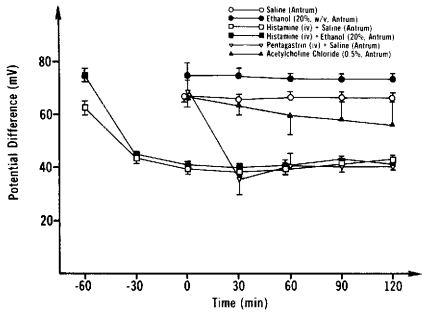

FIG. 3. Potential difference (mV) with time for the same six groups described in Fig. 2.

output, and serum gastrin with successive antral perfusions of TES buffer, 10 and 20% ethanol in TES, and glycine (1 *M*). The PD had a slight tendency to increase with ethanol and decrease with glycine, however, no changes were significant. The acid output, which was measured at 15-min intervals to detect even slight changes, was 7.7 ± 0.8 μEq during the control period (TES buffer). Following 30 min of antral perfusion with 10% ethanol, the acid output was 7.1 ± 0.1 μEq/15 min; with 20% ethanol it was 7.6 ± 0.4 μEq/15 min; and with glycine it was 10 ± 1.5 μEq/15 min. No changes were significant when compared to the control period. Serum gastrin remained essentially unchanged with 10% ethanol, showed a slight tendency to increase with 20% ethanol, and showed a more pronounced increase with glycine. No time periods were significantly different from the control value of 39.7 ± 2.2 pg/ml. When the peak values within each solution period were compared with control, there was no significant difference with 10% ethanol, 20% ethanol produced a significant increase with a peak value of 60.5 ± 5.2 pg/ml, and glycine significantly increased serum gastrin with a peak value of 79.6 ± 13.5 pg/ml. When peak values for PD and acid output were compared with control values, there were no significant changes with any of the solutions.

Discussion. Ethanol, when administered topically to the fundic mucosa, inhibits acid secretion and active transport of ions in the rat (6) and dog (7, 8) stomach. When administered intra-arterially into the fundic portion of the stomach at concentrations as high as 30%, there is no effect (7). However, ethanol may indirectly stimulate acid secretion by inducing gastrin release from the antral mucosa.

Becker *et al.* (11) reported an increase in antral venous gastrin of approximately 200 pg/ml when 10% ethanol perfused the isolated antral pouch. However, no values were reported for either peripheral venous serum gastrin concentrations or acid secretion from the fundic pouch. Jackson *et al.* (12), using a similar preparation, demonstrated that acetylcholine chloride antral pouch perfusion significantly increased antral venous serum gastrin levels by approximately 600 pg/ml, increased peripheral venous serum gastrin levels by 50 pg/ml, and increased gastric acid output from the fundic pouch. However, antral venous serum gastrin concentrations remained elevated, while acid secretion and peripheral venous serum gastrin concentrations returned to basal values. Cooke and Turtle (13) measured increased serum gastrin levels in human subjects in response to physiological stimuli, including glycine, but did not observe an increase in serum gastrin levels following ethanol meals (150 ml of 16% w/v).

The responsiveness of the preparation used in the present studies was evaluated via the pentagastrin, acetylcholine, and glycine experiments. Pentagastrin has been found to be a potent stimulator of acid secretion in the dog (19). Since in this preparation the acid output increased and the PD decreased

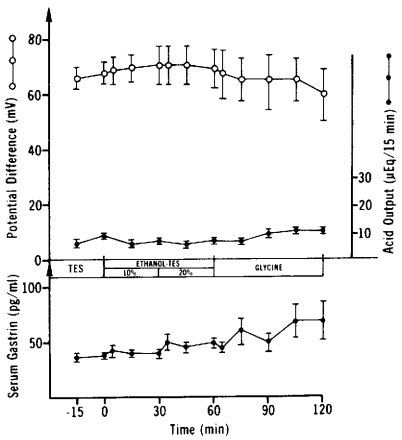

FIG. 4. Potential difference (mV), acid output (μEq/15 min), and serum gastrin (pg/ml) with time during antral perfusion with TES buffer, ethanol in TES buffer (10 and 20%), and glycine at pH 7.4. Means ± SEM for four experiments.

significantly during the pentagastrin iv infusion, the gastric chamber preparation is responsive to changes induced by exogenous gastrin. The results of the acetylcholine and glycine experiments verify the responsiveness of this preparation to endogenous gastrin. Both substances are stimulators of gastrin release and acid secretion (9, 12). Acetylcholine chloride increased acid output and decreased potential difference to a lesser degree than did pentagastrin. Elwin and Uvnäs (20) have postulated that chemical stimuli (meat extracts, peptones, glycine, and ethanol) release gastrin via mucosal receptors in the antral mucosa. Elevated serum gastrin concentrations with corresponding changes in PD and acid output directly measured during antral stimulation with glycine and 20% ethanol demonstrate antral mucosal reactivity in the preparation. However, the maximal increase in serum gastrin with 20% ethanol, 21 pg/ml, or with glycine, 40 pg/ml, was insufficient to significantly increase acid secretion, although there was a tendency toward an increase with glycine.

Ethanol (20%) perfusing the antral pouch for 120 min did not increase acid secretion or alter the potential difference in the resting stomach. In the histamine-stimulated stomach, ethanol also did not significantly change the acid output or potential difference when compared with its own control period or the histamine only group.

Summary. The possible stimulatory, inhibitory, or absent effect of antral perfusion with ethanol on gastric acid secretion and transmucosal potential difference was studied using an *in vivo* chambered fundic segment and isolated antral pouch preparation with intact blood supply. The preparation was proven responsive to exogenous gastrin with pentagastrin iv infusion (6 μg/kg-hr) and to endogenous gastrin with acetylcholine chloride (0.5%) and glycine (1 *M*) perfusion of the antral pouch. Peripheral venous serum gastrin concentration increased in response to antral perfusion with glycine with a slight increase in fundic acid secretion. A small increase in peripheral serum gastrin without a corresponding increase in acid output occurred during antral perfusion with 20% ethanol. No significant differences were found in either potential difference or acid output between saline and ethanol groups, or between histamine and histamine-plus-ethanol groups. These data suggest that ethanol-stimulated antral gastrin release is insufficient to stimulate or potentiate gastric acid secretion during acute conditions in the anesthetized dog.

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