The in Vivo Effect of Ethanol on Gastrointestinal Motility and Gastrointestinal Handling of Calcium in Rats

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Summary The acute effects of an intragastric administration of ethanol (2 g/kg body weight) (given as 13.3% w/v solution) on the in vivo gastrointestinal motility and gastrointestinal absorption and secretion of calcium were investigated in 20-h fasted rats. Gastric ethanol concentration remained high for 90 min while the concentration in the duodenum peaked at 30 min before declining to a range slightly higher than that in the mid- and distal intestine. Plasma ethanol peaked at 60 min. From the polyethylene glycol (PEG) distribution, ethanol was found to delay gastric emptying, and at 60 min, 40% of PEG was still retained in the stomach of the ethanol-treated group while gastric emptying had been completed in the controls. However, ethanol placed directly into the duodenum was found to enhance intestinal motility.

Under control conditions, exogenous calcium was completely absorbed by the time it reached the mid-small intestine (J3) and calcium found in more distal segments was of endogenous origin. Ethanol suppressed calcium absorption while markedly stimulating calcium secretion in the stomach and the distal small intestine, resulting in a net 32% increase in the total gastrointestinal calcium content. This effect on the distal small intestine was from ethanol that had reached this area from the circulation, and not from ethanol transit along the gastrointestinal tract.

Key Words ethanol, calcium absorption, calcium secretion, gastrointestinal motility

Previous in vitro investigations have demonstrated an inhibitory effect of ethanol on net calcium absorption (1, 2). Recently, we have shown using an in situ intestinal loop technique that acute intragastric ethanol administration reduced the net calcium absorption by inhibiting the lumen to plasma calcium flux in the duodenum, while stimulating the plasma to lumen flux of calcium in the ileum (3). Since changes in the rate of intestinal absorption of calcium may have resulted from changes in both the absorption rate per se and the rate of transit along the gastrointestinal tract, experimental results obtained from in vitro or in situ studies
do not necessarily represent the processes occurring in vivo. Especially relevant to this case is the fact that ethanol has been known to delay the gastric emptying rate (4, 5) which would delay intestinal calcium absorption. Thus, the present study aims to demonstrate the time-sequence distribution of ethanol in plasma and along the gastrointestinal tract following intragastric administration and the concomitant change in gastrointestinal motility. The study also demonstrates quantitatively the effect of ethanol on in vivo calcium absorption and secretion along the entire length of the gastrointestinal tract.

MATERIALS AND METHODS

Adult male Wistar rats each weighing between 180-200g, supplied by the Animal Centre, Salaya Campus, Mahidol University, and maintained on commercial rat chow (Gold Coin Ltd., Singapore) were fasted for 20h with access to tap water before the experiment.

Experimental protocol. Under light ether anesthesia, after the stomach was lavaged with 0.9% saline, 3 ml of a test solution was administered via a gastric tube. The test solution was composed of CaCl₂ dissolved in 0.9% saline (10 mg/100 ml) or in ethanol (13.3% w/v, 2 g/kg body weight) and each solution contained polyethylene glycol 4000 (PEG) (1 g/100 ml) and ⁴⁵Ca (Radiocentre, Amersham, UK) (approximately 2 μCi). After a period of 10, 20, 30, 60 or 120 min, the rats were anesthetized again with ether and an abdominal incision was made to expose the gastrointestinal tract. Seven segments including the stomach (S), a 5-cm duodenal segment (D), 4 equal small intestinal segments (J₁-J₄) and the cecum and colon segment (C) were isolated and removed as described by Poulakos and Kent (6). The contents of each segment were centrifuged and the supernatant was analyzed for volume and concentrations of PEG, ethanol, ⁴⁵Ca and total calcium.

In two separate groups of rats, the test solution (3 ml) containing PEG in 0.9% saline (1 g/100 ml) or ethanol (2 g/kg body weight) was instilled directly through a small puncture into the proximal part of the duodenum. Ten minutes later, the intestinal segments were prepared as before and the PEG content in each segment was determined.

To determine the plasma concentration of ethanol, another group of fasted rats was used. After being anesthetized with an intraperitoneal administration of sodium pentobarbital (50 mg/kg body weight), the rats were tracheostomized and the femoral artery was cannulated with polyethylene tubing (No. 50) for blood collection at 10, 20, 30, 60, and 120 min after intragastric administration of ethanol (2 g/kg body weight). The plasma was analyzed for ethanol concentration.

The amount of administered calcium that remained in the lumen of each segment at various time intervals was calculated as follows:

Suppose the specific activity of ⁴⁵Ca in the test solution = x/y cpm ⁴⁵Ca/µg.

At various time intervals after administration of the test solution, in each segment...
let

\[ V = \text{volume of luminal fluid}, \]

and

\[ c = {^{45}\text{Ca concentration (cpm/ml)}}. \]

Thus, the total ${^{45}\text{Ca}}$ content in the segment = \( VC \) cpm. Therefore, \( VC \) cpm \( {^{45}\text{Ca}} \) represents \( VC \cdot \frac{y}{x} \mu\text{g} \) Ca; and \( VC \cdot \frac{y}{x} \mu\text{g} \) Ca represents the amount of administered calcium remaining in the lumen of the segment.

Analyses. The calcium concentration in the luminal contents were measured by atomic absorption spectrophotometry (Varian 575)(7). The radioactivity of \( {^{45}\text{Ca}} \) was determined by the standard liquid scintillation technique (LKB Rackbeta 1219). Determination of the PEG concentration was performed by turbidimetric analysis(8). Ethanol concentration in plasma and the luminal fluid was determined enzymatically (Sigma Diagnostics, Sigma Chemical Co., Ltd., St. Louis, USA)(9).

Statistical analyses. Data were presented as means ± SE. Significance of difference was determined with the paired or unpaired Student's t-test.

RESULTS

Following an intragastric administration of ethanol, the gastric ethanol

![Fig. 1. Ethanol concentrations in plasma and contents from the gastrointestinal segments at various time intervals. Concentrations of ethanol (mg/100 ml) in the plasma, the stomach, duodenum and intestinal segments J1 and J4 of fasted rats (n = 6) which had received an intragastric administration of ethanol (2 g/kg body weight) are presented as means ± SE.](image)
Fig. 2. Distribution of polyethylene glycol in the gastrointestinal segments at various time intervals. Content of polyethylene glycol (% of administered dose) in the stomach (S), duodenum (D), intestinal segments J1–J4 and cecum and colon (C), 10, 20, 30, and 60 min after intragastric administration of test solutions in the control (n=5) and ethanol-treated groups (2 g/kg body weight, n=5). Values are means ± SE.

concentration remained between 6,000 and 3,000 mg/100 ml for 90 min before decreasing to 500 mg/100 ml at 120 min (Fig. 1). In the duodenum, the ethanol level peaked at 30 min (1,500 ± 200 mg/100 ml) and then abruptly dropped to 275 ± 20 mg/100 ml at 60 min. In J1, the concentration of ethanol gradually increased from 17 ± 6 mg/100 ml to peak at 165 ± 12 mg/100 ml at 30 min. After 60 min, the concentration dropped to 57 ± 5 mg/100 ml and 64 ± 11 mg/100 ml at 90 and 120 min, respectively. The ethanol level in segment J4 remained in the range of 40–50 mg/100 ml from 20 min throughout the remainder of the experiment. The plasma ethanol concentration rose from 45 ± 6 mg/100 ml at 10 min to peak at 110 ± 9 mg/100 ml at 60 min; thereafter, it gradually decreased but was still maintained at 73 ± 7 mg/100 ml at 120 min.

The PEG distributions in the gastrointestinal tract at various times after intragastric administration of saline or ethanol are shown in Fig. 2. In saline-administered controls, the leading edge of PEG appeared in segments J3 and J4 at 20 and 60 min, respectively, and the major portion found in the stomach at 10 min (62.3 ± 0.4% administered dose) and 20 min (41.5 ± 0.5%) moved to J3 (52.1 ± 0.7%) and J4 (48.2 ± 0.6%) at 30 and 60 min. In contrast, the leading edge of PEG in the

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Fig. 3. Distribution of polyethylene glycol in gastrointestinal segments 10 min after administration of test solutions. Contents of polyethylene glycol (% of administered dose) in the duodenum (D), and intestinal segments J1–J4 and cecum and colon (C), 10 min after intraduodenal administration of test solutions in the control (□, n=6) and ethanol-treated groups (2 g/kg body weight ■, n=6). ** p<0.01; *** p<0.001, when compared with corresponding controls. Values are means±SE.

ethanol-treated group only reached segment J3 at 60 min and the major portion of PEG was found in the stomach at 10 min (82.5±0.4%) and 20 min (75.7±0.5%) and 30 min (50.2±0.2%). At 60 min there was still 40.7±0.7% of PEG in the stomach with 18.5±0.3% in J2 and 30.0±0.5% in J3. However, when the test solutions were instilled directly into the duodenum, bypassing the stomach, ethanol was found to accelerate the transit time of PEG. As shown in Fig. 3, at 10 min the leading edge of PEG in the ethanol-treated group had reached J3 in contrast to J2 in the control group. The major portion of PEG was found in J2 (71.7±6.9%) in the ethanol-treated group as compared to J1 (62.5±4.0%) in controls.

Figure 4 shows the distribution of calcium along the gastrointestinal tract after intragastric administration of the control test solution. It can be seen that at 10 min there were two peaks of calcium content, one in the stomach (75.3±2.1 µg) and the other in J4 (90.1±10.2 µg) and C (92.3±5.2 µg). This pattern of total calcium distribution did not change much with time, except that the gastric calcium content gradually decreased to 42.4±3.3 µg at 60 min while that in the colon remained high at 102.1±4.4 µg. Figure 4 also shows that the amount of exogenous calcium still remaining in the lumen (as calculated from the luminal 45Ca) in the stomach was high at 10 min (71.2±1.5 µg) before it gradually decreased to 14.2±3.1 µg at 30 min and 2.0±0.5 µg at 60 min. This amount of exogenous calcium was very small or absent in other intestinal segments, except in J3 where 10.0±2.0 µg and 4.1±0.5 µg calcium were present at 30 and 60 min, respectively. These amounts of exogenous calcium in J3 were not due to the calculation from 45Ca which may have been
Fig. 4. Distribution of endogenous and exogenous calcium in the gastrointestinal tract at various time intervals in control rats. The contents (µg) of exogenous calcium (as calculated from the luminal ⁴⁵Ca content, □) and calcium (exogenous + endogenous) along the gastrointestinal tract (■) and the total exogenous calcium (■) and total calcium content (□) at 10, 20, 30, and 60 min after intragastric administration of test solution in the control group (n = 5). Values are means ± SE.

absorbed from the proximal intestine and resecreted into the distal parts of the small intestine, since a ligation between J2 and J3 before administration of test solution abolished it (data not shown). The last two columns on the right (Fig. 4) represent the total amount of exogenous calcium still remaining in the lumen and the total calcium in the entire gastrointestinal tract at various time intervals. In controls, the exogenous calcium gradually decreased with time from approximately 76 µg at 10 min to 8 µg at 60 min, whereas the total calcium content scarcely changed.

Figure 5 shows the effect of ethanol on the absorption of exogenous calcium and the total calcium content in the gastrointestinal tract. The general pattern of total calcium distribution was similar to that of the control group. However, when compared with the control group, there were marked increases in gastric calcium content at 10 min (112.5 ± 2.0 µg, p < 0.05), 20 min (120.5 ± 5.1 µg, p < 0.001), 30 min (122.6 ± 6.6 µg, p < 0.001) and 60 min (118.3 ± 2.4 µg, p < 0.001). The increase in the total calcium content in the distal intestine was also evident from 30 min to 60 min.
Fig. 5. Distribution of endogenous and exogenous calcium in the gastrointestinal tract at various time intervals in ethanol-treated rats. The contents (μg) of exogenous calcium (as calculated from the luminal 45Ca content, □) and calcium (exogenous + endogenous) along the gastrointestinal tract (■■) and the total exogenous calcium (■■) and total calcium content (■■) at 10, 20, 30, and 60 min after intragastric administration of test solution in the ethanol-treated group (2 g/kg body weight, n=5). Values are means ± SE.

Figure 5 also shows that ethanol administration resulted in a significant gastric retention of exogenous calcium from 20 min onwards. In both the control (Fig. 4) and the ethanol-treated group (Fig. 5), the exogenous calcium emptied from the stomach was completely absorbed during its transit along the duodenum and jejunum. However, in the ethanol-treated group, the total amount of unabsorbed exogenous calcium was 5 times that of control at 60 min, while the total calcium content was increased by about 35% and 32% at 30 and 60 min, respectively.

DISCUSSION

In the present study, acute intragastric administration of ethanol was found to markedly delay the gastric emptying of the test solution, which is consistent with previous reports (4, 5). However, the leading edge of the test solution in the ethanol-treated group was found in the same segments as in the control group, or was in
only one segment behind that of controls at 60 min, suggesting that once it reached the small intestine, ethanol seemed to have a stimulatory effect on intestinal motility. This was supported by results from an experiment that showed that when the test solutions were administered directly into the proximal duodenum, both the leading edge and the major portion of PEG, an index of intestinal motility, were found in more distal segments when compared with the control group. There have been only a few studies dealing with the effect of ethanol on intestinal motility. Studies in humans (10, 11) have shown that intestinal motility is increased by both oral and intravenous ethanol. Thus, under in vivo conditions, ethanol suppresses gastric emptying but stimulates intestinal motility.

It has been shown that the absorption of ethanol in the stomach and small intestine correlates with ethanol concentration in the lumen (12), suggesting that absorption is by simple diffusion. The rate of absorption is also influenced by other factors, such as the rate of gastric emptying, ingested food and the enterohepatic circulation of ethanol (13, 14). However, consistent with previous reports (15, 16) the plasma ethanol concentrations did not necessarily reflect the intraluminal concentrations and the peak plasma level usually occurred at a time when the intraluminal level had already started to decline. On the contrary, ethanol concentration in the distal small intestine seemed to correspond to the plasma concentration. The plasma ethanol concentration during the 120-min experimental period was always higher than that in the lumen of segment J4, suggesting that the luminal ethanol recovered in this segment was probably secreted from the ECF. Moreover, by 60 min after intragastric ethanol administration, the PEG distribution study demonstrated that the test solution containing ethanol had not yet reached segment J4. These findings also indicate that the ethanol found in segment J4 or the ileum was from the extracellular fluid rather than from transit along the small intestine. Thus, ethanol may have exerted its effect on the distal small intestine as early as 10–20 min after intragastric administration, even though the bolus had not yet been propelled to that part of the intestine.

Intestinal calcium transport in the present in vivo study was influenced by changes in the rate of gastric emptying, intestinal motility, blood flow distribution and humoral factors, some of which were absent in the in vitro and in situ preparations. Besides, the presence of serosal and muscularis layers as potential barriers to transepithelial movement in vitro did not interfere with in vivo transport processes (17). Under control conditions (Fig. 4) most of the exogenous calcium was absorbed in the proximal and mid-small intestine and the luminal calcium present along this part of intestine was largely represented by endogenous calcium, a large portion of which was of gastric origin. A substantial amount of endogenous calcium was also found in the distal small intestine which is the main site for calcium secretion (18, 19). Since the colon is a site of calcium absorption rather than calcium secretion (20, 21), the endogenous calcium present in the colon probably resulted from a transit from the distal small intestine. Consistent with our previous in situ investigation (3), the present in vivo study demonstrated that ethanol markedly
stimulates gastric calcium secretion and suppresses calcium absorption in the proximal small intestine (Fig. 5). The secretion of calcium in the distal small intestine was also significantly enhanced as early as 20 min by ethanol, which had evidently reached the distal small intestine by circulation. It is not known, however, how ethanol stimulates calcium secretion or whether the action is exerted from the luminal or the serosal side.

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