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Effects of Ethanol on Bicarbonate-Stimulated ATPase, ATP, and Cyclic AMP in Canine Gastric Mucosa[1] (39598)

LARRY L. TAGUE AND LINDA L. SHANBOUR[2]

Department of Physiology, The University of Texas Medical School at Houston, Houston, Texas 77025

Although the stomach is one of the primary sites of action of ingested alcohol, there have been few studies designed to explore the mechanisms by which alcohol affects the gastric mucosa. Inhibition of the active transport of chloride in the rat stomach by topical application of 20% ethanol has been demonstrated (1). Studies conducted on the isolated (2) and intact dog stomach (3) have also demonstrated inhibition of active ion transport and acid secretion. A model for active chloride transport has been described by Kasbekar and Durbin (4) which is operative through a bicarbonate-stimulated ATPase, coupling chloride movement with bicarbonate in a manner similar to ATPase-activated sodium and potassium transport. However, unlike the sodium plus potassium ATPase, chloride is not necessary for maximal bicarbonate ATPase activity (4). The only physiological base which is known to stimulate this ATPase is bicarbonate. Simon and Sachs (5) have suggested that this enzyme may play a role in bicarbonate transport in the pancreas.

To determine whether the ethanol-induced inhibition of chloride transport in the gastric mucosa is mediated by inhibition of the bicarbonate-stimulated ATPase, the effects of ethanol were evaluated on bicarbonate-stimulated ATPase activity, *in vitro* and *in vivo* ATP content, and *in vivo* cAMP content of dog gastric mucosa.

Methods. Magnesium and magnesium plus bicarbonate-stimulated ATPase activities were determined according to Blum *et al.* (6) using crude homogenates of canine gastric mucosa. Gastric segments were resected from dogs fasted for 24 hr and anesthetized with 0.68 g/kg of chloralose-urethane (1:10). The mucosa was separated from the muscular portion of the stomach using glass slides. The mucosal portion was homogenized (1:10) in 0.25 *M* ice-cold sucrose with a ground glass tissue grinder, diluted, and analyzed within 1 hr. Reaction mixtures contained the following final concentrations: 100 m*M* Tris-HEPES (pH 8.2), 3 m*M* $MgCl_2$, 3 m*M* ATP, homogenate equal to 2.0 mg of tissue per ml of reaction mixture, and 20 m*M* $NaHCO_3$ when bicarbonate stimulation was evaluated. The samples were incubated at 37° and the reaction was terminated by pipetting 0.2 ml of the reaction mixture into 0.4 ml of 5% TCA. Denatured protein was removed by centrifugation and the quantity of inorganic phosphate released during the reaction was determined by the method of Buell *et al.* (7).

Effects of ethanol on ATP were evaluated using thin mucosal slices incubated in dog plasma gassed with 95% O_2 and 5% CO_2 at 37° with and without 20% ethanol (v/v). At varying time intervals, the tissues were removed, blotted, frozen in liquid nitrogen, pulverized, and homogenized in 5% TCA. Wet weights remained stable when three mucosal slices were incubated with 20% ethanol in dog plasma for 10, 20, and 90 min and sequentially weighed. ATP was quantitated according to the bioluminescent method of Stanley and Williams (8).

In vivo effects of ethanol on ATP and cAMP contents of dop gastric mucosa (fasted 24 hr) were evaluated using a chambered segment previously described by Mao *et al.* (9). A segment of the fundic gastric mucosa (approximately 36 cm^2) was mounted in a Lucite chamber with blood supply maintained intact. The chamber was divided into experimental and control sides.

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Author to whom reprint requests should be addressed.

After a 30-min exposure to alcohol, freeze-clamped biopsies were taken of the mucosa from each side. These tissues were pulverized in liquid nitrogen, homogenized in 1.0 ml of 5% TCA, and 0.10-ml aliquots were removed for determination of ATP. The homogenates were then purified on cation-exchange columns prepared according to Krishna *et al.* (10), and the cAMP was quantitated by the protein-binding assay described by Gilman (11).

Results. The effects of various concentrations of ethanol on Mg^{2+} and Mg^{2+} HCO_3^- ATPase activities are illustrated in Fig. 1. Both activities were inhibited 50% at 13.7 and 13.2% ethanol (v/v), respectively. Only a slight but insignificant degree of inhibition was observed with concentrations of 10% or less. The activities were inhibited 96% with concentrations of 20% ethanol. Figure 2 illustrates the effects of ethanol on ATP content in the tissue slice incubation studies. Ethanol significantly ($P < 0.05$) reduced ATP levels for all time intervals tested from 10 to 30 min. At 30 min the ATP content, after treatment with ethanol, was 93% less than the initial level (0 min). The ATP content of the control tissue at 30 min showed a 40% reduction compared to its initial value. Results were essentially the same whether expressed as wet tissue weight or dry tissue weight.

In vivo studies confirmed the *in vitro* observations. After a 30-min *in vivo* exposure to 20% ethanol, ATP content was reduced 44% as compared to the saline control side of the chambered mucosal flap (Fig. 3).

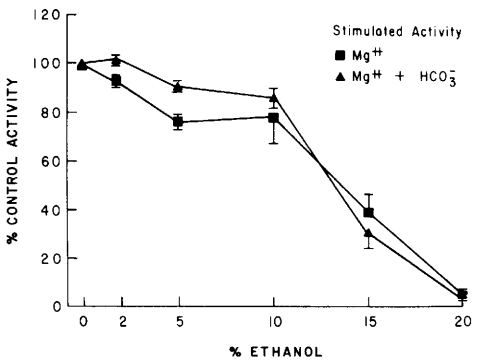

FIG. 1. Effects of various concentrations of ethanol on Mg^{2+}- and $Mg^{2+}HCO_3^-$-stimulated ATPase activities. Means ± SE for three experiments.

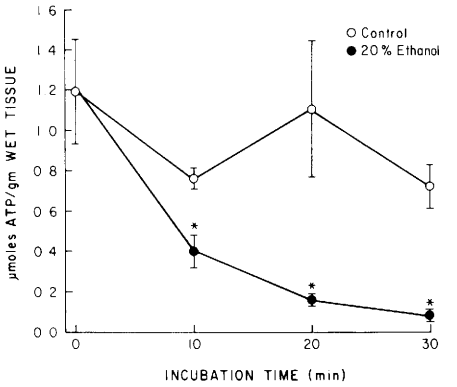

FIG. 2. Time-dependent effects of 20% ethanol on mucosal ATP content in tissue slice incubation studies. Asterisks indicate significant difference of $P < 0.05$ when compared with control at same time interval. Means ± SE for three experiments.

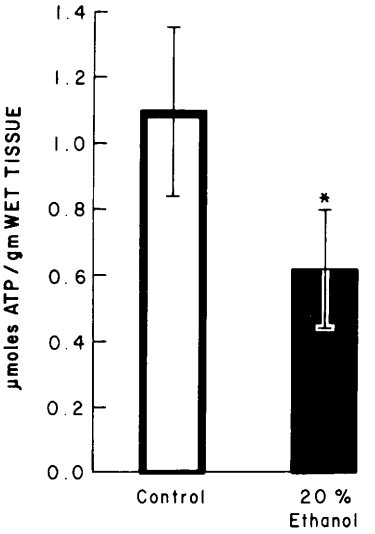

FIG. 3. *In vivo* effects of ethanol on gastric mucosal ATP content. Asterisk indicates significant difference of $P < 0.05$ when compared with control. Means ± SE for six experiments.

However, a 30-min exposure of the chambered flap to 20% ethanol did not significantly decrease cAMP content (Fig. 4).

Discussion. The demonstration of inhibition of magnesium and magnesium plus bicarbonate ATPases in the dog gastric mucosa by ethanol is in agreement with experiments conducted by Israel *et al.* (12), who showed a depression by ethanol of sodium plus potassium ATPase in rat and guinea pig brain. In addition, Tague and Shanbour

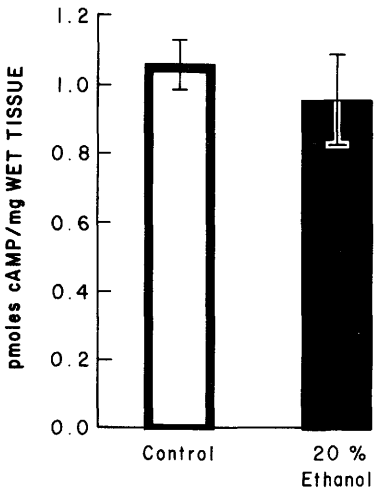

FIG. 4. *In vivo* effects of ethanol on cAMP content in gastric mucosa. Means ± SE for six experiments.

(13) have shown that ethanol inhibits adenylate cyclase, the enzyme which converts ATP to cyclic AMP, and phosphodiesterase, which converts cyclic AMP to 5'-AMP, with no change in cyclic AMP content which is probably due to the greater activity of phosphodiesterase as compared to adenylate cyclase.

The finding that the gastric mucosal content of ATP is decreased both *in vitro* and *in vivo* by 20% ethanol is in agreement with recent studies by Carter and Isselbacher (14). These investigators demonstrated a decrease in small intestinal and liver ATP content 18 hr after administering 7.5 g/kg of ethanol by stomach tube in the rat. They showed that the lowered ATP content was not due to ethanol metabolism by utilizing a blocking agent, 1 m*M* pyrazole. Similar observations have also been reported for the effects of ethanol on rat liver ATP content by Walker and Gordon (15) and Hyams and Isselbacher (16). However, acute studies conducted by French showed an increase in rat liver ATP at 8 hr after ethanol administration which returned to control levels at 24 hr, but a decrease in ATP content occurred after chronic ethanol feeding (17). The decrease in ATP content observed *in vivo* as well as *in vitro* could be an integral part of the mechanism by which ethanol inhibits active transport of H^+, Na^+, and Cl^- in the mammalian gastric mucosa (1–3). Electrophysiological studies have demonstrated that inhibition of active transport precedes permeability alterations which may lead to subsequent mucosal ulceration (1–3).

Inhibition by ethanol of active transport of sodium in frog skin has been demonstrated by Israel and Kalant (18). Mozsik (19) reported inhibition of Na^+K^+-dependent ATPase from human gastric mucosa by cAMP and 5'-AMP. Therefore, if ethanol stimulated adenylate cyclase activity, as reported by Greene *et al.* (20) for rat jejunum, the resultant increase in cAMP or metabolites could produce depressed active transport if ATPase enzymes are involved. However, since previous studies by Tague and Shanbour (13) and Puurunen and Karppanen (21) show inhibition of gastric adenylate cyclase by ethanol, rather than stimulation, and the present studies show no significant change in cAMP content, it is unlikely that ethanol-inhibited active transport of ions (1–3) in the gastric mucosa is mediated by cAMP.

Summary. Previous reports have demonstrated that ethanol inhibits acid secretion and active transport in the gastric mucosa. In the present studies, enzymes which have been implicated in gastric active transport (Mg^{2+} and Mg^{2+} HCO_3^- ATPases), as well as ATP and cAMP, were evaluated in response to ethanol. The activities of Mg^{2+} and $Mg^{2+}HCO_3^-$-stimulated ATPases were not altered at concentrations below 10% ethanol, but were significantly inhibited with 15 and 20%. Tissue slice incubations with 20% ethanol show significantly decreased ATP concentrations at 10, 20, and 30 min. Exposure of the dog gastric mucosa *in vivo* to 20% ethanol produced a significant decrease in ATP content but did not alter the cAMP level. These studies suggest that the ethanol-produced decrease in gastric ATP content may be involved in the inhibition of active ion transport observed with ethanol, but that cyclic AMP is probably not involved.

1. Shanbour, L. L., Miller, J., and Chowdhury, T. K., Amer. J. Dig. Dis. **18,** 311 (1973).
2. Kuo, Y. J., Shanbour, L. L., and Sernka, T. J., Amer. J. Dig. Dis. **19,** 818 (1974).

3. Sernka, T. J., Gilleland, C. W., and Shanbour, L. L., Amer. J. Physiol. **226,** 397 (1974).
4. Kasbekar, D. K., and Durbin, R. P., Biochim. Biophys. Acta **105,** 472 (1965).
5. Simon, R. K., and Sachs, G., Biochim. Biophys. Acta **282,** 293 (1972).
6. Blum, A. L., Shah, G., St. Pierre, T., Helander, H. F., Sung, C. P., Wiebelhaus, V. D., and Sachs, G., Biochim. Biophys. Acta **249,** 101 (1971).
7. Buell, M. V., Lowry, D. H., Roberts, N. R., Chang, M. L. W., and Kapphahn, J. L., J. Biol. Chem. **232,** 979 (1958).
8. Stanley, P. E., and Williams, S. G., Anal. Biochem. **29,** 381 (1969).
9. Mao, C. C., Shanbour, L. L., Hodgins, D. S., and Jacobson, E. D., Gastroenterology **63,** 427 (1972).
10. Krishna, G., Weiss, B., and Brodie, B. B., J. Pharmacol. Exp. Ther. **163,** 379 (1968).
11. Gilman, A. G., Proc. Nat. Acad. Sci. USA **67,** 305 (1970).
12. Israel, Y., Kalant, H., and Laufer, I., Biochem. Pharmacol. **14,** 1803 (1965).
13. Tague, L. L., and Shanbour, L. L., Life Sci. **14,** 1065 (1974).
14. Cartaer, E. A., and Isselbacher, K. J., Proc. Soc. Exp. Biol. Med. **142,** 1171 (1973).
15. Walker, J. E. C., and Gordon, E. R., Biochem. J. **119,** 511 (1970).
16. Hyams, D. E., and Isselbacher, K. J., Nature (London) **204,** 1196 (1964).
17. French, S. W., Proc. Soc. Exp. Biol. Med. **121,** 681 (1966).
18. Israel, Y., and Kalant, H., Nature (London) **200,** 476 (1963).
19. Mozski, G. Y., Eur. J. Pharmacol. **9,** 207 (1970).
20. Greene, H. L., Herman, R. H., and Kraemer, S., J. Lab. Clin. Med. **71,** 336 (1971).
21. Puurunen, J., and Karppanen, H., Life Sci. **16,** 1513 (1975).

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