Pharmacology Biochemistry & Behavior, Vol. 18, Suppl. 1, pp. 337–340, 1983. © Ankho International. Printed in the U.S.A.

Colonic Cyclic AMP Metabolism Following Chronic Ethanol Consumption in the Rat: Effect of Hormonal Secretagoques

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SEITZ, H. K., B. SIMON, P. CZYGAN AND B. KOMMERELL. *Colonic cyclic AMP metabolism following chronic ethanol consumption in the rat: Effect of hormonal secretagoques.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 337–340, 1983.—The colonic cyclic AMP system is known to be involved in intestinal secretion and can be stimulated by a variety of gastrointestinal hormones including prostaglandins. We have investigated the effect of chronic ethanol ingestion on the activity of the key enzymes in cyclic AMP metabolism—adenylate cyclase and cyclic AMP phosphodiesterase—in the colonic mucosa of the rat. Chronic ethanol consumption by feeding a nutritionally adequate liquid diet enhanced basal colonic adenylate cyclase activity significantly by 168% ($p<0.01$), but had no effect on colonic low K_m cyclic AMP phosphodiesterase activity. In addition, various hormonal secretagoques were used to stimulate colonic adenylate cyclase. Colonic adenylate cyclase exhibited a significantly greater sensitivity and efficacy to prostaglandins and vasoactive intestinal peptide after chronic ethanol ingestion. Since increased intestinal cyclic AMP production due to an increased activity of intestinal adenylate cyclase is known to promote intestinal secretion of water and electrolytes, the frequently observed diarrhea in alcoholics may be explained at least in part by an enhanced production of colonic cyclic AMP.

Ethanol Colonic mucosa Adenylate cyclase Prostaglandins Phosphodiesterase Vasoactive intestinal peptide

THE cyclic AMP system is known to be involved in gastrointestinal secretion and certain types of diarrhea are caused by an increase of intracellular mucosal cyclic AMP due to a stimulation of intestinal adenylate cyclase activity [8].

In man, chronic ethanol administration results in enhanced secretion of water and electrolytes in the jejunum [15]. This enhancement of intestinal secretion was thought to be mediated by an increase of jejunal adenylate cyclase activity leading to elevated intracellular cyclic AMP concentrations.

Indeed, such an increase of jejunal cyclic AMP was found in vitro by the addition of ethanol to organ cultures from the rabbit jejunum [25]. In addition, experimental evidence indicates that not only ethanol but also other alcohols can stimulate adenylate cyclase activity in vitro in animals and in humans [10]. However, no data exist on the effect of ethanol on the colonic cyclic AMP system.

Since colonic adenylate cyclase activity can also be stimulated by a variety of gastrointestinal hormones including prostaglandins [22] and since alcoholics exhibit an increased incidence of diarrhea [1], we investigated the effect of chronic ethanol consumption on colonic cyclic AMP metabolism in the rat.

METHOD

Adult male Sprague Dawley rats were pair fed nutritionally adequate liquid diets containing 36% of total calories either as ethanol or isocaloric carbohydrates [13]. Protein (18% of calories) and lipid (35% of calories) contents were thus the same, but the diets had different amounts of carbohydrates (11% in the alcohol fed rats and 46% in the control rats). Ethanol was introduced gradually into the diet reaching the full concentration of 5 g per 100 ml on the 5th day, and the total duration of feeding was 4 weeks.

Thereafter, the animals were sacrificed and the colonic mucosa was scraped from the muscle layer and homogenized in a teflon glass homogenizer (Colora Meßtechnik, Zell Homogenisator, Lorch, Württemberg, F.R.G.) in a medium containing 50 mmol/l Tris-HCl buffer, pH 7.4, 3 mmol/l $MgCl_2$ and 3 mmol/l mercaptoethanol [23]. Adenylate cyclase activity was determined by the method of Salomon *et al.* [19] at 30°C. The assay mixture contained 25 mmol/l Tris-HCl, pH 7.6, 5 mmol/l $MgCl_2$, 20 mmol/l creatinine phosphate, 100 U/ml creatine phosphokinase, 1 mmol/l 3′,5′-cyclic adenosine monophosphate (c-AMP) and 1 mmol/l ATP containing 40–50 cpm/pmol of a ^{32}P ATP. The reaction was initiated by addition of 20 μl of membrane protein and terminated by addition of 0.1 ml stopping solution composed of 2% (w/v) lauryl sulphate, 1 mmol/l c-AMP and 40 mmol/l ATP. c-AMP formation was linear up to 25 minutes using 40–100 μg of protein per assay. Cyclic ^{32}P-c-AMP was purified by column chromatography using Dowex AG 50W-X4 and neutral alumina [16].

Cyclic AMP-phosphodiesterase activity was determined by the method of Pöch [16]. This method is based on the use of radioactive labelled cyclic 3',5'-AMP as substrate and the quantitative removal of the labelled product, 5'-AMP.

After the colonic mucosa was prepared for the adenylate cyclase assay, the homogenate was centrifuged at 20,000 g for 10 min. One hundred μl of supernatant containing 200–400 μg of protein, were added to 200 μl of incubation mixture. The final composition of the incubation mixture (300 μl) was 50 mmol/l Tris-HCl (pH 7.5) 3 mmol/l $MgCl_2$, 1 mmol/l 5'-AMP and 1 μmol/l cyclic 3',5'-AMP (specific activity 70–100 counts/min/pmol). The hydrolysis of cyclic AMP was stopped after 10 min by the addition of 200 μl $ZnSO_4$ (0.17 mol/l). The reaction product, 5'-AMP, was removed by precipitation using 200 μl of $Ba(OH)_2$ (0.15 mol/l).

The protein content of the samples was measured according to Lowry *et al.* [14]. The activity of the adenylate cyclase is given as pmol c-AMP formed per mg protein per 15 minutes. The activity of phosphodiesterase is expressed as the amount of 3',5'c-AMP hydrolyzed per mg protein per minute.

Statistical analysis was performed by the Student's *t*-test for paired samples. Each enzyme determination was done in triplicate. The coefficient of variation averages 4–8%.

A ^{32}P-ATP and ^{3}H-c-AMP were obtained from the Radiochemical Center Amersham Bucks, U.K. VIP (highly purified porcine) was purchased from Calbiochem, Switzerland through Fa. Paesel, Frankfurt/Main, F.R.G. Prostaglandins were kindly given to us by Upjohn GmbH, Heppenheim, F.R.G. All other chemicals and reagents were of the highest grade commercially available.

RESULTS

Basal adenylate cyclase activity in the colon mucosa was increased significantly by 168% after chronic ethanol consumption when compared to controls (Fig. 1), whereas no such effect was observed with respect to basal low K_m c-AMP phosphodiesterase (Fig. 1). Figure 2 illustrates the effect of increasing concentrations of prostaglandin E_2 and prostaglandin I_2 on the colonic adenylate cyclase activity. Both prostaglandins stimulate colonic adenylate cyclase; however, sensitivity and efficacy of colonic adenylate cyclase to both prostaglandins was significantly increased following chronic ethanol administration. A significant stimulation ($p<0.05$) of colonic adenylate cyclase following ethanol administration was observed after the addition of 10^{-3} M prostaglandin I_2 to the incubation system, whereas no such stimulation of colonic adenylate cyclase was found in the control animals ($p>0.05$).

In addition, prostaglandin E_2 in a concentration of 10^{-5} M stimulated colonic adenylate cyclase activity from ethanol fed rats ($p<0.05$), whereas in the control rats a significant stimulation of colonic adenylate cyclase started at the level of 10^{-4} M ($p<0.05$). Maximal response of colonic adenylate cyclase to prostaglandin E_2 was also increased significantly after chronic ethanol feeding (205±35 vs. 142±10% above basal activity).

Figure 3 illustrates the effect of increasing concentrations of vasoactive intestinal peptide (VIP) on colonic adenylate cyclase activity. VIP stimulates colonic adenylate cyclase after ethanol feeding at a concentration of 10^{-7}M ($p<0.05$) and maximal stimulation was reached at 10^{-6} M. However, in ethanol-treated rats, more VIP was needed (10^{-6} M) to stimulate adenylate cyclase activity significantly ($p<0.02$).

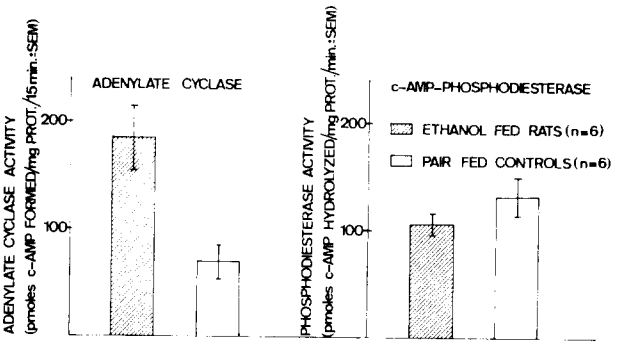

FIG. 1. Effect of chronic ethanol consumption on colonic adenylate cyclase (AC) and cyclic AMP phosphodiesterase (PD) activity in the rat. AC activity was significantly increased after ethanol feeding ($p<0.01$), whereas PD activity was unchanged ($p>0.05$).

VIP is the most potent stimulator of colonic adenylate cyclase when compared to other compounds. Maximal response of adenylate cyclase to VIP was increased significantly after chronic ethanol ingestion (455±137 vs. 232±82% above basal activity).

DISCUSSION

The data presented here show a significant increase in basal and stimulated adenylate cyclase activity of the colonic mucosa following chronic ethanol consumption. On the other hand, colonic basal low K_m cyclic AMP phosphodiesterase activity was not affected by ethanol feeding. Since these two key enzymes of cyclic AMP metabolism are known to control intracellular cyclic AMP levels, a significant increase in intracellular cyclic AMP might be expected in the colon after chronic ethanol ingestion probably leading to an enhanced secretion of water and electrolytes.

Indeed, data exist demonstrating that ethanol administration results in increased cyclic AMP levels in the small intestinal mucosa. It was observed that in vitro addition of 1.52 M ethanol to rabbit jejunal mucosa maintained in organ culture increased cyclic AMP levels more than 3-fold within 15 minutes [25]. Furthermore, in vitro addition of ethanol (0.2–33%) to rat and human jejunum caused a significant enhancement of adenylate cyclase activity in a dose-related manner [10].

Thus, it seems of interest that in man the intake of ethanol (2–10 g/100 ml) for a two week period produced either a net reduction in sodium and water absorption or a net secretion of sodium and water [15].

Our data show an additional effect of chronic ethanol ingestion on the sensitivity and efficacy of colonic adenylate cyclase to gastrointestinal hormones. Ethanol enhances the maximal response of the enzyme to prostaglandin E_2, I_2 and VIP. This is in accordance with the ethanol-mediated augmentation of cholera enterotoxin-stimulated small intestinal adenylate cyclase [25]. Furthermore, colonic adenylate cyclase activity of chronically ethanol fed rats was more sensitive to hormonal secretagoques exhibiting increased stimulation at lower hormone concentrations when compared to control animals.

Prostaglandin I_2, which usually does not stimulate rat small intestinal adenylate cyclase activity [24], also did not

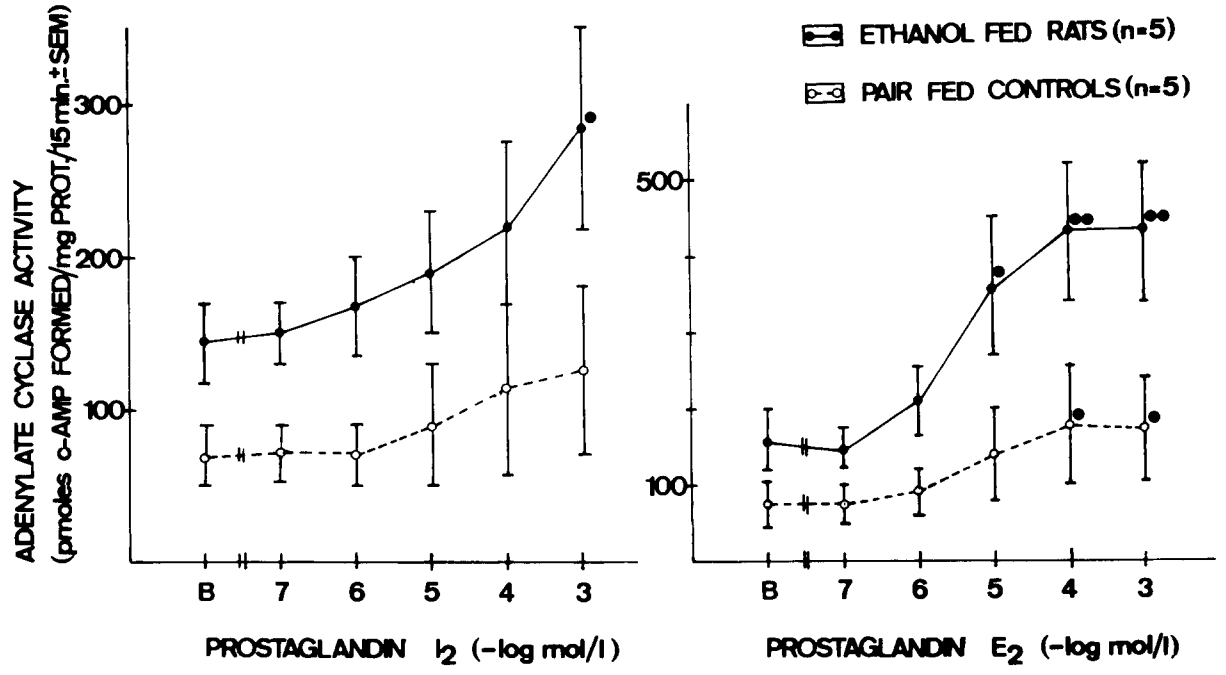

FIG. 2. Stimulation of colonic adenylate cyclase (AC) activity by increasing concentrations of prostaglandins in vitro. Colonic AC activity of chronically ethanol fed rats showed a significantly greater sensitivity and efficacy to prostaglandin I_2 and E_2 when compared to controls. No significant stimulation of AC was observed by prostaglandin I_2 in control animals. Closed circles represent a statistically significant increase in AC activity compared to basal values (B): (●) $p<0.05$; (●●) $p<0.02$.

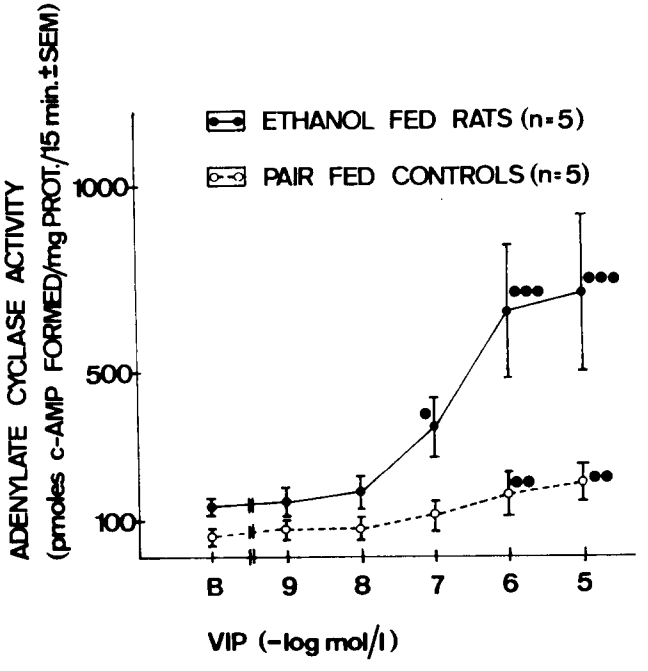

FIG. 3. Stimulation of colonic adenylate cyclase (AC) activity by increasing concentrations of vasoactive intestinal peptide (VIP) in vitro. Colonic AC of chronically ethanol fed rats shows a significantly greater sensitivity and efficacy to VIP when compared to controls. Symbols as in Fig. 2. (●) $p<0.05$; (●●) $p<0.02$; (●●●) $p<0.001$.

enhance colonic adenylate cyclase activity of control rats. However, in ethanol fed rats, prostaglandin I_2 stimulated adenylate cyclase significantly.

The mechanism by which ethanol alters colonic adenylate cyclase activity and its sensitivity and efficacy to gastrointestinal hormones is not clear. One possible explanation may be an ethanol induced change in the availability of the numbers of brush border membrane receptors to specific stimulation.

Changes in receptor availibility have been attributed to changes in receptor position within the membrane when the fluidity of red cell membranes is altered [2]. Ethanol is known to alter membrane fluidity in a variety of tissues [9,20].

It is known that hormone and prostaglandin exposure can result in various affinity states of the receptors, in the uncoupling of the adenylate cyclase system from hormonal receptors and in general refractriness [4]. Plasma membranes of turkey [12] erythrocytes as well as rat cardiac membranes are good examples [5]. When exposed to isoproterenol, these membranes exhibited decreased responsiveness to isoproterenol, glucagon and secretin with no change in the membrane β-receptors. This fully reversible phenomenon is observed relatively shortly after hormone exposure [11].

Since no data exist on the plasma or intestinal concentrations of prostaglandins and VIP after chronic ethanol consumption, it is not known to what extent ethanol-induced changes in hormone levels may influence the responsiveness of colonic adenylate cyclase. In a recent study no changes in prostaglandin E_2 concentrations in the jejunal fluid between chronic alcoholics and control persons could be detected [3].

Subsequently, various bile acids are known as stimulators of colonic adenylate cyclase in vitro [7] and in vivo [6]. We have found an increased fecal bile acid excretion after chronic alcohol administration in the rat [21]. Thus, bile acids may be responsible, at least in part, for the enhancement of colonic adenylate cyclase after alcohol.

Prostaglandins and VIP may play a role in the pathogenesis of certain types of diarrhea in humans [8,18] and ethanol may increase prostaglandin formation as suggested by Puurunen *et al.* [17] for the stomach. In addition, alcoholics exhibit an increased incidence of diarrhea [1] and an enhanced activity and efficacy of colonic adenylate cyclase after chronic ethanol consumption may explain this phenomenon, at least in part, although other causes such as pancreatic insufficiency, maldigestion, lactase defficiency and changes in intestinal motility may also contribute.

ACKNOWLEDGEMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (Se 333-4/1). The authors wish to thank Mrs. S. Veith and Mr. R. Kessler for their expert technical assistance.

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