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EFFECT OF CHRONIC ETHANOL INGESTION ON INTESTINAL METABOLISM AND MUTAGENICITY OF BENZO(α)PYRENE

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Summary: Chronic ethanol ingestion in male rats causes an increase in cytochrome P-450 content and in the activity of microsomal benzo(α)pyrene hydroxylase in the upper intestinal mucosa. Intestinal microsomes from ethanol fed rats also exhibit an enhanced capacity to activate the ubiquitous procarcinogen benzo(α)pyrene to a mutagen. These findings could be of relevance with respect to the increased incidence of cancer in the alcoholic.

INTRODUCTION

Chronic ethanol consumption is known to increase cytochrome P-450 content and microsomal enzyme activity in the liver (1), resulting in an accelerated metabolism of drugs (2), hepatotoxins (3), carcinogens (4) and ethanol itself (2, 5). The effect of chronic ethanol ingestion on microsomal enzyme activities of the intestine, however, has not been investigated. Intestinal mixed function oxidase activity can be influenced by drugs, environmental pollutants and dietary factors (6, 7). Chronic ethanol administration produces ultrastructural changes including proliferation of the smooth endoplasmic reticulum in intestinal epithelial cells both in man and in rats (8). We report here the influence of chronic ethanol ingestion on intestinal microsomal cytochrome P-450 content, benzo(α)pyrene hydroxylase activity and activation of benzo(α)pyrene to a mutagen.

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MATERIALS AND METHODS

Soybean trypsin inhibitor and benzo(α)pyrene were purchased from SIGMA Chemical Co., St. Louis, Mo. Heparin was purchased from Fisher Scientific Co. 3-hydroxybenzo(α)pyrene was kindly supplied by Dr. A. Patel, NIH, Bethesda, Md. Male Sprague Dawley rats (250-400g) were pair fed adequate liquid diets containing 36% of total calories either as ethanol or as isocaloric carbohydrates (9). Following 3-4 weeks of feeding the rats were sacrificed by decapitation after an overnight fast which was established to avoid excess bile flow into the duodenum. Intestinal microsomes were obtained by the method of Stohs et al.(10). An intestinal segment consisting of the first 25cm distal to the pylorus was excised, washed with ice cold Tris HCl buffer (pH 7.8) and the mucosa was removed by scraping with the edge of a glass slide. The mucosa was suspended in the same buffer containing soybean trypsin inhibitor , glycerol and heparin, which decrease degradation and agglutination of the mixed function oxidase system. After homogenization microsomes were isolated from the 10 000 x g supernatant by centrifugation at 105 000 x g for 60 min. The pellet was washed with 0.15M KCl solution. The reduced carbonmonoxide-bound cytochrome P-450 difference spectrum was determined according to Omura and Sato (11). Intestinal microsomal benzo(α)pyrene hydroxylase activity was measured fluorimetrically according to Dehnen et al.(12) using 3-hydroxybenzo(α)pyrene as a standard. Protein was determined by the method of Lowry et al.(13) using bovine serum albumin as a standard. Microsomal activation of benzo(α)pyrene to its mutagenic derivatives was assayed with the Salmonella typhimurium His^- strain TA 100 as described by Ames et al (14), using purified microsomes and an NADPH generating system for metabolic activation (15). In this assay, activation of benzo(α)pyrene to a mutagen results in an increase in the number of His^+ revertants, which are counted as colonies growing on histidine defficient medium. All the mutagenesis assays were performed in duplicate and the colony counts and reversion frequencies presented are averages of replicate plates. The variation in colony counts on replicate plates was within 10% of the total number of colonies counted. Within individual experiments the standard deviation of the observed spontaneous mutation frequency in the absence of added mutagen was ± 10%.

RESULTS AND DISCUSSION

A 3-fold increase in intestinal microsomal cytochrome P-450 content was observed in the ethanol fed rats compared to their pair fed controls (26.0 ± 4.6 vs. 8.9 ± 2.1 pmoles/mg microsomal protein, Fig. 1). This increase in microsomal cytochrome P-450 was evident whether related to microsomal protein, length of bowel or total protein. Intestinal benzo(α)pyrene hydroxylase activity was also increased 3-fold in the ethanol fed rats compared to their pair fed controls (18.1 ± 3.2 vs. 6.6 ± 1.0 pmoles 3-hydroxybenzo(α)pyrene/mg microsomal protein/min, Fig. 1). When benzo(α)pyrene was tested for mutagenic activity in the presence of microsomes

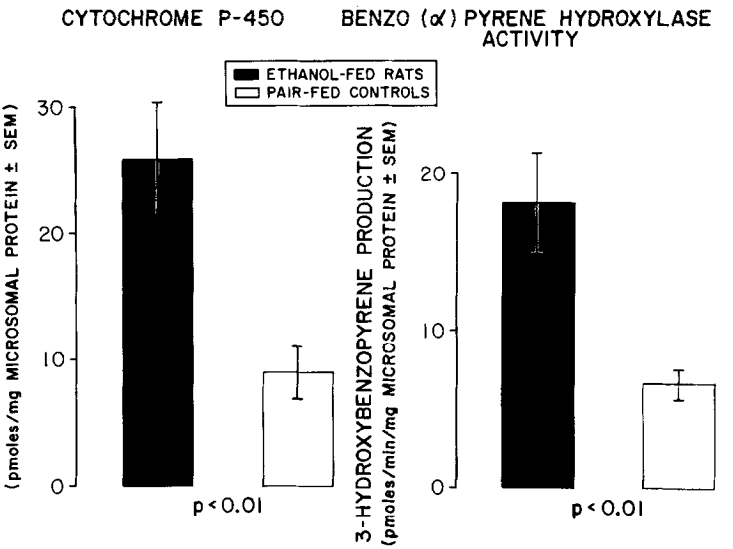

Fig. 1 Microsomal cytochrome P-450 content and benzo (α) pyrene hydroxylase activity in the mucosa of the proximal small intestine after 25 days of ethanol administration in 7 pairs of rats.

from ethanol fed and control animals, clear dose response curves were observed (Fig. 2). Over the entire range of benzo(α)pyrene concentrations studied, the levels of revertants were highest with microsomes of ethanol fed animals. With 10ug benzo(α)-pyrene per plate (an amount saturating for the levels of microsomal protein used) the observed differences in the levels of revertants when analyzed by Student's paired t-test were statistically significant ($p<0.01$). Intestinal cytochrome P-450 levels measured in chow fed animals were similar to those in rats fed the control diet. A depression of cytochrome P-450 by the control diet could therefore be ruled out. Intestinal cytochrome P-450 levels in control animals were comparable to those found by Stohs et al. (10) and by Correia and Schmid (16), but the activity of benzo(α)pyrene hydroxylase was somewhat lower. This could be explained by the absence of cholesterol in our diet. Indeed, it has been shown recently by Hietanen et al. (17) that intestinal benzo(α)pyrene hydroxylase activity is significantly enhanced by adding cholesterol to the diet.

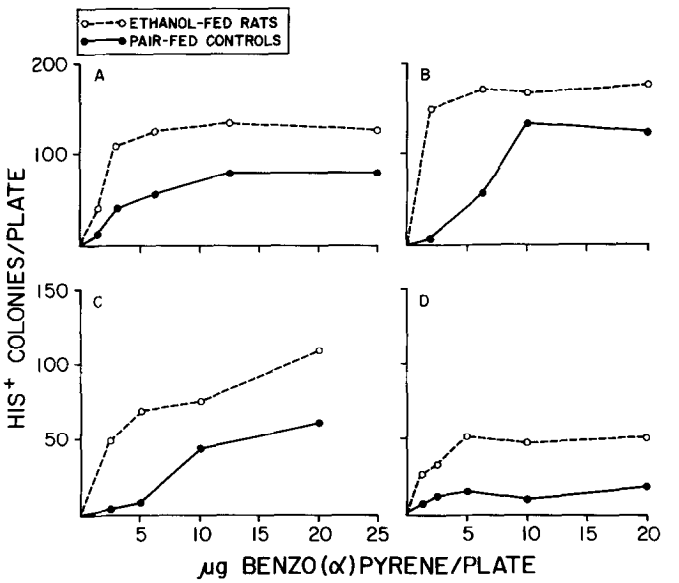

Fig. 2 Metabolic activation of benzo (α) pyrene to a mutagen by intestinal microsomes isolated from ethanol fed and controls rats. Each point is an average of the number of His^+ colonies of S. typhimurium strain TA 100 present on duplicate plates. The levels of spontaneous His^+ revertants have been subtracted. In each case the microsomes were from pools of 3 animals pair fed either the ethanol containing or the control diet. The microsomal protein concentrations per plate were as follows: A 0.5 mg; B 0.5 mg; C 1.0 mg; D 1.4 mg.

Although the liver is the principal organ of xenobiotic metabolism, intestinal metabolism of ingested drugs or other chemicals is also important. While the metabolic capacity of the intestine is relatively small compared to the liver (18), this metabolism could influence both the absorption and biological activity of xenobiotic compounds. In addition such compounds are present in the gastrointestinal tract in great variety and in relatively high concentrations. In contrast to the liver where chronic ethanol ingestion increases microsomal cytochrome P-450 only by 40% (4), we found a 200% increase in intestinal cytochrome P-450 in ethanol fed rats compared to their controls. A variety of inducers of the mixed function oxidase system has been shown to increase the intestinal metabolism of benzo(α)pyrene (6, 7). We now find ethanol to have a similar effect. Furthermore, among all these inducers ethanol may be considered of particular importance because

of its widespread use. It is noteworthy that an intake of alcohol of 36% of total calories, which leads to ethanol blood concentrations of 63.7 ± 12.6 mg per 100ml (19) (levels comparable with those of so called social drinkers), produces a significant increase of intestinal xenobiotic metabolism.

Several studies have indicated that the microsomal cytochrome P-450 dependent biotransformation system is involved in chemical carcinogenesis and that the metabolic activation of many procarcinogens can be correlated with microsomal cytochrome P-450 levels (20, 21, 22). Chronic ethanol consumption in man is associated with an increased incidence of oropharyngeal, esophageal, gastrointestinal, pancreatic and liver malignancies (23, 24, 25). The results presented here suggest that this increased incidence of cancer seen among alcoholics may be due, at least in part, to the enhanced capacity of these individuals to activate procarcinogens in the intestine.

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