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Ethanol-induced changes in membrane ATPases: inhibition by iron chelation

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Abstract—The effect of chronic ethanol intake, with and without an iron chelator, on the activity of rat membrane ATPases was investigated. Using the intragastric feeding model, male Wistar rats (250 g) were fed a liquid diet and ethanol for 1 month. In control pair-fed animals, ethanol was isocalorically replaced by dextrose. In addition to the above groups, two groups of animals (dextrose or ethanol-fed) also received an oral iron chelator (1,2-dimethyl-3-hydroxypyrid-4-one, L_1) (25 mg/kg/day for 30 days). The blood ethanol levels were maintained between 150 and 300 mg/dL. Red cells were washed immediately with ice-cold saline, membranes were prepared, and ATPases were measured. The mean Ca^{2+} pump ATPase in animals fed ethanol was lower than in dextrose-fed controls. In contrast, Na^+/K^+ pump ATPase was enhanced following chronic ethanol treatment. The addition of L_1 to the diet prevented the changes in both the Ca^{2+}-ATPase and Na^+/K^+-ATPase in ethanol-fed rats. Although the exact mechanism for the prevention of changes in ATPase activity by L_1 is unknown, it is not a result of non-specific interaction between the chelator and membranes. Incubation of purified membranes with different concentrations of L_1 for 60 min at 37° had no effect on the activity of the ATPase. In conclusion, chronic intake of ethanol specifically inhibited Ca^{2+} pump ATPase and enhanced Na^+/K^+-ATPase in rat red blood cell membranes. The iron chelator, L_1, corrected both of these ethanol-induced changes.

Key words: iron chelator; plasma membrane; ATPase; ethanol

Chronic intake of ethanol is associated with several inflammatory and degenerative disorders that affect many organs [1]. Although the exact mechanisms of action of ethanol in different systems are still unknown, the generalized pathological effects may be due to its deleterious effects on plasma membrane and cellular transport systems [2]. One group of transport systems that may be affected include membrane ATPases.

Chronic exposure to ethanol has been shown to result in increase in Na^+/K^+-ATPase activity [3]. Acute exposure to ethanol has been shown to inhibit Na^+/K^+- and Ca^{2+}/Mg^{2+}-ATPase activity, while the effects of chronic intake of ethanol on the activity of Ca^{2+}-ATPase are variable in different tissues [4, 5]. Mobilization of iron has been proposed as one mechanism responsible for alcohol-induced tissue damage [6]. Since free iron and iron-containing compounds can act as catalysts in the formation of lipid peroxides [7] and since it is recognized that lipid peroxides can affect ATPases in cell membranes [8], we also examined the effect of an oral iron chelator, 1,2-dimethyl-3-hydroxypyrid-4-one (L_1) [9] on membrane ATPases. L_1, a member of the 3-hydroxypyridin-4-one family of identate iron chelators, is a neutral molecule that forms a 3:1 chelator–iron complex [9] at pH 7.4. L_1 has been shown to be an effective chelator in patients with myelodysplasia and thalassemia [10, 11].

The present study was designed to evaluate: (a) the effect of chronic ethanol intake on erythrocyte membrane-bound ATPases, and (b) the effect of L_1 on ethanol-mediated changes in the cellular transport systems.

Materials and Methods

Male Wistar rats, weighing 225–250 g, were fed by continuous infusion of a liquid diet through permanently implanted gastric cannulas, as described previously [12]. The diet contained corn oil as the source of fat (25% of calories). The amount of ethanol fed was initially 8 g/kg/day and increased up to 17 g/kg/day as tolerance developed. Blood alcohol levels were maintained at between 150 and 300 mg/dL. L_1 (25 mg/kg/day) was added to the diets of both ethanol-fed and control groups. The animals were killed 1 month after the start of feeding. For each group, the pair-fed dextrose–corn oil controls were killed at the same time.

For ATPase determinations, blood was collected from the aorta into heparinized tubes and centrifuged immediately at 4° to separate the red blood cells (RBC). RBC membranes were prepared according to the method of Farrance and Vincenzi [13]. Na^+/K^+-ATPase and Ca^{2+}-ATPase were measured simultaneously using a microtiter plate assay [14].

Results and Discussion

Figure 1A shows the effect of chronic ethanol intake on erythrocyte membrane Na^+/K^+-ATPase activity. Chronic ethanol consumption resulted in an increase in the activity of Na^+/K^+-ATPase. Our results are consistent with those of other investigators who observed a similar effect of ethanol [15]. The exact mechanism(s) by which ethanol enhances Na^+/K^+-ATPase activity is unknown. However, several hypotheses, including an increase in the number of the pumps [16], "improper processing of the pump" [15], and changes in membrane fluidity [17], have been proposed. Israel *et al.* [18, 19] originally postulated that the increase in Na^+/K^+-ATPase in the liver after ethanol administration was due to increased oxidative phosphorylation and oxygen consumption to supply ATP for ATPase activity. Studies by Johnson and Crider [3] suggest that the increased enzyme activity in treated cells is due to the enzyme being less efficient in coupling ion pumping to ATP hydrolysis than the enzyme in normal cells [3].

The importance of Ca^{2+} in cellular physiology and organ injury is well established [20]. For this reason, we chose to investigate the effect of chronic ethanol consumption on the Ca^{2+} pump ATPase. As shown in Fig. 1B, feeding ethanol for 1 month decreased the activity of Ca^{2+} pump ATPase. This inhibition of Ca^{2+}-ATPase may account for the accumulation of intracellular calcium observed in tissues of ethanol-fed rats [4].

Chronic ethanol intake has also been shown to result in accumulation and mobilization of iron [21]. This catalytic free iron can catalyze the formation of reactive oxygen

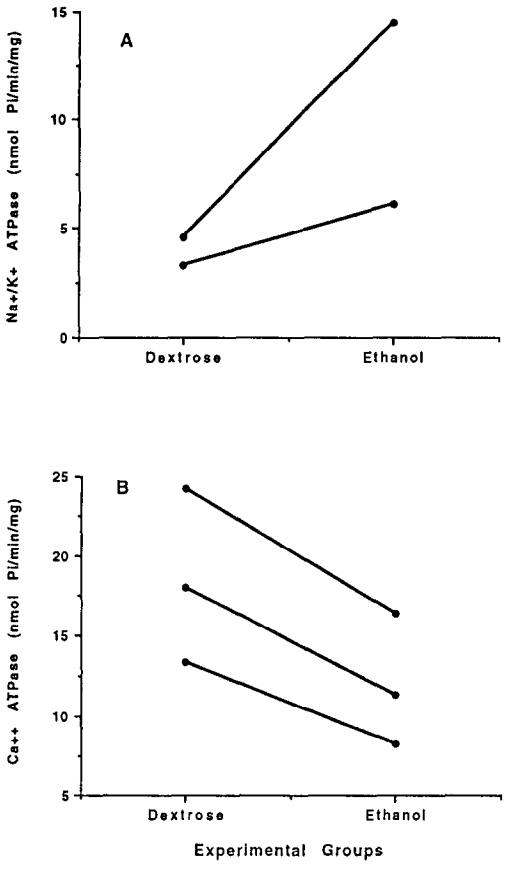

Fig. 1. Effect of chronic ethanol feeding on red cell membrane Na^+/K^+-ATPase (A) activity and Ca^{2+}-ATPase (B) activity. Animals were fed ethanol for 30 days. Each point represents a single animal.

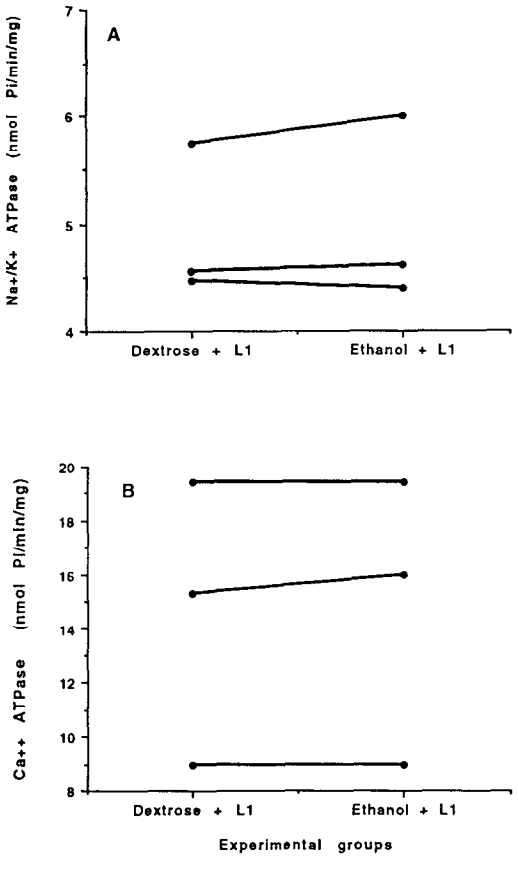

Fig. 2. Effect of L_1 treatment on ethanol-induced changes in Na^+/K^+-ATPase (A) and Ca^{2+}-ATPase (B) activities. The drug was given over a 1-month period of feeding. Each point represents a single animal.

species that are hypothesized to be important in the pathogenesis of alcohol-induced tissue damage [6]. Whether iron is involved in ethanol-induced changes in the ATPase activity is unknown. In other experimental systems, there is evidence that non-heme iron promotes lipid peroxidation and leads to inhibition of Ca^{2+}-ATPase activity [22]. Our results show that when animals were treated with a powerful iron chelator, L_1, the effect of ethanol on erythrocyte membrane ATPases was prevented (Fig. 2). This result is consistent with the hypothesis that iron chelation leads to decreased lipid peroxidation and the prevention of inhibition of Ca^{2+}-ATPase activity. It is important to note that the effect of L_1 on membrane-bound ATPases is not a non-specific effect, since incubation of membranes with different concentrations of L_1, for 60 min at 37°, had no effect on the activities of the ATPases (Fig. 3). The fact that treatment with L_1 prevented the effect of ethanol indicates that iron is involved in ethanol-induced changes in membrane ATPases. However, a non-iron-mediated effect of L_1 cannot be ruled out completely.

In conclusion, our results indicate that chronic ethanol intake results in an increase in the activity of Na^+/K^+-ATPase and a decrease in the activity of Ca^{2+}-ATPase in rat erythrocyte membranes. L_1, an oral iron chelator, prevented this effect of ethanol. Further studies are needed to understand the mechanisms of action by which iron affects cell membrane ATPases in ethanol-fed rats.

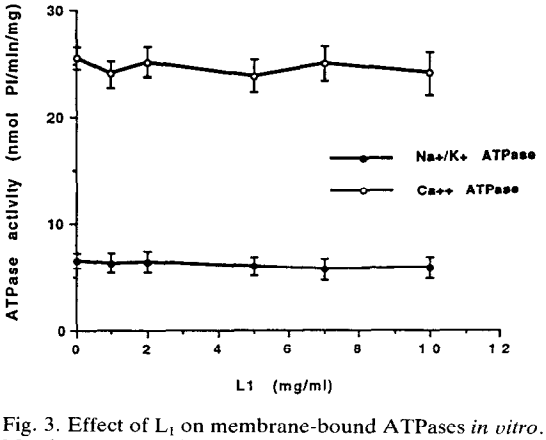

Fig. 3. Effect of L_1 on membrane-bound ATPases *in vitro*. Membranes were incubated with L_1 at 37° for 60 min. Each point represents the mean ± SD of five separate experiments.

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