CHANGES IN LIVER INORGANIC PYROPHOSPHATE CONTENT DURING ETHANOL METABOLISM

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ABSTRACT

The injection of ethanol (70 mmol/kg body weight) into male Wistar rats fed ad libitum caused an increase within 15 min in the liver inorganic pyrophosphate (PPi) content from $0.012 \pm 0.001 \mu\text{mol/g}$ (wet weight) to $0.029 \pm 0.001 \mu\text{mol/g}$. The injection of acetate (20 mmol/kg body weight) increased the liver PPi to $0.157 \pm 0.075 \mu\text{mol/g}$ in 15 min. The alcohol dehydrogenase inhibitor 4-methylpyrazole blocked the accumulation of acetate and the increase in PPi was prevented. Disulfiram, an inhibitor of aldehyde dehydrogenase, did not prevent changes in redox state, but the accumulation of acetate in the liver was decreased and the increase in liver PPi content was diminished ($0.019 \pm 0.003 \mu\text{mol/g}$). These data suggest that the increase in PPi observed after injection of ethanol may be due to the activation of the acetate produced during ethanol and acetaldehyde oxidation.

INTRODUCTION

The oxidation of ethanol to acetaldehyde by alcohol dehydrogenase and the further oxidation of acetaldehyde to acetate by aldehyde dehydrogenase is followed to some extent in liver by conversion of acetate to acetyl-CoA by acetyl-CoA synthetase.
The latter reaction involves the cleavage of ATP to produce AMP and inorganic pyrophosphate (PP$_i$). According to the commonly held view (Kornberg, 1962; White et al., 1973; Lehninger, 1975; Metzler, 1977) the PP$_i$ should be rapidly hydrolyzed by an inorganic pyrophosphatase. Calculation of the cytosolic concentration of PP$_i$, however, has revealed that it was three orders of magnitude higher than predicted from the pyrophosphatase equilibrium (Guynn et al., 1974). Furthermore, the total liver content of PP$_i$ is 13 nmol/g (wet weight), another order of magnitude higher than the value calculated for free cytosolic PP$_i$ (Cook et al., 1978). The present study was initiated to determine whether the production of PP$_i$ during ethanol metabolism by the liver could produce changes in the total liver content of PP$_i$.

MATERIALS AND METHODS

Animals used in these experiments were male, Wistar rats weighing 180 to 220 grams purchased from Charles River Laboratories (Wilmington, Massachusetts). They received food (NIH stock diet) and water ad libitum. Stomach contents were checked after killing animals to be certain they were well fed.

Disulfiram was purchased from Sigma Chemical Company (St. Louis, Missouri). Sodium acetate was obtained from J.T. Baker Chemical Co. (Phillipsburg, New Jersey) and 4-methylpyrazole was from Polysciences, Inc. (Warrington, Pennsylvania). Pyrophosphate:fructose 6-phosphate 1-phosphotransferase was a gift of Dr. W.E. O'Brien, Baylor College of Medicine, Houston, Texas. Other enzymes were purchased from Boehringer-Mannheim (Indianapolis, Indiana).

Rats used in ethanol experiments were injected intraperitoneally with ethanol (either 10 mmol/kg or 70 mmol/kg body weight) in saline 15 min before killing. Control animals were injected with saline. In some experiments 4-methylpyrazole was injected (11 mmol/kg body weight) intraperitoneally 60 min before ethanol administration. In other experiments disulfiram (2 mmol/kg body weight) was given orally 24 hrs before ethanol injection. Animals injected with acetate (20 mmol/kg body weight, i.p.) were killed 15 min after injection.