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BINDING OF ACETALDEHYDE TO RAT LIVER MICROSOMES: ENHANCEMENT AFTER CHRONIC ALCOHOL CONSUMPTION

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SUMMARY

Rat hepatic microsomal preparations were assessed for their capacity to bind exogenous ^{14}C-acetaldehyde and ^{14}C-acetaldehyde formed endogenously from ^{14}C-ethanol in the microsomal ethanol-oxidizing system (MEOS). Exogenous acetaldehyde was found to bind to rat liver microsomes. Blocking of the free amino groups and thiol groups with site specific reagents (pyridoxal 5'-phosphate and p-hydroxymercuribenzoate) reduced the binding of acetaldehyde. Acetaldehyde formed endogenously from ethanol also bound to hepatic microsomes. This was increased after chronic alcohol consumption, in association with enhanced MEOS activity. The binding of acetaldehyde produced by MEOS was significantly greater than that of an equivalent amount of exogenous acetaldehyde. Thus, acetaldehyde produced in situ may exert local toxic effects on the endoplasmic reticulum.

INTRODUCTION

Acetaldehyde is the first toxic metabolite of ethanol and a number of studies have documented its significant involvement in the toxic effects of ethanol (1-3). It has also been shown that binding of reactive metabolites to liver macromolecules is related to liver injury (4,5). To test whether acetaldehyde, a reactive metabolite of ethanol, has the potential to play such a role in alcoholic liver injury, rat hepatic microsomal preparations were assessed for their capacity to bind low concentration of exogenous ^{14}C-acetaldehyde and ^{14}C-acetaldehyde formed endogenously from ^{14}C-ethanol by the microsomal ethanol-oxidizing system (MEOS).

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MATERIALS AND METHODS

[1,2-^{14}C] Acetaldehyde (5 or 10mCi/mmol), [1-^{14}C] ethanol (7.9mCi/mmol) were obtained from New England Nuclear Corp. As suggested (6), radioactive acetaldehyde was diluted to 1mCi/ml by the manufacturer to avoid the polymerization to paraldehyde. Male Sprague-Dawley rats (100g-250g body weight) were purchased from Charles River Breeding Laboratories, North Wilmington, MA. These experiments were conducted in three groups of animals: animals fed Purina Laboratory chow and tap water ad lib, animals maintained on a liquid diet containing ethanol as 36% of caloric intake and their pair-fed controls (7). Hepatic microsomes were prepared as previously described (8). In 2.0ml of reaction mixture containing 0.1M potassium phosphate buffer (pH 7.4), microsomes (3mg protein/ml) prepared from each group of animals were incubated with 200μM of [1, 2-^{14}C] acetaldehyde (5 or 10mCi/mmol) at 37°C for 60 min. The binding of acetaldehyde, produced by MEOS, was assessed in hepatic microsomes (3mg protein/ml) prepared from pair-fed animals in the presence of [1-^{14}C] ethanol (0.5mCi/mmol). The incubation medium (final volume 2.0ml) contained 1mM sodium azide, 1mM Na_2-EDTA, 6mM $MgCl_2$ and 50mM of the ratioactive ethanol in 0.1M potassium phosphate buffer. The reaction was started by adding NADPH (final concentration 1mM) and carried out for 60 min. Tubes without NADPH were incubated as blanks. The total amount of acetaldehyde produced during 60 min was measured and another aliquot of the same microsomes was incubated with an equivalent amount of exogenous ^{14}C-acetaldehyde in the reaction mixture described above. All incubations were carried out in 25ml closed glass bottles designed for use in a Perkin Elmer gas liquid chromatograph. After incubation, unbound acetaldehyde was removed by either dialysis as described by Scheulen et al. (9) with slight modification or organic solvent extractions (10). In additional experiments, after dialysis, microsomes (about 1mg protein) solubilized by sodium dodecyl sulphate (1%/W/V) were applied to a Sephadex G-25 gel column (1x9cm) equilibrated with 50mM sodium phosphate buffer (pH 7.4) and then eluted by the same buffer. The protein amount and the radioactivity of the column eluate were determined. In the reaction systems described above (but without ethanol), the disappearance of acetaldehyde (200μM) was followed by measuring the acetaldehyde concentration of aliquots obtained during 60 min. Microsomes (about 20mg protein) prepared from chow-fed animals were incubated with 4μmoles of p-hydroxymercuribenzoate (PHMB) in 50mM sodium phosphate buffer (pH 7.4) at 37°C for 60 min. The mixture was dialyzed against the buffer to remove the excess PHMB. As a control, another aliquot of the same microsomes was incubated without PHMB and dialyzed. The effect of this treatment was assessed by measuring total thiol groups of the microsomes according to Boyne and Ellman (11). Binding of exogenous acetaldehyde to the treated microsomes and the control was assessed as described above. When indicated, pyridoxal 5'-phosphate (PLP) was added to the incubation mixture of a final concentration of 40mM together with acetaldehyde and incubations were carried out in the dark. Direct interaction between acetaldehyde and PLP was assessed by following the concentration of acetaldehyde during the incubation. The activity of the microsomal ethanol-oxidizing system was measured as described elsewhere (12) using 0.5mg microsomal protein. The concentration of acetaldehyde was measured with a Perkin-Elmer F-40 gas liquid chromatograph as previously described (13). Protein was determined according to Lowry et al. (14). Radioactivity was assessed in a Beckman LS-250 liquid scintillation counter using Aquasol scintillation fluid and radioactivity was corrected for background and for quenching (external standardization). The statistical significance of the results were assessed by the Student's t-test.

TABLE 1

Comparison of the binding to rat liver microsomes of exogenous acetaldehyde and acetaldehyde formed endogenously from ethanol by MEOS. (Values are expressed as nanomoles of bound acetaldehyde/mg protein/hr).

Endogenous binding	Exogenous binding
0.38	0.18
0.19	0.15
0.41	0.13
0.44	0.22
0.29	0.14
0.13	0.08
0.33	0.16
0.18	0.12
Mean ± SEM	
0.29 ± 0.04	0.15 ± 0.02
$p<0.005$	

RESULTS

It was found that acetaldehyde, whether added exogenously or formed endogenously from ethanol by MEOS, binds to rat liver microsomes as shown in table 1. When solubilized microsomes were subjected to Sephadex G-25 gel column chromatography, the elution pattern of protein paralleled that of radioactivity (Fig. 1) and the recovery of protein (90%) was in good agreement with that of radioactivity, indicating that the radioactivity was associated with macromolecules and that unbound acetaldehyde had been effectively removed by the dialysis. Furthermore, the values obtained by two different methods (dialysis and organic solvent extractions) agreed well, suggesting that the macromolecules with bound acetaldehyde was mostly protein rather than microsomal lipid.

Binding of acetaldehyde formed endogenously from ethanol by MEOS was consistently greater than that of an equivalent amount of exogenous acetaldehyde (Table 1). The binding of endogenously formed acetaldehyde was significantly enhanced after chronic alcohol consumption (0.36 ± 0.03 vs 0.24 ± 0.03 nmole/mg protein/hr, $N=8$, $p<0.02$). This increase was associated with a 71% rise in MEOS activity as shown in table 2. The binding of exogenous acetaldehyde (200μM) to microsomes

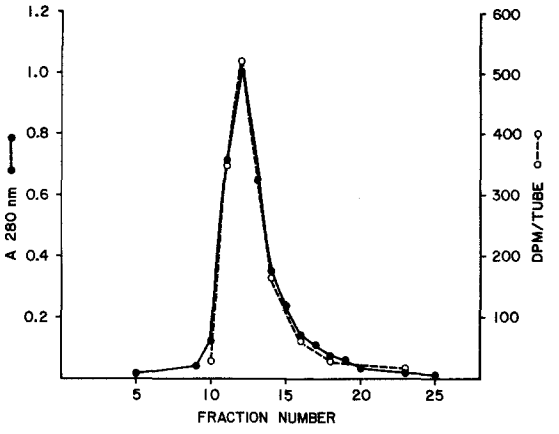

Figure 1: Sephadex G-25 gel filtration of solubilized microsomes after incubation with ^{14}C-acetaldehyde and dialysis. Solubilized microsomes were applied and eluted as described in Methods. The elution pattern of protein (solid line) paralleled that of radioactivity (dotted line). The recovery of protein (90%) was in agreement with that of radioactivity.

obtained from ethanol-fed rats and their pair-fed controls were comparable (0.11 ± 0.02 vs 0.10 ± 0.01 nmole/mg protein/hr, N=5). When microsomes were treated by p-hydroxymercuribenzoate, which is known to modify protein thiol groups, total thiols were decreased by 82%. This change in the available thiol groups resulted in a 28% decrease in the binding of acetaldehyde (Fig. 2). The binding of acetaldehyde was also inhibited by 51% in the presence of pyridoxal 5'-phosphate which is known to bind to free amino groups (Fig. 2). Those two effects were found to be additive (Fig. 2). The direct interaction between acetaldehyde and PLP was found to be negligible. When the disappearance of acetaldehyde was followed as described in Methods, there was no measurable change. Therefore, it is unlikely that a sufficient amount of acetate (oxidation product of acetaldehyde) was produced during the incubation to account for the protein bound radioactivity.

DISCUSSION

This study reveals that acetaldehyde formed from ethanol binds to hepatic microsomal protein; furthermore, this process is enhanced after

TABLE 2

Effect of chronic alcohol consumption on MEOS activity and the binding of acetaldehyde formed by MEOS from ^{14}C-ethanol to rat liver microsomes in eight pairs of rats.

MEOS activity (nmole acetaldehyde formed/mg protein/min)		Binding of acetaldehyde (nmole bound/mg protein/hr)	
Ethanol-fed	Controls	Ethancl-fed	Controls
9.7	5.9	0.46	0.19
17.4	7.1	0.38	0.28
13.3	10.2	0.30	0.36
16.9	7.7	0.19	0.13
13.4	9.7	0.41	0.33
16.1	11.9	0.29	0.27
15.6	10.0	0.44	0.18
18.0	7.6	0.37	0.17
Mean ± SEM		Mean ± SEM	
15.1 ± 0.98	8.8 ± 0.7	0.36 ± 0.03	0.24 ± 0.03
$p<0.001$		$p<0.02$	

chronic ethanol consumption. It has been known that aldehydes react with thiol compounds such as cysteine and glutathione (15-17). Acetaldehyde also has been shown to condense with amines such as catecholamines and tryptamines (18, 19). Therefore, it is likely that acetaldehyde binds to functional groups of protein. Furthermore, it has been postulated that binding of reactive metabolites to liver macromolecules may be one of the important mechanisms of liver injury (4, 5). It is tempting to speculate that acetaldehyde, a reactive metabolite of ethanol could play such a role in alcoholic liver injury. The results of this study indicate that acetaldehyde has a potential to play that role. Although alcohol dehydrogenase in the cytosolic fraction is an important pathway of ethanol oxidation, it is well known that ethanol is also oxidized in the endoplasmic reticulum by the microsomal ethanol-oxidizing system (MEOS). In vivo, the local concentration of acetaldehyde could be high in the endoplasmic reticulum. Indeed, it has been shown that chronic alcohol consumption enhances MEOS activity (20). Two related interesting observations were made in this study. First, the binding to rat liver microsomes of acetaldehyde formed endogenously from ethanol by MEOS was found to be significantly increased after

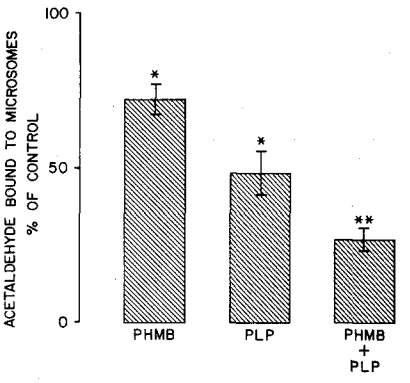

Figure 2: Effects of p-hydroxymercuribenzoate (PHMB) treatment and pyridoxal 5'-phosphate (PLP) on the binding of exogenous acetaldehyde to rat liver microsomes. Microsomes were treated by PHMB as described in Methods. Binding of exogenous acetaldehyde (200μM) to the treated microsomes and their controls were determined as described above. The effect of PLP on acetaldehyde binding was assessed by determining the binding in the presence of PLP (40mM). The results were expressed as % of controls (PHMB untreated and in the absence of PLP) ±SEM.
*$p<0.01$
**$p<0.001$

chronic alcohol consumption. This was associated with the increase in MEOS activity. Since binding of exogenous acetaldehyde was unaffected by chronic alcohol consumption, it is likely that the increase in binding is due to the increased production of acetaldehyde by MEOS rather than to changes in available binding sites. Secondly, the binding of acetaldehyde produced by MEOS was greater than that of an equivalent amount of exogenous acetaldehyde. The reason for the greater binding of endogenous acetaldehyde is not known, but it is reasonable to speculate that acetaldehyde produced at the surface of the membrane of the endoplasmic reticulum may have greater accessibility to the binding sites. It is also conceivable that acetaldehyde produced in situ may exert greater local toxic effects on the endoplasmic reticulum than acetaldehyde originating from the other cellular site of acetaldehyde production, namely the cytosol. As in the case of other hepatotoxic drugs, part of the toxicity of ethanol may be mediated by the binding of its reactive metabolite, namely acetaldehyde, to liver

macromolecules, but the specific effects of the binding of acetaldehyde on functional and morphological aspects of alcohol induced liver damage remain to be investigated.

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