NADPH-DEPENDENT OXIDATION OF METHANOL, ETHANOL, PROPANOL AND BUTANOL BY HEPATIC MICROSOMES

Rolf Teschke, Yasushi Hasumura and Charles S. Lieber

Section of Liver Disease and Nutrition, Bronx Veterans Administration Hospital, and the Department of Medicine, Mount Sinai School of Medicine (CUNY)
New York, New York 10468

Received August 15, 1974

Summary

Hepatic microsomes catalyze the oxidation of methanol, ethanol, propanol and butanol to their respective aldehydes. The reaction requires molecular oxygen and NADPH and is inhibited by CO, sharing thereby properties with other microsomal drug oxidations. This microsomal alcohol oxidizing system increases in activity after chronic ethanol consumption and operates independently from catalase as well as alcohol dehydrogenase. It appears responsible, at least in part, for the alcohol metabolism by the alcohol dehydrogenase independent pathway of the liver.

It is well recognized that hepatic microsomes are capable of oxidizing ethanol to acetaldehyde in a reaction requiring NADPH and molecular oxygen (1-3). The oxidation of ethanol is relatively insensitive to catalase inhibitors (2) suggesting that the bulk of the ethanol oxidizing activity in whole microsomes is catalase independent. More recently, this microsomal ethanol oxidizing system (MEOS) was solubilized and separated from both alcohol dehydrogenase and catalase activities by DEAE-cellulose column chromatography (4-6). Other groups, however, have attributed the ethanol oxidizing activity in microsomes exclusively to a reaction involving peroxidatic activity of catalase (7, 8).

In view of the finding that propanol and butanol function poorly or not at all as substrates for catalase-H_2O_2 (9, 10), we assessed whether substrate specificity could serve to differentiate the microsomal system from a process involving peroxidatic activity of catalase.

Materials and Methods

Male Sprague-Dawley rats (280-380 g BW) were purchased from Charles River Breeding Laboratories, North Wilmington, MA, and fed Purina Laboratory chow and tap water ad lib. When indicated, female rats (starting BW of 140-160 g) were used which were pair-fed nutritionally adequate liquid diets (11) containing either ethanol (36 per cent of total calories) or isocaloric carbohydrate (dextrin) as controls for 6-8 weeks prior to sacrifice. The livers were homogenized in 0. 15 M KCl, and washed microsomes were prepared as described (12).

The activity of the microsomal alcohol oxidizing system (MAOS) was determined in hepatic microsomes (6 mg protein/flask) in the presence of methanol (100 mM), ethanol (50 mM), propanol (50 mM) or butanol (50 mM) as described (6). The incubation media (final volume 3. 0 ml) contained 1. 0 mM Na_2-EDTA and 5. 0 mM $MgCl_2$ in 0. 1 M phosphate buffer (pH 7. 4), and the reaction was started by adding a NADPH generating system (0. 4 mM $NADP^+$, 8 mM sodium isocitrate and 2 mg per ml of isocitrate dehydrogenase). No aldehydes were produced when the alcohol, the NADPH generating system or microsomes were omitted from the medium, or when boiled microsomes were used. In some instances, a H_2O_2 generating system was employed consisting of 10 mM glucose and 0. 7 μg per ml of glucose oxidase (Type I, Boehringer Mannheim GmbH, Germany). With each of the latter incubation sets, experiments were run in which the microsomes were replaced by 0. 1 M phosphate buffer (pH 7. 4), and the observed blank values thus obtained were subtracted from the corresponding experimental result. All reactions were carried out for 0, 5 and 10 min and terminated by adding 0. 5 ml of 70 per cent of trichloroacetic acid (w/v). Formaldehyde produced upon oxidation of methanol was determined according to Nash (13). The incubations with ethanol,

propanol and butanol were carried out in the main chamber of closed 50 ml Erlenmeyer flasks with center wells containing 0.6 ml of 15 mM semicarbazide hydrochloride in 0.1 M phosphate buffer (pH 7.4) (2). After an overnight diffusion period the aldehydes bound to the semicarbazide were measured at 224 nm (14). Qualitative identification and quantitative assessment of the produced aldehydes trapped by the semicarbazide were performed by gas liquid chromatography in a number of experiments. Losses during the microsomal preparation were corrected for according to Greim et al. (15). The statistical significance of the results was assessed by the Student's t-test for pairs.

Results

In the presence of a NADPH generating system hepatic microsomes actively oxidize all four alcohols, methanol, ethanol, propanol and butanol to their respective aldehydes (Table 1). By contrast, virtually no aldehydes were produced with propanol and butanol as substrates in the presence of a H_2O_2 generating system whereas methanol and ethanol were oxidized (Table 1). Thus, microsomes contain a NADPH dependent alcohol oxidizing system which operates

Table 1

SUBSTRATE SPECIFICITY OF THE MICROSOMAL ALCOHOL OXIDIZING SYSTEM

The incubations were carried out with systems generating either NADPH (0.4 mM $NADP^+$, 8 mM sodium isocitrate and 2 mg per ml of isocitrate dehydrogenase) or H_2O_2 (10 mM glucose and 0.7 μg per ml of glucose oxidase). The values represent the average results of three experiments.

Substrate	NADPH	H_2O_2
	nmoles aldehyde/min/mg protein	
Methanol	7.7	7.8
Ethanol	9.5	9.2
Propanol	4.6	0.2
Butanol	3.9	0

independently from a process involving catalase-H_2O_2. Theoretically, contaminating catalase combined with NADPH oxidase, a microsomal enzyme capable of generating H_2O_2 (16), could enhance the NADPH dependent alcohol oxidizing activity for methanol as well as ethanol in vitro. To test this, microsomes were incubated with sodium azide (1.0 mM), a strong catalase inhibitor (2): under these experimental conditions, the NADPH dependent alcohol oxidizing activity was decreased by 20-30% with respect to methanol and ethanol, but there was no inhibitory effect on the oxidation of propanol and butanol.

The effect of chronic ethanol administration on the activity of the microsomal alcohol oxidizing system was studied in rats pair-fed the liquid diets containing either ethanol or dextrin as controls for 6-8 weeks. Chronic ethanol consumption resulted in a striking enhancement of MAOS activity whether expressed per mg of microsomal protein or per 100 g BW (Table 2). Of particular interest was the finding that this increase in specific activity was observed with all four alco-

Table 2

EFFECT OF CHRONIC ETHANOL CONSUMPTION (6-8 WEEKS) ON THE ACTIVITY OF MAOS

Female rats were pair-fed nutritionally adequate liquid diets containing either ethanol or dextrin as controls. The values represent means (±SEM) of six pairs.

Substrate	Control rat	Ethanol fed rat	P	Control rat	Ethanol fed rat	P
	nmoles aldehyde/min/ mg micros. protein			nmoles aldehyde/min/ 100 g BW		
Methanol	7.3±0.6	12.7±1.0	<0.01	983±45	1650±83	<0.001
Ethanol	9.9±0.4	14.9±0.8	<0.01	1364±57	1948±83	<0.01
Propanol	5.8±0.6	11.1±0.9	<0.01	808±102	1461±150	<0.02
Butanol	4.4±0.3	7.4±0.7	<0.01	575±49	966±95	<0.01

hols as substrates (Table 2). These experiments therefore show that the adaptive response observed after chronic ethanol consumption can be ascribed predominantly to a catalase-H_2O_2 independent mechanism since it was also demonstrated with propanol and butanol as substrates (Table 2) which virtually fail to react peroxidatically with catalase-H_2O_2 (Table 1).

With all four alcohols, MAOS was found to be most active with NADPH (1.0 mM) or a NADPH generating system. With NADH as cofactor, the activity was less than 25% of that obtained with NADPH. Optimum pH for MAOS activity was determined to be in the physiological range (pH 6.9 - 7.5). The K_m for MAOS activity (Lineweaver-Burk plot) varied, depending on the substrate used: methanol (22 mM), ethanol (9 mM), propanol (6 mM), and butanol (5 mM). Compared to control incubations, the alcohol oxidation was significantly ($p<0.02$) inhibited under an atmosphere containing CO ($CO:O_2$ = 10:1): methanol (41%), ethanol (41%), propanol (52%), and butanol (47%). Replacement of air by nitrogen abolished MAOS activity almost completely. Pyrazole (0.1 mM), a potent inhibitor for alcohol dehydrogenase (17), had no effect on MAOS activity.

Discussion

The results of the present study show that hepatic microsomes catalyze the NADPH dependent oxidation of methanol, ethanol, propanol and butanol to their respective aldehydes (Table 1 and 2). This microsomal alcohol oxidizing system (MAOS) requires molecular oxygen and NADPH and is inhibited by CO, sharing thereby properties with other microsomal drug metabolizing enzymes (18). Previously, only the oxidation of methanol and ethanol has been reported in hepatic microsomes (2, 19) but not that of alcohols with higher aliphatic chains (19). This was considered by some as evidence for an obligatory role of catalase in microsomal alcohol oxidation (8), since higher aliphatic alcohols including propanol and butanol have been reported to be extremely poor sub-

strates for catalase (9,10). Whereas the present study confirms the extremely low affinity of propanol and butanol for catalase-H_2O_2 (Table 1), it demonstrates that hepatic microsomes are capable of oxidizing propanol and butanol at significant rates in the presence of NADPH (Table 1 and 2), indicating that microsomes contain an alcohol oxidizing system which is independent from catalase.

The finding that hepatic microsomes actively metabolize methanol, ethanol, propanol and butanol suggests that this microsomal alcohol oxidizing system could account, at least in part, for the alcohol dehydrogenase independent pathway of alcohol metabolism in vivo (12,20) as well as in vitro in liver slices (2), perfused liver (21) and isolated parenchymal liver cells (22,23). Finally, since chronic ethanol consumption strikingly enhances the NADPH dependent MAOS activity (Table 2) in a manner similar to that of other microsomal drug metabolizing enzymes (24,25) it is conceivable that this microsomal system is involved, at least in part, in the enhanced ethanol clearance observed in vivo following chronic ethanol consumption (11).

Acknowledgements

The authors wish to thank Miss Nancy Lowe for her skillful technical assistance. This work was supported in part by USPHS grants AA 00224, AM 12511 and VA project No. 5251-02.

References

1. Lieber, C.S., and DeCarli, L.M., Science 162, 917 (1968).
2. Lieber, C.S., and DeCarli, L.M., J. Biol. Chem. 245, 2505 (1970).
3. Hildebrandt, A.G., Speck, M., and Roots, I., Naunyn Schmiedeberg's Arch. Pharmacol. 281, 371 (1974).
4. Teschke, R., Hasumura, Y., Joly, J.-G., Ishii, H., and Lieber, C.S. Biochem. Biophys. Res. Commun. 49, 1187 (1972).
5. Mezey, E., Potter, J.J., and Reed, W.D., J. Biol. Chem. 248, 1183 (1973).
6. Teschke, R., Hasumura, Y., and Lieber, C.S., Arch. Biochem. Biophys. 163, 404 (1974).
7. Khanna, J.M., and Kalant, H., Biochem. Pharmacol. 19, 2033 (1970).
8. Thurman, R.G., Ley, H.G., and Scholz, R., Eur. J. Biochem. 25, 420 (1972).
9. Chance, B., Acta Chem. Scand. 1, 236 (1947).

10. Chance, B., and Oshino, N., Biochem. J. 122, 225 (1971).
11. DeCarli, L.M., and Lieber, C.S., J. Nutr. 91, 331 (1967).
12. Lieber, C.S., and DeCarli, L.M., J. Pharmacol. Exp. Ther. 181, 278 (1972).
13. Nash, T., Biochem. J. 55, 416 (1953).
14. Gupta, N.K., and Robinson, W.G., Biochim. Biophys. Acta 118, 431 (1966).
15. Greim, H., Schenkman, J.B., Klotzbucher, M., and Remmer, H., Biochim. Biophys. Acta 301, 20 (1970).
16. Gillette, J.R., Brodie, B.B. and La Du, B.N., J. Pharmacol. Exp. Ther. 119, 532 (1957).
17. Theorell, H., and Yonetani, T., Biochem. Z. 338, 537 (1963).
18. Conney, A.H., Pharmacol. Rev. 19, 317 (1967).
19. Orme-Johnson, W.H., and Ziegler, D.M., Biochem. Biophys. Res. Commun. 21, 78 (1965).
20. Lester, D., and Benson, G.D., Science 169, 282 (1970).
21. Papenberg, J., von Wartburg, J.P., and Aebi, H., Enzym. Biol. Clin. 11, 237 (1970).
22. Thieden, H.I.D., Acta Chem. Scand. 25, 3421 (1971).
23. Grunnet, N., Quistorf, B., and Thieden, H.I.D., Eur. J. Biochem. 40, 275 (1973).
24. Rubin, E., and Lieber, C.S., Science 162, 690 (1968).
25. Joly, J.-G., Ishii, H., Teschke, R., Hasumura, Y., and Lieber, C.S. Biochem. Pharmacol. 22, 1532 (1973).