HEPATIC MICROSOMAL ETHANOL OXIDIZING SYSTEM (MEOS): DIFFERENTIATION FROM ALCOHOL DEHYDROGENASE AND NADPH OXIDASE*

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Summary

Washed hepatic microsomes contain an active ethanol oxidizing system (MEOS) but no detectable ADH activity, even when 3-AP-NAD is used as a cofactor. ADH inhibitors (pyrazole and DMSO) failed to affect MEOS activity at concentrations which markedly reduced ADH activity. Therefore ADH is not a component of MEOS. Cholate, which inhibits the activities of both microsomal NADPH oxidase and MEOS, also strikingly diminishes that of other microsomal enzymes such as aniline hydroxylase and aminopyrine demethylase. Furthermore cholate inactivates both total and enzymatically reducible microsomal cytochrome P450. Thus, because of the non-specific nature of the inhibition by cholate,one cannot conclude from its effects that NADPH oxidase is a component of MEOS.

Recently, a hepatic microsomal ethanol oxidizing system (MEOS) has been described which has optimum activity at physiological pH, requires O_2 and NADPH, and adaptively increases in activity upon ethanol feeding[1,2]. On the basis of these and other characteristics, MEOS was considered to be distinct from hepatic alcohol dehydrogenase (ADH). This conclusion has been challenged recently in studies using the ADH inhibitors dimethylsulfoxide (DMSO) and pyrazole[3]. Moreover, hepatic microsomes display NADPH oxidase activity capable of generating H_2O_2 from NADPH and O_2[4]. They also contain some catalase which, in the presence of H_2O_2, can oxidize ethanol to acetaldehyde[5]. Therefore, it was postulated that ethanol oxidation by MEOS may be due to a NADPH oxidase-catalase mechanism[6,7]. NADPH oxidase

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can be inhibited by cholate[4], and the observation that cholate also diminishes MEOS activity was recently presented as evidence in favor of the NADPH oxidase-catalase hypothesis[3]. These observations are at variance with our studies on the relationship of MEOS to ADH and NADPH oxidase-catalase. We found that MEOS activity can be fully differentiated from that of ADH, both by its cofactor requirements and the effects of inhibitors, and that at the concentration used, cholate inhibition of MEOS is a nonspecific effect.

Experimental Procedure

Sprague-Dawley rats (CD) were purchased from Charles River Breeding Laboratories (North Wilmington, Mass.) and fed Purina laboratory chow and tap water ad libitum. They were killed by decapitation, exsanguinated and the livers were excised. Hepatic cytosol (100,000 g supernatant) and washed microsomes were prepared as described previously[2]. ADH activity was measured in both fractions either with NAD[8] or with acetylpyridine-NAD (3-AP-NAD)[9]. NADPH oxidase activity of the microsomes was determined according to Gillette et al.[4] and MEOS activity was measured as described previously[2], except that in some experiments, the NADPH generating system (NADP, isocitrate, isocitrate dehydrogenase and nicotinamide) was replaced by either NADPH or 3-AP-NAD (0.3 mM).

Effects of pyrazole (1, 2, 4 mM) and DMSO (10, 50, 100 mM) on the activity of ADH, and the action of these compounds and that of sodium cholate (14 mM) on the activities of MEOS, NADPH oxidase, aniline hydroxylase[10], and aminopyrine demethylase[11] were studied. In addition, the effect of sodium cholate on microsomal cytochrome-P450[12] and the rate of reduction of cytochrome-P450 were determined[13].

Results and Discussion

The effects of various concentrations of pyrazole on the activities of ADH and

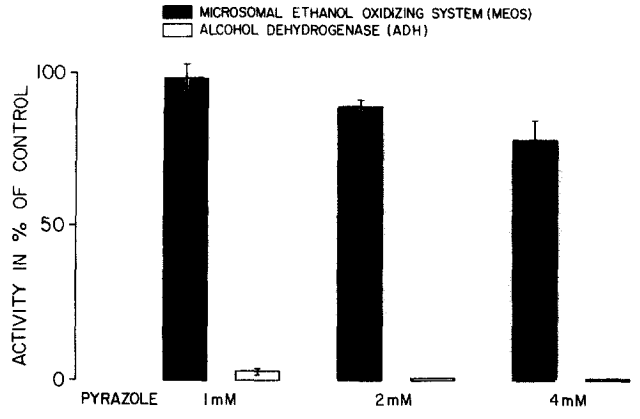

Figure 1. Effect of various concentrations of pyrazole on the activities of the hepatic microsomal ethanol oxidizing system (MEOS) and that of alcohol dehydrogenase (ADH) in the cytosol. No detectable ADH activity was present in these washed microsomes.

MEOS are indicated in Figure 1. Whereas 4 and 2 mM pyrazole abolished ADH activity, they reduced MEOS activity by only 22 and 11 percent respectively; 1 mM pyrazole diminished ADH activity by 98 percent, but had no significant effect on MEOS activity. These results are at variance with those of Isselbacher and Carter[3] who reported that 1 mM pyrazole inhibits MEOS by 48 percent. The reasons for this discrepancy are not apparent, but it must be pointed out that other investigators also failed to find an inhibition of MEOS by 1 mM pyrazole[14,15] or even by larger pyrazole concentrations[16]. The small decrease in MEOS activity we observed with the larger pyrazole concentrations in our study may be related to the inhibition of ethanol binding to microsomal heme protein and interference with the reduction of cytochrome-P450 by this compound[17,18]. With DMSO, another ADH inhibitor[19], a similar discrepancy can be found between our data and those of Isselbacher and Carter[3]. These authors reported that in addition to ADH inhibition, DMSO (14 mM) significantly reduced the activity of MEOS. By contrast, we found no diminution of MEOS activity by 10, 50 or even 100 mM DMSO, whereas ADH activity of the

cytosol was reduced 80% by 100 mM DMSO. The lack of effect of ADH inhibitors on MEOS activity is not surprising, since in washed microsomes, we reported no detectable ADH activity using NAD as a cofactor[2]. This was confirmed in the present study which also showed no microsomal ADH activity even with 3-AP-NAD. This cofactor increased ADH activity of the cytosol fourfold, confirming previous observations[9]. We conclude from these results that MEOS activity found in washed microsomes cannot be attributed to ADH activity. This conclusion is strengthened by the observation that replacement of NADPH or the NADPH generating system by 3-AP-NAD resulted in a loss of 88 percent of MEOS activity. Therefore, neither we nor others[14] could confirm the recent report that 3-AP-NAD can replace NADPH for MEOS activity[3]. Isselbacher and Carter[3] postulate that ADH can utilize NADP as a cofactor. Although this is the case, to a limited extent, with purified enzyme and cofactors[20], it probably plays no significant role in biological systems, as shown by the failure of Isselbacher and Carter[3], using NADP or NADPH, to detect ADH activity in the cytosol, despite its abundance in that subcellular compartment.

It has been shown previously that cholate inhibits both microsomal NADPH oxidase[3,4] and MEOS[3]; this was considered as evidence that NADPH oxidase is a component of MEOS[3]. We wondered however about the specificity of the effect of cholate on NADPH oxidase since Hutterer et al.[21] found recently that even low concentrations of bile acids inhibit a variety of other microsomal enzymes and that cholate (4 mM) in particular exerts a nonspecific detergent effect[22]. We repeated these studies, using a concentration of cholate (14 mM) identical to that of Isselbacher and Carter[3]. In addition to inhibition of NADPH oxidase and MEOS, we found striking reductions in activities of other microsomal enzymes. Cholate (14 mM) reduced not only the activities of NADPH oxidase and MEOS by

approximately 80%, but it also diminished to the same extent that of aminopyrine demethylase and almost abolished that of aniline hydroxylase(at aniline concentrations from 0.02 to 0.2 mM). A Lineweaver Burk plot showed the inhibition of aminopyrine demethylase to be uncompetitive (Figure 2). We confirmed the recent observation of Hutterer et al.[22] that cholate decreases enzymatically

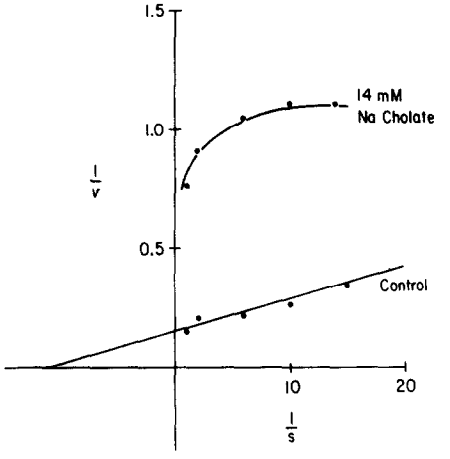

Figure 2. Lineweaver-Burk plot showing uncompetitive inhibition of aminopyrine demethylase by 14 mM Na cholate. Enzyme activity does not conform to saturation kinetics at higher concentrations of substrate. V - nmoles formaldehyde formed per mg protein per min. S - concentration of aminopyrine (mM).

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reducible cytochrome P450. Cholate (14 mM) inactivates cytochrome P450, as shown by the quantitative conversion to cytochrome P420 (Figure 3) and the interference with the reduction of cytochrome P450 (Figure 4). The inactivation of cytochrome P450 by cholate has obvious implications for microsomal oxidation of ethanol, because ethanol has been shown to bind to microsomal heme protein in a fashion similar to that of other drugs[18]. Thus, the observation that cholate inhibits both NADPH oxidase and MEOS cannot be considered as evidence that NADPH oxidase is a component of MEOS, since, at the concentration used, cholate is a nonspecific inhibitor which affects many microsomal functions. More-

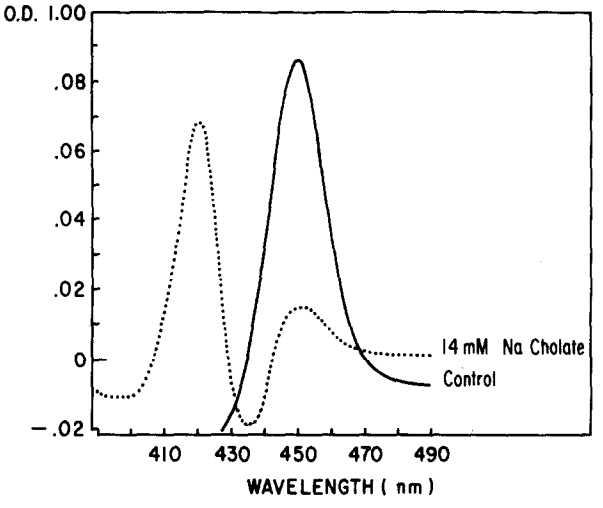

Figure 3. Effect of 14 m M Na cholate on hepatic microsomal cytochrome P450. Difference spectrum was recorded after washed microsomes were treated with dithionite and CO. After addition of 14 mM cholate to the sample cuvette, the magnitude of the peak at 450 nm is decreased by 85 percent, and a new peak, whose magnitude is equivalent to the loss at 450 nm, appears at 420 nm. The microsomal suspension contained 1 mg protein per ml.

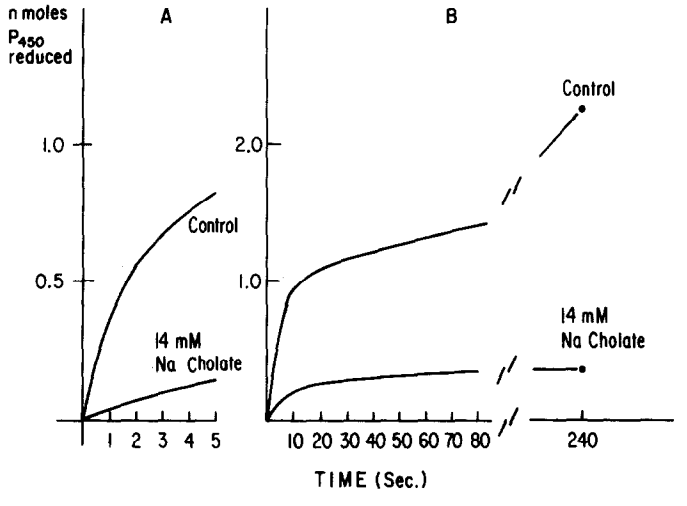

Figure 4. Effect of 14 mM Na cholate on the rate of reduction of hepatic microsomal cytochrome P450. Initial velocity, shown on the expanded scale (A), is 0.35 nmoles P450 reduced per second for the control, and 0.04 in the presence of 14 mM Na cholate. Total enzymatically reduced P450 (B), measured after 4 minutes, was about 7 times greater in the control than in the suspension containing 14 mM Na cholate. Protein concentration of the microsomal suspension was 3 mg per ml.

over, for the H_2O_2 generated by microsomal NADPH oxidase to contribute to alcohol oxidation, catalase is needed[4]. When catalase activity however was abolished by addition, in vitro, of azide to microsomes obtained from animals given pyrazole in vivo, there was almost complete inhibition of ethanol oxidation by a H_2O_2 generating system[2]. By contrast, the bulk of MEOS activity persisted[2], thus indicating that the postulated pathway plays no significant role in MEOS activity. Furthermore, MEOS can be inhibited by CO, whereas NADPH oxidase is not[2,23].

In conclusion, the failure of the ADH inhibitors, pyrazole and DMSO, to affect significantly MEOS activity at concentrations which conspicuously inhibit ADH, and the lack of detectable ADH or MEOS activity in washed microsomes when NAD or 3-AP-NAD is used as a cofactor, support the thesis that ADH plays no role in the hepatic microsomal ethanol oxidizing system (MEOS). We also found no evidence to link the activity of MEOS with that of microsomal NADPH oxidase.

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. Isselbacher, K. J. and Carter, E. A. Biochem. Biophys. Res. Commun., 39, 530 (1970).
4. Gillette, J. R., Brodie, B. B. and La Du, B. N. J. Pharmacol. Exp. Ther., 119, 532 (1957).
5. Keilin, D. and Hartree, E. F. Biochem. J., 39, 293 (1945).
6. Roach, M. K., Reese, Jr., W. N. and Creaven, P. J. Biochem. Biophys. Res. Commun., 36, 596 (1969).
7. Tephly, T. R., Tinelli, F. and Watkins, W. D. Science, 166, 627 (1969).
8. Bonnichsen, R. K. and Brink, N. G. In S. P. Colowick and N. O. Kaplan (Editors), Methods in enzymology, Vol. 1, Academic Press, New York, p. 495 (1955).
9. Kaplan, N. O., Ciotti, M. M. and Stolzenbach, F. E. J. Biol. Chem., 221, 833 (1956).
10. Imai, Y. and Sato, R. Biochem. Biophys. Res. Commun., 25, 80 (1966).
11. Schenkman, J. B., Remmer, H. and Estabrook, R. W. Mol. Pharmacol., 3, 113 (1967).

12. Omura, T. and Sato, R. J. Biol. Chem., 239, 2370 (1964).
13. Gigon, P. L., Gram, T. E. and Gillette, J. R. Mol. Pharmacol., 5, 109 (1969).
14. Roach, M. K. Personal communication.
15. Mezey, E. Personal communication.
16. Khanna, J. M. and Kalant, H. In Proceedings of the Symposium on Biological Aspects of Alcohol, University of Texas Press, in press, (1970).
17. Rubin, E., Gang, H. and Lieber, C. S. Fed. Proc. 29, 275 (1970).
18. Rubin, E., Lieber, C. S., Alvares, A. P., Levin, W. and Kuntzman, R. Biochem. Pharmacol., in press (1970).
19. Perlman, R. L. and Wolff, J. Science, 160, 317 (1968).
20. Dalziel, K. and Dickinson, F. M. Biochem. J., 95, 311 (1965).
21. Hutterer, F., Denk, H., Bacchin, P. G., Schenkman, J. B., Schaffner, F. and Popper, H. Life Sci., 9, 877 (1970).
22. Hutterer, F., Bacchin, P. G., Denk, H., Schenkman, J. B., Schaffner, F. and Popper, H. Personal communication.
23. Orrenius, S. J. Cell Biol., 26, 713 (1965).