Vol. 133, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 27, 1985 Pages 44-51

Fatty Acid-Dependent Ethanol Metabolism

Jeffrey A. Handler and Ronald G. Thurman*

Department of Pharmacology
University of North Carolina at Chapel Hill
Chapel Hill, N.C. 27514

Received September 9, 1985

Rates of ethanol oxidation by perfused livers from fasted female rats were decreased from 82 ± 8 to 11 ± 7 μmol/g/hr by 4-methylpyrazole, an inhibitor of alcohol dehydrogenase. The subsequent addition of fatty acids of various chain lengths in the presence of 4-methylpyrazole increased rates of ethanol uptake markedly. Palmitate (1 mM) increased rates of ethanol oxidation to 95 ± 8 μmol/g/hr, while octanoate and oleate increased rates to 58 ± 11 and 68 ± 15 μmol/g/hr, respectively. Hexanoate, a short-chain fatty acid oxidized predominantly in the mitochondria, had no effect. Addition of oleate also increased the steady-state level of catalase-H_2O_2. Pretreatment of rats for 1.5 hours with 3-amino-1,2,4-triazole (1.0 g/kg), an inhibitor of catalase, prevented the ethanol-dependent decrease in the steady-state level of catalase-H_2O_2 completely. Under these conditions, aminotriazole decreased rates of ethanol oxidation by about 50% and blocked the stimulation of ethanol oxidation by fatty acids. Oleate decreased rates of aniline hydroxylation by about 50%, indicating that cytochrome P_{450} is not involved in the stimulation of ethanol uptake by fatty acids. Furthermore, oleate stimulated ethanol uptake in livers from ADH-negative deermice indicating that fatty acids do not simply displace 4-methylpyrazole from alcohol dehydrogenase. It is concluded that the stimulation of ethanol oxidation by fatty acids is due to increased H_2O_2 supplied by the peroxisomal ß-oxidation of fatty acids for the catalase-H_2O_2 peroxidation pathway.

The interactions between ethanol metabolism and fatty acid oxidation in the liver have been studied intensively (1,2). It is known that ethanol decreases rates of hepatic fatty acid oxidation (1) and it has been demonstrated that fatty acids decrease ethanol oxidation by about 30% (2). These interactions between ethanol and fatty acid metabolism are believed to be due to competition between the two pathways for available NAD^+.

Keilin and Hartree (3) established that ethanol can be peroxidized by catalase-H_2O_2 . The rate of the reaction is dependent upon the concentration of ethanol and the amount of catalase heme (4) and is limited by the supply of H_2O_2 (5). Substrates for peroxisomal flavoproteins which increase the generation of H_2O_2, such as urate and

*To whom all correspondence should be addressed.

0006-291X/85 $1.50

glycolate, can stimulate catalase-dependent peroxidation of ethanol in the perfused liver significantly (6).

In 1976, Lazarow and DeDuve (7) demonstrated that peroxisomes can oxidize fatty acyl-CoA compounds. Unlike mitochondrial ß-oxidation, peroxisomes produce one molecule of H_2O_2 for every acetyl-CoA generated (8). In the present study, we present evidence that the peroxisomal ß-oxidation of fatty acyl-CoA compounds can stimulate catalase-dependent peroxidation of ethanol in the perfused liver at high rates. Preliminary accounts of this work have been presented elsewhere (9).

EXPERIMENTAL PROCEDURES

Materials. Aniline hydrochloride was purchased from Aldrich Chemical Co., Milwaukee, Wis. 3-amino-1,2,4-triazole (aminotriazole), bovine serum albumin, and other chemicals were purchased from Sigma Chemical Company, St. Louis, Mo. Bovine serum albumin was defatted by the procedure of Chen (10).

Animals. Female Sprague-Dawley rats (200-300 g; Zivic-Miller, Allison Park, PA) were fasted for 20-24 hours prior to use. In some experiments rats were injected 1.5 hours before perfusion with either saline or aminotriazole (1.0 g/kg i.p.). Deermice genetically deficient in alcohol dehydrogenase (ADH-negative deermice) were obtained from a colony maintained at the University of North Carolina from breeding stock generously provided by Dr. M. Felder, University of South Carolina.

Liver Perfusion. Rat livers were perfused with oxygen-saturated Krebs-Henseleit bicarbonate buffer maintained at 37 C in either a recirculating (11) or non-recirculating (12) system. Deermouse livers were perfused by a modification of the above techniques (Handler, J.A., Bradford, B.U., and Thurman, R.G., manuscript in preparation). Livers from rats and deermice were perfused via the portal vein at rates around 4 or 8 ml/min/g, respectively. Oxygen concentration in the effluent perfusate was monitored continuously with a Teflon-shielded, Clark-type O_2 electrode. Ethanol was determined enzymatically (13) and p-aminophenol was determined colorimetrically (14) in samples of effluent perfusate. Rates were calculated from the influent minus effluent concentration differences, the flow rate and tissue wet weight.

Determination of pyridine nucleotide fluorescence and the steady-state level of catalase-H_2O_2. The steady-state level of catalase-H_2O_2 compound I was detected spectrophotometrically (660-640 nm) through a lobe of the liver as described by Sies and Chance (14). A large-tipped fiber-optic light guide was used for the determination of NADH fluoresence from the surface of the liver. Briefly, one end of a bifurcated light guide was connected to a 100 W mercury arc lamp fitted with a 366 nm transmittance filter. The other end was connected to a photomultiplier filtered to detect 450 nm light, and the output was amplified and recorded as described elsewhere (15).

RESULTS AND DISCUSSION

In livers from normal, fasted rats, ethanol (25 to 35 mM) was taken up at rates of about 80 µmol/g/hr (Figure 1A). The subsequent addition of 4-methylpyrazole (4 mM), an inhibitor of alcohol dehydrogenase, decreased rates to 11 $\pm$ 7 µmol/g/hr. Several fatty acids of various chain lengths were then added to evaluate their effect on ethanol uptake. Hexanoate (1 mM) did not alter rates of ethanol uptake significantly; however, octanoate and oleate increased rates to 58 $\pm$ 11 and 68 $\pm$ 15 µmol/g/hr, respectively (Figure 1A). The largest increase was observed with palmitate which increased rates to

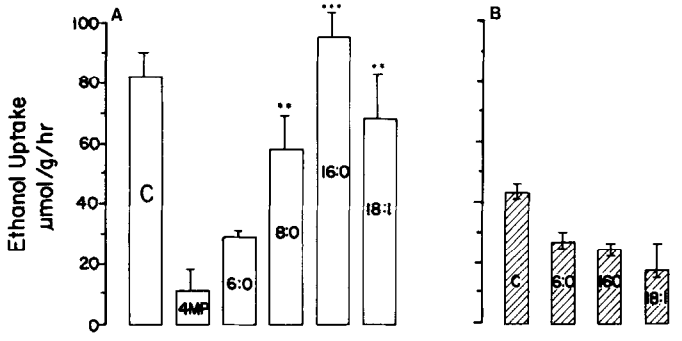

Figure 1. Stimulation of ethanol uptake in perfused livers by fatty acids. Livers from fasted female rats were perfused in a recirculating system as described in Methods and Materials. Ethanol (25 to 35 mM) was added and rates of ethanol uptake were calculated from the disappearance of ethanol from the perfusate after correction for vaporization from the system (about 5%). A, rates of ethanol uptake after infusion of 4-methylpyrazole (4 mM) and various fatty acids. 4-methylpyrazole was present in all experiments with fatty acids. 6:0, hexanoate; 8:0, octanoate; 16:0, palmitate; 18:1, oleate. B. Conditions are as in A except that rats were injected with aminotriazole (1.0 g/kg) i.p. 1.5 hours before perfusion. Aminotriazole (11.8) was also present in the perfusate. Data are mean ± S.E.M. for 3-7 livers per group. $^{**}p<0.05$, $^{***}p<0.01$ compared to rates in the presence of 4-methylpyrazole.

95 ± 8 µmol/g/hr (Figure 1A). There are several possible explanations for this stimulation of ethanol metabolism by fatty acids. First, it is possible that fatty acids displace 4-methylpyrazole from the lipophilic binding site on alcohol dehydrogenase. Second, fatty acids could activate cytochrome P_{450}-dependent monooxygenation of ethanol. Third, ß-oxidation of fatty acids in peroxisomes could provide H_2O_2 for peroxidation of ethanol via catalase-H_2O_2.

To test the hypothesis that fatty acids displace 4-methylpyrazole from alcohol dehydrogenase, experiments were performed with livers from deermice genetically deficient in alcohol dehydrogenase (17). Oleate (0.5 mM) added in the absence of 4-methylpyrazole increased rates of ethanol metabolism in perfused livers from ADH-negative deermice by 28 ± 3 µmol/g/hr (n = 4). Since fatty acids increase rates of ethanol metabolism in the absence of alcohol dehydrogenase, it is unlikely that displacement of 4-methylpyrazole from alcohol dehydrogenase can explain the stimulation of ethanol oxidation by fatty acids.

Purified cytochrome P_{450} form 3a has a similar turnover number for aniline and ethanol and has been shown to be largely responsible for the metabolism of ethanol by rabbit (18) and deermouse (Koop, D., Coon, M.J., Bradford, B.U., Handler, J.A. and Thurman, R.G., unpublished) liver microsomes. To test whether fatty acids stimulate

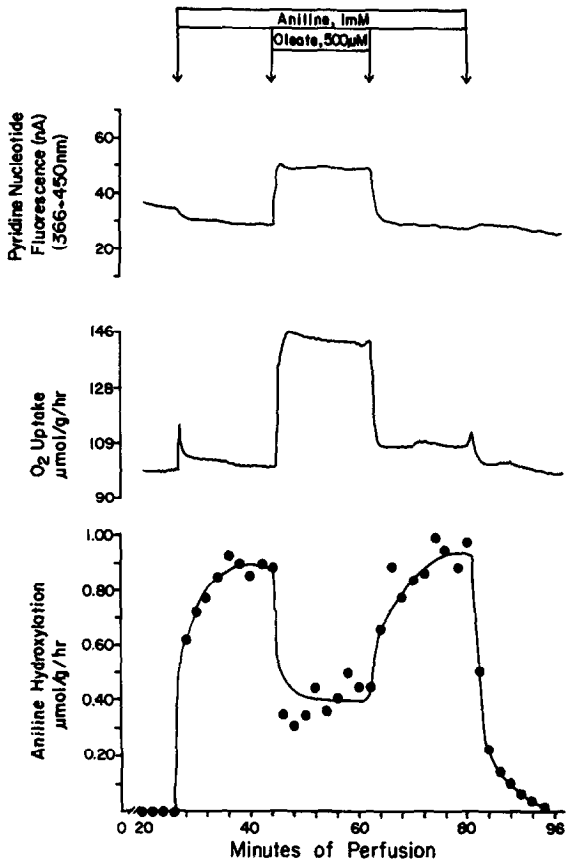

Figure 2. Inhibition of aniline hydroxylation in perfused rat liver by oleate. Livers were perfused in a non-recirculating system. The concentration of O_2 was monitored with a Teflon-shielded, Clark-type electrode and pyridine nucleotides were monitored fluorometrically. Aniline hydroxylation was measured from the production of p-aminophenol as described in Materials and Methods. Oleate (500 μM) was infused bound to fat-free bovine serum albumin (fatty acid : albumin ratio, 4:1). Typical experiment.

cytochrome $P_{450_{3a}}$-dependent mixed-function oxidation, aniline hydroxylation was monitored. Aniline (1 mM) had little effect on pyridine nucleotide fluorescence or oxygen uptake (Figure 2) but was converted into p-aminophenol at rates of 0.9 ± 0.4 μmol/g/hr. The subsequent infusion of oleate produced predictable increases in pyridine nucleotide fluorescence and O_2 uptake. Under these conditions, oleate (0.5 mM) decreased the cytochrome $P_{450_{3a}}$-dependent metabolism of aniline by about 60% in a reversible manner. This inhibition is most likely due both to a decrease in the supply of NADPH and to a direct inhibition of cytochrome P_{450} by acyl-CoA compounds (19). Since aniline hydroxylation was not increased by fatty acids, we conclude that

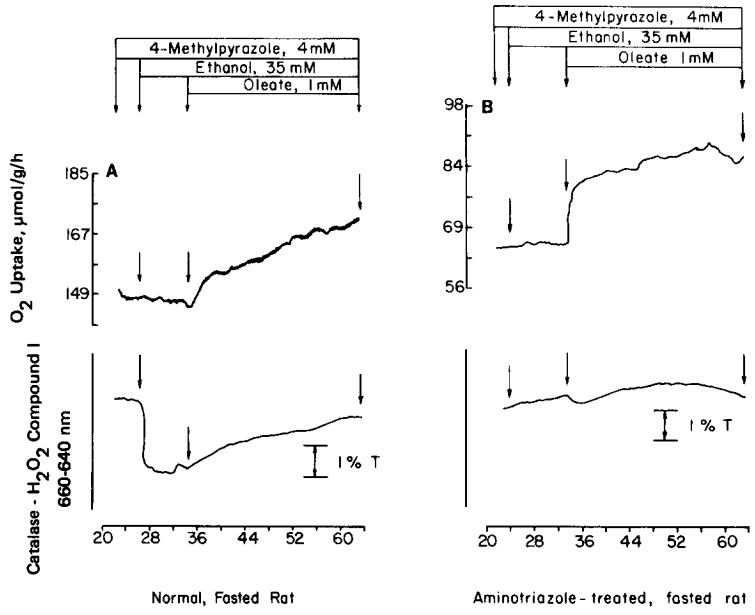

Figure 3. Effect of ethanol and oleate on oxygen uptake and catalase-H_2O_2 in perfused rat liver. Livers were perfused as described in Figure 1 and oxygen uptake and the steady-state level of catalase-H_2O_2 compound I were measured as described in the text. 4-methylpyrazole (4 mM), ethanol (35 mM) and oleate (1 mM) were added at the times designated by the vertical arrows and horizontal bars. A, liver from a normal, fasted rat. B, liver from a fasted rat injected with aminotriazole (1.0 g/kg) 1.5 hours before perfusion. Perfusate also contained aminotriazole (11.8 mM). Typical experiments.

cytochrome P_{450} is not involved in the stimulation of ethanol oxidation by oleate observed in the perfused liver (Figure 1A).

Sies and Chance demonstrated previously that the steady-state level of catalase-H_2O_2 can be monitored spectrophotometrically through a lobe of the liver (15). When ethanol is infused into the liver, the steady-state level of catalase-H_2O_2 decreases characteristically due to the peroxidation of ethanol by catalase-H_2O_2 (Figure 3A;15). In addition, when fatty acids are infused into the perfused liver, the steady-state level of catalase-H_2O_2 is increased presumably due to increased H_2O_2 supply (Figure 3A;20). We tested the hypothesis that the stimulation of ethanol uptake by fatty acids was due to an increase in H_2O_2 supplied by the peroxisomal ß-oxidation of acyl-CoA compounds from fatty acids. When rats were pretreated with aminotriazole, ethanol had no effect on the steady-state level of catalase-H_2O_2 (Figure 3B), providing physical evidence that catalase was inhibited under these conditions. Oleate also had no effect on the steady-state level of catalase-H_2O_2 in livers from aminotriazole-treated

rats (Figure 3B). Aminotriazole treatment decreased rates of ethanol oxidation by perfused livers from fasted rats by about 50% (Figure 1B). Moreover, it blocked the stimulation of ethanol uptake by fatty acids. When either octanoate, palmitate or oleate were infused into livers from aminotriazole-treated rats in the presence of 4-methylpyrazole, rates of ethanol uptake were only between 20-30 μmol/g/hr (Figure 1B). Therefore, it is concluded that the stimulation of ethanol uptake by fatty acids is due to the peroxidation of ethanol via catalase-H_2O_2. Most likely, H_2O_2 is supplied by the peroxisomal ß-oxidation of acyl-CoA compounds formed from fatty acids.

It is generally accepted that catalase-dependent metabolism of ethanol is minimal. This is based on the fact that measured rates of H_2O_2 production are less than rates of ethanol oxidation and on the observation that ethanol oxidation in some studies is insensitive to the catalase inhibitor aminotrizole. These conclusions are, however, inconsistent with the findings of this study that catalase-H_2O_2-dependent ethanol oxidation is stimulated by fatty acids (Figure 1A). Rates of H_2O_2 generation by perfused livers have been estimated based on the destruction of catalase-H_2O_2 compound I by methanol (21). Rates of about 10 μmol/g/hr were observed in livers from fed rats following infusion of 0.3 mM octanoate (21) and about 8 μmol/g/hr following infusion of 0.065 mM oleate (20). Since at least one mole of H_2O_2 must be generated for the peroxidation of one mole of ethanol via catalase-H_2O_2, rates of fatty acid-stimulated ethanol uptake should be roughly equivalent to rates of H_2O_2 generation due to peroxisomal ß-oxidation. In sharp contrast to the studies referred to above (20,21), rates of fatty acid-stimulated ethanol oxidation were 40-80 μmol/g/hr in this study (Figure 1A). There are several experimental differences between previous work and our study which may explain the discrepencies between measured rates of H_2O_2 generation and rates of fatty acid-stimulated ethanol oxidation. First, the study by Oshino et al (21) was performed at 30°C while our study was carried out at 37°C. Second, both the studies of Oshino et al (21) and Foerster et al (20) utilized livers from fed rats, while our study used livers from fasted rats. It is well known that rates of ß-oxidation are increased by fasting and a component of this increase involves the peroxisomal system (22,23). Third, the fatty acid concentrations used in the present study (1 mM) are considerably higher than those employed by Oshino et al (0.3 mM) and Foerster et al

(0.02-0.065 mM). Indeed, when livers from fed female rats were perfused with oleate (0.12 mM) and ethanol (30 mM) in the presence of 4-methylpyrazole (4 mM), rates of fatty acid-stimulated ethanol oxidation of 12 $\pm$ 7 μmol/g/hr (n = 4) were observed. Thus, we observed rates of ethanol oxidation similar to rates of H_2O_2 generation measured by Foerster et al when the experiments were performed under similar conditions. Therefore, the high rates of H_2O_2 generation, as indicated by rates of fatty acid-stimulated ethanol oxidation, were not observed in previous studies probably due to variations in experimental conditions and not to differences in detection of H_2O_2 between the two methods. Indeed, fatty acid-stimulated ethanol oxidation may be a simple way to estimate rates of peroxisomal H_2O_2 generation qualitatively.

Several studies have failed to demonstrate an effect of aminotriazole on ethanol oxidation in vivo (24,25,26) and in isolated hepatocytes (27). In contrast, if care is taken to verify spectrophotometrically that the peroxidatic reaction is actually inhibited, aminotriazole decreases rates of ethanol oxidation by about 50% at ethanol concentrations employed in this study (Figure 1B). Oshino et al showed that H_2O_2 supply is rate-limiting for the oxidation of ethanol via catalase. Indeed, when exogenous fatty acids are added to the perfused liver, as would occur in vivo after a meal, rates of catalase-H_2O_2-dependent ethanol metabolism are quite high. Thus, it is not surprising that rates of catalase-H_2O_2-dependent ethanol oxidation were not very high in previous studies with in vitro preparations where fatty acids were not added. Accordingly, catalase may be more important in ethanol oxidation than has been thought previously, particularly following ingestion of fat.

ACKNOWLEDGMENTS

This work was supported by grants AA-03624 from the National Institute on Alcohol Abuse and Alcoholism and NCARA 8404 from the N.C. Alcoholism Research Authority.

REFERENCES

1. Williamson, J.R., Scholz, R., Browning, E.T., Thurman, R.G. and Fukami, M.H. (1969) J. Biol. Chem. **244**, 5044-5054.
2. Thurman, R.G. and Scholz, R. (1975) Fed. Proc. **24**, 634.
3. Keilin, D. and Hartree, E.F. (1945) Biochem. J. **39**, 293-305.
4. Oshino, N., Jamieson, D., Sugano, T. and Chance, B. (1975) Biochem. J. **146**, 67-77.
5. Thurman, R.G., Ley, H.G. and Scholz, R. (1972) Eur. J. Biochem. **25**, 420-430.
6. Thurman, R.G. and McKenna, W. (1974) Hoppe-Seylers Z. Physiol. Chem. **355**, 336-340.

7. Lazarow, P.B. and de Duve, C. (1976) Proc. Natl. Acad. Sci. USA **73**, 2043-2046.
8. Bremer, J. and Osmundsen, H. (1984) in Fatty Acid Metabolism and Its Regulation. ed. S. Numa. pp.113-154, Elsevier Science Publishers.
9. Handler, J.A. and Thurman, R.G. (1985) Fed. Proc. **44**, 1486.
10. Chen, R.F. (1967) J. Biol. Chem. **242**, 173-181.
11. Scholz, R., Thurman, R.G., Williamson, J.R. and Chance, B. (1969) J. Biol. Chem. **244**, 2317-2324.
12. Thurman, R.G. and Scholz, R. (1977) Eur. J. Biochem. **75**, 13-21.
13. Bergmeyer, H.U., ed. (1970) Methoden der Enzymatischen Analyse, Verlag Chemie, Weinheim.
14. Eacho, P.I. and Weiner, M. (1980) Drug Metabol. Disp. **8**, 385-389.
15. Sies, H. and Chance, B. (1970) FEBS Letters **11**, 172-176.
16. Chance, B., Legallis, V., Sorge, J. and Graham, N. (1975) Anal. Biochem. **66**, 498-514.
17. Burnett, K.G. and Felder, M.R. (1978) Biochem. Genet. **16**, 1093-1105.
18. Koop, D.R., Nordbloom, G.D. and Coon, M.J. (1984) Arch. Biochem. Biophys. **235**, 228-238.
19. Danis, M., Kauffman, F.C., Evans, R.K., Holtzclaw, D., Reinke, L.A. and Thurman, R.G. (1985) Biochem. Pharmacol. **34**, 609-616.
20. Foerster, E.C., Fährenkemper, T., Rabe, U., Graf, P. and Sies, H. (1981) Biochem. J. **196**, 705-712.
21. Oshino, N., Chance, B., Sies, H. and Bücher, T. (1973) Arch. Biochem. Biophys. **154**, 117-131.
22. Ishii, H., Horie, S. and Suga, T. (1980) J. Biochem. **87**, 1855-1858.
23. van den Branden, C., Kerckaert, I. and Roels, F. (1984) Biochem. J. **218**, 697-702.
24. Bartlett, G.R. (1952) Quart. J. Stud. Alc. **13**, 583-589.
25. Kinard, F.W., Nelson, G.H., and Hay, M.G. (1956) Proc. Soc. Exp. Med. Biol. **92**, 772-3.
26. Nelson, G.H., Kinard, F.W., Hall, J.C. and Hay, M.J. (1956) Quart. J. Stud. Alc. **18**, 343-348.
27. Berry, M.N., Fanning, P.C., Grivell, A.R. and Wallace, P.G. (1980) Biochem. Pharmacol. **29**, 2161-2168.