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ROLE OF PEROXISOMAL FATTY ACID BETA-OXIDATION IN ETHANOL METABOLISM

Nobuhiro Inatomi, Shinzo Kato, Daisuke Ito and Charles S. Lieber*

Alcohol Research and Treatment Center, Bronx VA Medical Center, and Mt. Sinai School of Medicine, CUNY, New York, NY 10468

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The contribution of peroxisomal fatty acid ß-oxidation to ethanol metabolism was examined in deermice hepatocytes. Addition of 1 mM oleate to hepatocytes isolated from fasted alcohol dehydrogenase (ADH)-positive deermice in the presence of 4-methylpyrazole or to hepatocytes from fasted or fed ADH-negative deermice produced only a slight and statistically not significant increase in ethanol oxidation. Lactate (10 mM), which is not a peroxisomal substrate, showed a greater effect on ethanol oxidation. There was also a lack of oleate effect on the oxidation of ethanol by hepatocytes of ADH-positive deermice. Furthermore, in ADH-negative deermice, the catalase inhibitor azide (0.1 mM) did not inhibit the increase in ethanol oxidation by oleate and lactate. The rate of oleate oxidation by hepatocytes from fasted ADH-negative deermice was much lower than that of ethanol. These results indicate that in deermice hepatocytes, peroxisomal fatty acid oxidation does not play major role in ethanol metabolism.

Alcohol dehydrogenase (ADH) is considered to play a major role in ethanol oxidation. Non-ADH pathways are also contributory, as indicated by the observation of ethanol metabolism in deermice lacking the low Km hepatic ADH (1,2). There is a controversy, however, on the respective role of two such non-ADH pathways, i.e. the cytochrome P-450 dependent microsomal ethanol oxidizing system (MEOS) and catalase. The contribution of the catalase-H_2O_2 system to ethanol oxidation has been considered to be minimal, because H_2O_2 generation in living systems is very low (3) and ethanol oxidation is insensitive to the catalase inhibitor aminotriazole (2). However, Handler and Thurman (4,5) found that long chain fatty acids, such as oleate and palmitate, stimulate ethanol uptake by perfused livers of fasted rats in the presence of 4-methylpyrazole (4-MP), an ADH inhibitor, or of ADH-negative deermice. More recently, they also reported (6) that oleate stimulated the uptake of methanol (a substrate of catalase-H_2O_2) by livers of fasted, but not of fed rats, whereas oleate inhibited the uptake of butanol (a substrate of ADH) by livers of fasted and fed rats.

*To whom all correspondence should be addressed at Alcohol Research & Treatment Center, Bronx VA Medical Center, 130 West Kingsbridge Road, Bronx, New York 10468.

Abbreviations: 4-MP, 4-methylpyrazole; MEOS, microsomal ethanol oxidizing system.

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From these data obtained with methanol and butanol, they concluded that an increased supply of H_2O_2 due to peroxisomal ß-oxidation of fatty acids can explain the non-ADH pathway of ethanol metabolism. It is noteworthy, however, that methanol is also a substrate for ADH and that both methanol and butanol are also substrates for the microsomal system (7). In the present study, we tested the hypothesis of Handler and Thurman by examining the effect of oleate on ethanol oxidation in deermice hepatocytes. We also compared the effect of oleate with those of lactate and glucose which are not peroxisomal substrates, but which, like oleate, can serve as an energy source.

EXPERIMENTAL PROCEDURES

Animals. Female deermice, 6 to 15 months old, Peromyscus maniculatus, with genotypes for ADH, ADH^F/ADH^F (ADH-positive, ADH^+) and ADH^N/ADH^N (ADH-negative, ADH^-) were used. These animals were bred in our laboratory from an original stock kindly provided by Dr. M. Felder. Deermice were fed Lab-Blox (Wayne Food Division, Continental Grain Co., Chicago, IL), and given water ad lib.

Materials. Collagenase (type IV), bovine serum albumin, fatty acid free bovine albumin, oleate, lactic acid, sodium azide, and hyamine hydroxide were purchased from Sigma Chemical Co. (St. Louis, MO). KCN was obtained from J.T. Baker Chemical Co. (Phillipsburg, N.J.). 4-MP and petroleum ether were purchased from the Aldrich Chemical Co. (Milwaukee, WI), and [1,2-^{14}C]ethanol from Research Products International Corp. (Mount Prospect, IL). [1-^{14}C]Oleic acid, obtained from ICN Radiochemicals (Irvine, CA), was converted to its sodium salt according to the method of Debeer et al. (8).

Experimental procedure. Hepatocytes were isolated from fed or 18-20 hr fasted animals as described previously (9), except that glucose free Krebs bicarbonate buffer was used as isolation medium and the hepatocytes were suspended in Krebs Henseleit buffer (pH 7.4) containing 4% fatty acid free albumin. The average viability (as assessed by the trypan blue exclusion test) was 84%. The rate of ethanol oxidation by hepatocytes was determined as described before (10). Cells (0.5-1.0 X 10^6) and substrate were placed in small air tight vials and the reaction was started by adding [1,2-^{14}C]ethanol containing 35-40 dpm/nmole at 50 mM final concentration. The glass vials were capped and oxygenated and shaken at 37°C for 30 min. The reaction was stopped by adding 0.5 ml of 5N NaOH. The solution was transferred to a scintillation vial, the contents of the vials were evaporated to dryness, and the radioactivity was determined in a Beckman liquid scintillation counter (LS3801).

Oxidation of oleate by deermice hepatocytes was measured as described by Mannaerts et al. (11). Hepatocytes prepared from fasted ADH^- deermice were suspended in Krebs Henseleit buffer containing 4% fatty acid free albumin, and incubations were carried out in the main chamber of 10 ml Erlenmeyer flasks. The incubation mixtures had a final volume of 1 ml and contained [^{14}C]oleate (specific activity 0.5 Ci/ mol). The flasks were gassed with O_2:CO_2 (95:5), stoppered, and incubated at 37°C for 30 min with shaking. Reactions were terminated by adding 0.5 ml of 6% perchloric acid, and labeled CO_2 was trapped in hyamine hydroxide. The reaction mixture was centrifuged, and an aliquot of the supernatant was neutralized to pH 8 with 0.5N KOH. The tubes were placed in ice for 30 min and then centrifuged to remove $KClO_4$. An aliquot of the supernatant was analyzed for the ^{14}C content of the ketone bodies (ß-hydroxybutyrate plus acetacetate). Another aliquot of the supernatant was adjusted to pH 4 with 3M acetate buffer, extracted with petroleum ether to remove traces of [^{14}C]oleate, and part of the aqueous phase was counted for acid soluble oxidation products. Statistical significance was determined by paired Student t-test.

RESULTS

The ethanol oxidation rate was highest in hepatocytes from fed ADH^+ deermice (5.37 ± 1.14 nmol/10^6 cells/min ; n=4), followed by fasted ADH^+ (4.11 ± 0.77 nmol/10^6 cells/min; n=11), fed ADH^- (3.40 ± 1.16 nmol/10^6 cells/min; n=10), and fasted ADH^- (2.11 ± 0.39 nmol/10^6 cells/min; n=10) deermice hepatocytes.

Table 1. Effect of oleate and lactate on ethanol oxidation by hepatocytes of fasted ADH$^+$ deermice

Addition	Ethanol oxidation (nmol/10^6cells/min)
None	3.26 ± 0.81
4 MP (4 mM)	0.86 ± 0.33
4MP + oleate (0.5 mM)	0.66 ± 0.30
4MP + oleate (1 mM)	1.25 ± 0.64
4MP + lactate (10 mM)	1.35 ± 0.75

Data represent mean ± S.E. of 5 experiments. Ethanol oxidation was significantly inhibited by 4MP ($p<0.001$). In the presence of 4-MP (4 mM), no significant stimulation of ethanol oxidation was observed with addition of oleate (0.5 and 1 mM) or lactate (10 mM).

4-MP (4 mM) inhibited ethanol oxidation by 74% in hepatocytes from fasted ADH$^+$ deermice (Table 1). In the presence of 4-MP (4 mM), addition of oleate (0.5 mM) to hepatocytes did not increase ethanol oxidation. Oleate (1 mM) and lactate (10 mM) increased ethanol oxidation slightly but not significantly.

Addition of 1 mM oleate stimulated ethanol oxidation by hepatocytes from fasted ADH$^-$, fed ADH$^-$ and fasted ADH$^+$ deermice by 15-24%, but the effect was not statistically significant (fig. 1). Stimulation by oleate was not observed in hepatocytes from fed ADH$^+$ deermice. On the other hand, lactate (10 mM) significantly stimulated ethanol oxidation by

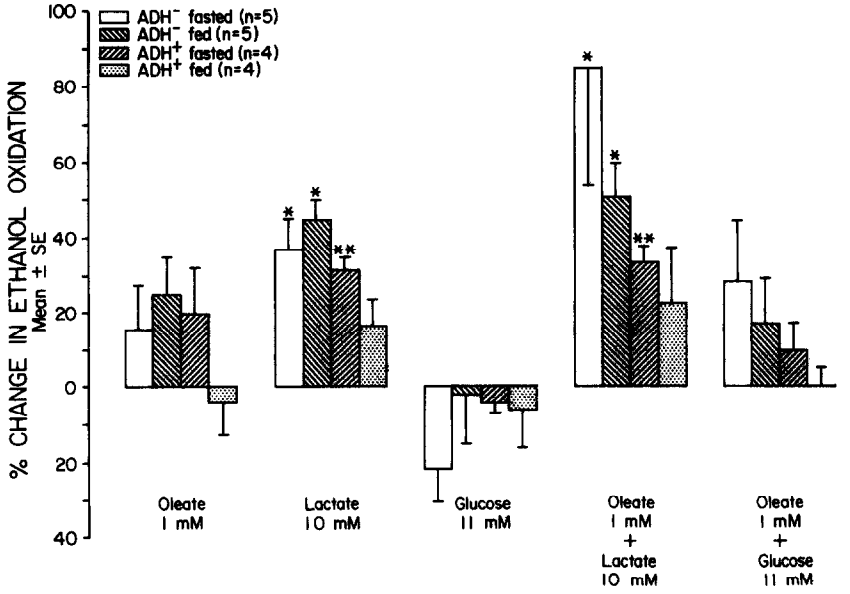

Fig. 1 Effect of oleate, lactate and glucose on ethanol oxidation by hepatocytes obtained from ADH$^-$ and ADH$^+$ deermice. Data represent mean ± S.E. of 4 or 5 experiments. * $P<0.05$, ** $P<0.01$ as compared to control.

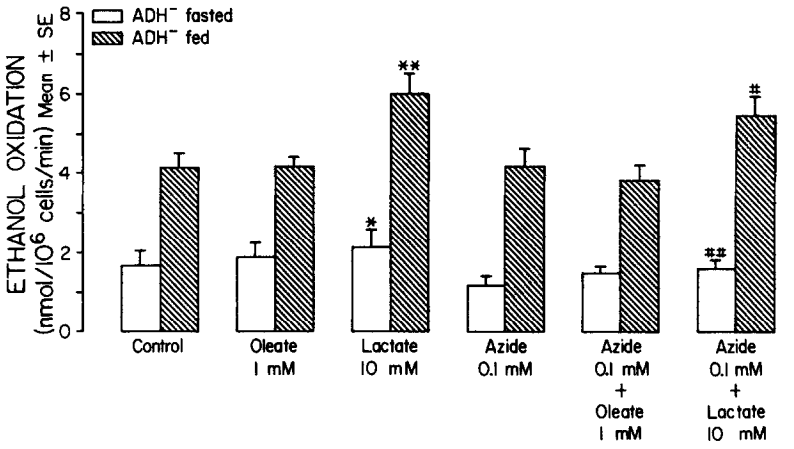

Fig. 2 Effect of oleate and lactate on ethanol oxidation by hepatocytes obtained from fasted and fed ADH⁻ deermice in the presence or absence of azide. Data represent mean ± S.E. of 5 experiments.
* P<0.05, ** P<0.01 as compared to control.
# P<0.05, ## P<0.01 as compared to the group treated with 0.1 mM azide.

hepatocytes from fasted ADH⁻, fed ADH⁻ and fasted ADH⁺ deermice by 31-44% (P<0.05), whereas the stimulation by lactate was not significant in hepatocytes from fed ADH⁺ deermice. Glucose (11 mM) did not stimulate ethanol oxidation by hepatocytes from both ADH⁻ and ADH⁺ deermice. Although a marked increase in ethanol oxidation by hepatocytes from fasted ADH⁻ deermice was observed with a combination of oleate (1 mM) and lactate (10 mM), the potentiation was not observed in hepatocytes from fed ADH⁻ and fasted ADH⁺ deermice. Glucose (11 mM) did not affect the stimulation by oleate.

Treatment of hepatocytes from fasted ADH⁻ deermice with 0.1 mM azide, a catalase inhibitor, slightly decreased ethanol oxidation (fig. 2). In the presence of azide, significant stimulation of ethanol oxidation by lactate (10 mM) but not by oleate (1 mM), was observed. In hepatocytes of fed ADH⁻ deermice, 0.1 mM azide did not decrease the ethanol oxidation rate (fig. 2). There was no significant stimulation of ethanol oxidation by oleate (1 mM) in the absence or presence of 0.1 mM azide. Lactate (10 mM) stimulated ethanol oxidation even in the presence of 0.1 mM azide.

The rate of oleate oxidation was much lower than that of ethanol oxidation (Tables 1 and 2). Oleate oxidation was not affected by 50 mM ethanol, but it was decreased 97% in the presence of 2 mM KCN, an inhibitor of mitochondrial ß-oxidation.

DISCUSSION

Handler and Thurman (4,5) reported that oleate or palmitate markedly increase ethanol uptake by perfused livers from fasted ADH⁻ deermice and by perfused rat livers treated with 4-MP (4 mM). They also reported that oleate (1 mM) stimulates methanol uptake by livers of fasted, but not of fed rat (6). However, in the present study, there was no significant increase in ethanol oxidation rate by oleate (0.5 and 1 mM) in hepatocytes from fasted ADH⁺ deermice in the presence of 4-MP. Oleate (1 mM) slightly increased ethanol

Table 2. Effect of cyanide and ethanol on oleate oxidation by hepatocytes of fasted ADH⁻ deermice

Addition	CO_2	Ketones	Acid-soluble oxidation products
	nmol/10^6 cells/min		
None	0.063 ± 0.015	0.651 ± 0.085	0.727 ± 0.108
KCN (2 mM)	0.001 ± 0.001*	0.019 ± 0.003*	0.025 ± 0.005*
Ethanol (50 mM)	0.052 ± 0.004	0.674 ± 0.102	0.760 ± 0.133

* P<0.01 as compared to no KCN.
Hepatocytes from fasted ADH⁻ deermice were incubated with [1-^{14}C]oleate (1 mM) at 37°C for 30 min. All results are expressed in terms of oleate equivalents oxidized per 10^6 cells per minute and are means ± S.E. of 5 experiments.

oxidation rate in hepatocytes from fasted or fed ADH⁻ deermice as well as from fasted ADH⁺ deermice, however, the effect of oleate did not reach statistical significance and was less than that by lactate (10 mM). The discrepancy between our results and those of Handler and Thurman (4,5) seems to be partly due to the difference of experimental conditions, i.e. we used isolated hepatocytes, whereas they used perfused livers. Furthermore, Handler and Thurman simply measured the decrease in ethanol concentration of the perfusate, which does not necessarily reflect ethanol metabolism, whereas the method used in the present study is based on the fact that acetate is a principal end product of ethanol metabolism.

In hepatocytes of fasted and fed ADH⁻ deermice, the catalase inhibitor azide (0.1 mM) did not produce a significant inhibition of ethanol metabolism. Kato et al. (12) also reported that hepatocytes prepared from deermice treated with aminotriazole, another catalase inhibitor, showed only a slight decrease in ethanol metabolism, whereas 1-butanol, a competitive inhibitor of MEOS, exerted a dose-dependent inhibition of ethanol oxidation in deermice hepatocytes. These findings suggest that MEOS, but not catalase, plays an important role in non-ADH ethanol metabolism. In the present study, stimulation of ethanol metabolism by oleate and lactate was observed in hepatocytes from fasted ADH⁻ deermice in the presence of azide. These results indicate that the stimulatory effect of oleate should not be ascribed to catalase-H_2O_2.

The rate of oleate oxidation by hepatocytes of ADH⁻ deermice was much lower than that of ethanol metabolism. In contrast with mitochondrial ß-oxidation, peroxisomal ß-oxidation is known to be insensitive to KCN (8). In this study, oleate oxidation was inhibited by 97% with 2 mM KCN. Mannaerts et al. (11) have also shown in rat hepatocytes that the contribution of peroxisomes to fatty acid oxidation was less than 10%, and that H_2O_2 produced by rat isolated hepatocytes in the presence of fatty acid was very low. According to their data, H_2O_2 productions by rat hepatocytes were 0.04 and 0.2 nmol/10^6 cells/min in the presence of 1 mM palmitate and 1 mM oleate, respectively. These findings indicate that if there is a contribution to ethanol oxidation of H_2O_2 produced by peroxisomal ß-oxidation of fatty acids, it must be very small.

It has been reported by several investigators that ethanol oxidation by rat liver is inhibited by long-chain fatty acids, probably because both ethanol and fatty acids provide reducing equivalents to the liver, and may compete for available NAD (13,14). In our study, using hepatocytes from fasted and fed ADH^+ deermice, oleate did not produce significant inhibition of ethanol oxidation, suggesting that the supply of NAD was not a limiting factor under these conditions.

Lactate stimulated ethanol oxidation in hepatocytes from fasted or fed ADH^+ and ADH^- deermice. Crow et al. (15) reported that the stimulation of ethanol oxidation by lactate in isolated rat hepatocytes was due to increased rate of transport of reducing equivalents into mitochondria *via* the malate-aspartate shuttle. This mechanism can explain the increased ethanol oxidation in hepatocytes from ADH^+ deermice in the absence, but not in the presence, of 4-MP, which blocks the generation of reducing equivalents. This explanation also does not apply to hepatocytes from ADH^- deermice in which NAD is not the cofactor of ethanol metabolizing enzymes. The stimulation of ethanol oxidation by lactate may be partly due to an increased energy demand, for instance for glucose synthesis (16).

In summary, oleate had only a slight and non significant effect on ethanol oxidation by deermice hepatocytes, whereas lactate had a significant stimulatory action. The slight oleate effect was unaffected by azide. Furthermore, the rate of peroxisomal ß-oxidation was too low to account for appreciable ethanol oxidation. From these findings, we conclude that peroxisomal fatty acid ß-oxidation does not play an important role in ethanol metabolism by hepatocytes.

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