INCREASED SUSCEPTIBILITY OF HEPATIC MITOCHONDRIA TO THE TOXICITY OF ACETALDEHYDE AFTER CHRONIC ETHANOL CONSUMPTION

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Summary

Acetaldehyde at concentrations known to occur in vivo significantly depresses respiration with NAD-dependent substrates and oxidation of fatty acids to CO_2 in mitochondria of rats fed ethanol chronically. This toxic effect of acetaldehyde may explain, in part, the mechanism of progressive liver injury in the alcoholic.

Chronic ethanol consumption causes liver injury associated with morphological evidence of damage of mitochondria in man (1, 2) and in experimental animals (3, 4). Functional disturbances of these mitochondria have also been reported in recent years including their fragility (5), decreased respiration (6) and depressed fatty acid oxidation (7, 8).

However even at concentrations found after heavy drinking, ethanol itself is not toxic to mitochondria in vitro (9, 10). Therefore it has been postulated that acetaldehyde, the first metabolite of ethanol, is the toxic substance responsible for injury. Indeed toxic effects of acetaldehyde on mitochondrial respiratory functions (8-10) and fatty acid oxidation (11) have been described. But acetaldehyde concentrations required to achieve these effects (0.5 - 20 mM) were much higher than the levels which are known to occur in vivo, which range from approximately 50 μM in the blood of alcoholics (12) to 200 μM in rat liver tissue (13) and in hepatic venous blood (14); at these lower "physiological" concentrations, acetaldehyde did not affect functions of normal mitochondria. We wondered however whether mitochondria impaired by chronic ethanol consumption can sustain their functions in the

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presence of acetaldehyde even at these low levels. To study this question, effects of acetaldehyde on hepatic mitochondrial respiration and fatty acid oxidation were studied by using rats fed ethanol chronically and their pair-fed controls, an experimental model in which liver mitochondria were shown before to be altered by ethanol (3).

Methods

Female Sprague-Dawley rat littermates were pair-fed nutritionally adequate liquid diets with 36% of total calories as ethanol or isocaloric carbohydrate for 4-6 weeks as described in detail elsewhere (15). Liver mitochondria were prepared (16). The isolated hepatic mitochondria were suspended in a medium consisting of 225 mM mannitol, 75 mM sucrose, 10 mM $MgCl_2$, 0.5 mM Na ethylenediaminetetraacetic acid (EDTA) and 10 mM phosphate buffer (pH 7.4). Mitochondrial oxygen consumption was determined polarographically with a Clark oxygen electrode at 26°C using a 3 ml reaction system in the medium mentioned above with 1.5 - 3 mg mitochondrial protein and 3.3 mM glutamate, β-hydroxybutyrate, or succinate. To initiate State 3 conditions (17), 675 nmoles adenine 5' -diphosphate (ADP) was added with 15 μl of the buffer. In some experiments 2, 4-dinitrophenol, (DNP, an uncoupler of oxidative phosphorylation) was employed at final concentrations of 50 and 10μM. Acetaldehyde was included at final concentrations of 200 μM and 1 mM, and after 3 min of preincubation ADP and DNP were added.

Respiratory control ratio (RCR) and ADP/O ratio were determined as described (18). Fatty acid oxidation was studied by measuring CO_2 production at 37°C using 1-^{14}C-labeled palmitic and oleic acids, adjusted to approximately 1250 dpm per nmole. The reaction system consisted of 3 ml of the medium without EDTA, 0.2% defatted bovine albumin, 0.4 mM malate, 3mM carnitine, 3 mM adenine triphosphate (ATP), 1.5 mM ADP, 3-5 mg of mitochondrial protein and 100 μM of substrate. The incubation was conducted for 30 min in the absence or presence of acetaldehyde added at an initial concentration of 200 μM. To replace the acetaldehyde oxidized in this system, 300 nmoles were supplied at 10 and 20 min; measurements of acetaldehyde concentrations showed that the initial concentration was not exceeded.

The CO_2 produced was trapped by 0.3 ml of hyamine hydroxide in the center-well and the content of the center-well was collected in a scintillation fluid (Aquasol). The radioactivity was determined in a liquid scintillation counter. The concentrations of acetaldehyde were measured by gas liquid chromatography. The results were expressed as means ± SEM and the statistical significance of the difference of means was calculated by the paired Student t-test.

Results and Discussion

After chronic ethanol treatment, rates of mitochondrial oxygen consumption with NAD-dependent substrates were depressed under both State 3 and 4 conditions as re-

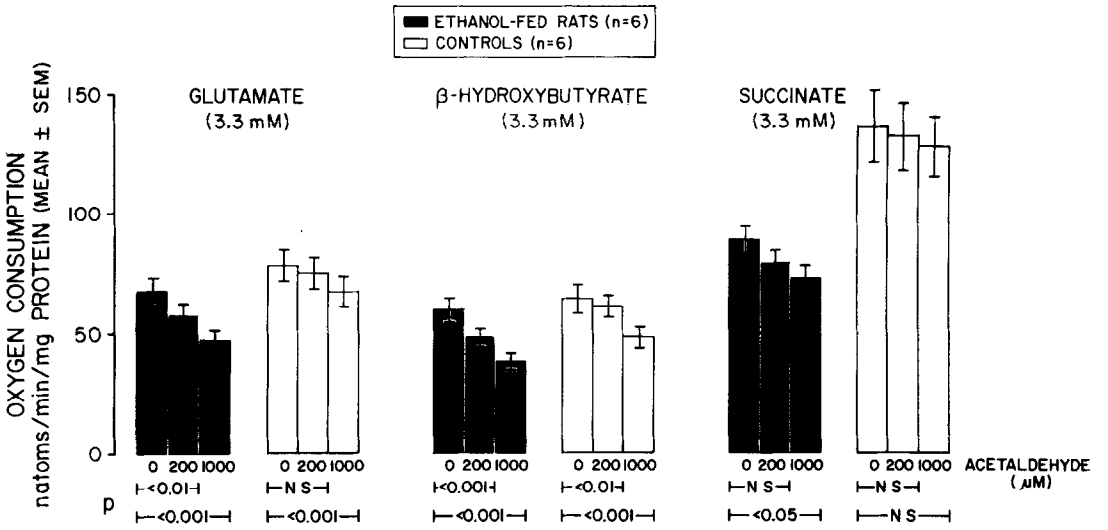

Fig. 1: Effect of acetaldehyde and chronic ethanol treatment on State 3 mitochondrial oxygen consumption.

1.5 - 3 mg of mitochondrial protein was incubated at 26°C as described in detail in the text. Increasing acetaldehyde concentrations resulted in progressive impairment of oxygen consumption, particularly in mitochondria of alcohol fed rats and with glutamate or β-hydroxybutyrate as substrate.

ported previously (6). In the absence of acetaldehyde the respiratory control ratios (RCR) did not significantly differ in both groups (5.0 ± 0.3 in ethanol-treated vs. 5.2 ± 0.3 in controls with glutamate, 4.2 ± 0.2 vs 4.3 ± 0.1 with β-hydroxybutyrate, 4.2 ± 0.2 vs 4.4 ± 0.1 with succinate respectively). In the ethanol-treated group the oxygen consumption with glutamate and β-hydroxybutyrate as substrates (which supply electrons to energy coupling Site I of the respiratory chain) was significantly depressed by adding 200 μM acetaldehyde under State 3 condition (Fig. 1). No decrease of oxygen consumption was observed with succinate (a Site II substrate). In the control group no effect was observed at the low acetaldehyde concentration with glutamate and β-hydroxybutyrate, but a depression was obtained at high acetaldehyde level (1 mM).

Table 1. Effect of acetaldehyde on hepatic mitochondria after chronic ethanol treatment in 6 pairs of rats.

	Ethanol-treated rats			Control rats		
	without acetaldehyde	with acetaldehyde 200 μM	with acetaldehyde 1000μM	without acetaldehyde	with acetaldehyde 200 μM	with acetaldehyde 1000 μM
ADP/O ratio						
glutamate (3.3mM)	2.47 ± 0.07	2.35 ± 0.05*	2.29 ± 0.06**	2.59 ± 0.05	2.52 ± 0.04	2.52 ± 0.05
β-hydroxybutyrate (3.3mM)	2.40 ± 0.03	2.21 ± 0.05**	2.09 ± 0.07**	2.64 ± 0.06	2.60 ± 0.07	2.48 ± 0.07*
succinate (3.3mM)	1.77 ± 0.04	1.70 ± 0.05	1.72 ± 0.03	1.71 ± 0.04	1.72 ± 0.05	1.70 ± 0.02
O_2 consumption after DNP stimulation (natoms O_2/min/mg protein)						
glutamate (3.3mM)	80.8 ± 7.4	78.5 ± 6.3	72.1 ± 6.4*	72.2 ± 5.7	72.8 ± 4.7	71.7 ± 3.3
β-hydroxybutyrate (3.3mM)	35.8 ± 3.5	36.8 ± 3.5	34.8 ± 2.2	44.8 ± 7.8	44.2 ± 6.8	43.6 ± 6.8
Fatty acid oxidation ($^{14}CO_2$ produced dpm/min/mg protein)						
palmitate	318.9 ± 35.3	192.1 ± 32.0**	———	376.0 ± 65.5	327.1 ± 58.6	———
oleate	287.9 ± 63.0	193.0 ± 58.1**	———	339.4 ± 80.0	291.0 ± 71.7	———

* and ** indicate p values less than 0.05 and 0.01 respectively, as determined by the comparison of the means in the presence and absence of acetaldehyde.

This was associated with a decreased ADP/O ratio in the ethanol-treated group (Table 1).

These results indicate that acetaldehyde, even at low levels, affects the respiratory chain of impaired mitochondria, particularly at energy coupling Site I. Acetaldehyde itself is known to be a NAD-dependent substrate for aldehyde dehydrogenase. When oxygen consumption was measured with 200 μM acetaldehyde itself as a substrate, the rate of oxygen consumption under State 3 condition was again lower in the ethanol-treated group (17.6 ± 1.1 nanoatoms per min per mg protein) than in controls (21.6 ± 2.1) ($p<0.05$), and the RCR was significantly decreased (2.05 ± 0.11 vs 2.46 ± 0.14 respectively; $p<0.001$).

Furthermore after dissociation of the respiratory chain from the oxidative phosphorylation by an uncoupler (2,4-dinitrophenol), the effects of acetaldehyde on oxygen consumption with Site I substrates were completely abolished even in the ethanol-treated group (Table 1).

Rates of CO_2 production from 1-^{14}C-palmitate and oleate were slightly but not significantly lowered in mitochondria of ethanol-treated rats (Table 1). Acetaldehyde at a concentration of 200 μM (and less) significantly depressed rates of fatty acid oxidation in ethanol-treated rats (Table 1), whereas there was no significant effect in the controls. The inhibition of fatty acid oxidation by acetaldehyde could contribute significantly to the development of the fatty liver after chronic ethanol consumption.

The increase by chronic ethanol consumption of the toxicity to mitochondria may be of particular relevance to the pathogenesis of alcohol induced liver injury in view of our prior observation of higher blood acetaldehyde levels in alcoholics than in controls (12), secondary, in part, to decreased acetaldehyde oxidation by the mitochondria (19). Increased blood levels probably reflect higher liver acetaldehyde

content. The development of both higher acetaldehyde levels and increased mitochondrial susceptibility to acetaldehyde toxicity after chronic ethanol consumption may represent a particularly pernicious combination leading to progressive injury to the liver, if our in vitro results can be extrapolated to the in vivo situation.

Thus, the theory of a "vicious cycle" (20) induced by chronic alcohol consumption whereby higher acetaldehyde levels lead to mitochondrial injury (including decreased capacity to metabolize acetaldehyde which in turn elevates acetaldehyde levels even further) has now been given experimental support by the demonstration that after chronic ethanol consumption acetaldehyde becomes indeed toxic to mitochondria even at the low concentrations which occur in vivo after ethanol ingestion.

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