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Control of Adenine Nucleotide Translocation in Liver Mitochondria from Ethanol-Fed Rats

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CUNNINGHAM, C. C., P. I. SPACH, R. E. BOTTENUS AND S. FILUS. *Control of adenine nucleotide translocation in liver mitochondria from ethanol-fed rats.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 63–66, 1980.—Male rats developed fatty liver after being fed an ethanol-containing diet for 31 days. Liver mitochondria from these animals (ethanol mitochondria) catalyzed ATP synthesis at a slower rate than did mitochondria from pair-fed control rats (control mitochondria). Furthermore, ATP translocation was decreased in ethanol mitochondria and parameters influencing such were investigated. Several experiments indicated that ADP uptake into ethanol mitochondria is not decreased due to inhibition of the adenine nucleotide translocase by either long chain acyl CoA derivatives or unesterified fatty acids. Analyses of endogenous adenine nucleotides in ethanol mitochondria revealed lower ATP concentrations, but no decrease in total adenine nucleotides. In experiments where endogenous ATP was shifted to higher concentrations by incubation with BSA, the rate of ADP translocation was increased, with a linear correlation being observed between endogenous ATP concentrations and the rate of ADP translocation. The depressed ATP concentration in ethanol mitochondria suggests that the ATP synthetase complex is replenishing endogenous ATP at a slower rate. A decrease in the rate of ATP synthesis in ethanol mitochondria is sufficient to explain the decreased ADP translocation.

Chronic ethanol feeding Liver mitochondria ATP synthesis ADP translocation

SEVERAL studies, including the present investigation, have demonstrated that liver mitochondria from rats fed an ethanol-containing diet synthesize ATP at a slower rate [1,11]. This decreased rate of ATP synthesis may be related to the observation that ATP levels are lowered approximately 50% in hepatic tissue of rats maintained on an ethanol-containing diet [4]. In this communication we report an investigation of those factors which influence the rate at which ATP is synthesized. This study, which focuses on control mechanisms for ADP transport into mitochondria, suggests that the ATP synthetase complex [8] functions at a slower rate in mitochondria from rats that have developed ethanol-induced fatty liver.

METHOD

Male Sprague-Dawley rats and most of the reagents were obtained from sources listed previously [7,10]. All other reagents were of the highest purity commercially available.

Male rats weighing 150–250 g were fed for 31 days a nutritionally adequate diet [3] in which ethanol provided 36% of the calories. Pair-fed controls received the same diet, but with maltose/dextrin isocalorically substituted for ethanol. Preparation of tightly coupled mitochondria, protein determinations, and measurements of respiratory rates, unesterified fatty acid levels, and the fatty acid compositions of mitochondrial phospholipids were carried out as described previously [10]. The rate of ATP synthesis was determined using the procedure for P/O ratio measurements [7]. Succinate (20 mM) was the oxidizable substrate for respiratory rate and ATP synthesis rate measurements. Translocation of ADP was measured at 0°C as described previously [7].

The endogenous adenine nucleotide (ATP, ADP, and AMP) content of mitochondria was measured by the method of Williamson and Corkey [13] in freshly prepared mitochondria and in mitochondria incubated with defatted bovine serum albumin (BSA) or oxidizable substrates. These incubations resulted in an elevation of the endogenous levels of ATP. Long chain acyl CoA thioesters were measured as described by Tubbs and Garland [12].

RESULTS

As is shown in Table 1, the rate of ATP synthesis with succinate as oxidizable substrate is decreased approximately 30% in mitochondria isolated from ethanol-fed animals. This decrease in the rate of ATP synthesis is accompanied by a comparable drop in the state 3 respiratory rate. The data in Table 1 demonstrate a major decrease in respiration in the presence of ADP, which is not observed when ethanol mitochondria are uncoupled. It suggests that the adenine nucleotide transport mechanism, rather than the electron transport chain, is responsible for the decreased respiratory activity under conditions where ATP is being synthesized at

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TABLE 1

EFFECTS OF ETHANOL ADMINISTRATION ON ATP SYNTHESIS, ELECTRON TRANSPORT PROPERTIES, AND ADP TRANSLOCATION IN RAT LIVER MITOCHONDRIA

	Liquid diet control	Ethanol-fed	% Decrease	p Values
Experiment 1*				
ATP synthesized (μmol/min/mg protein)	0.35 ± 0.03	0.25 ± 0.02	29	<0.01
Experiment 2†				
Rate of electron transport‡				
State 4 (−ADP)	0.058 ± 0.003	0.050 ± 0.003	14	<0.05
State 3 (+ADP)	0.27 ± 0.01	0.17 ± 0.01	36	<0.001
Experiment 3§				
Rate of electron transport‡ + Uncoupler	0.28 ± 0.01	0.29 ± 0.01	—	N.S.¶
Experiment 4#				
ADP translocation	1.93 ± 0.25	1.14 ± 0.12	41	<0.05

*Fifteen animals in each group. Values reported are average ± S.E.M. in this table and for data following. p values were obtained from the Student's t test for 2 means.

†Forty-eight animals in each group.

‡μG atoms 0/min/mg protein.

§Six control animals and five ethanol animals; one preparation of the pair-fed ethanol animals did not exhibit respiratory control and was omitted. Carbonyl cyanide p-trifluorophenylhydrazone (0.56 μM) was used as an uncoupler.

¶N.S., not significant.

#Amount of ADP translocated into mitochrondria in 2 min at 0°C; units=nmol/mg mitochondrial protein. Nine animals in each group.

TABLE 2

EFFECT OF CHRONIC ETHANOL CONSUMPTION ON THE ADENINE NUCLEOTIDE CONTENT OF MITOCHONDRIA*

Animal group	ATP	ADP	AMP	Total adenine nucleotides
Liquid diet control	4.96 ± 0.35	2.23 ± 0.19	4.49 ± 0.40	11.06 ± 0.85
Ethanol-fed	2.94 ± 0.26	2.66 ± 0.34	6.54 ± 0.39	12.14 ± 0.67
p	$p<0.001$	N.S.†	$p<0.005$	N.S.

*nmol/mg mitochondrial protein ± the S.E.M.; n=12 for both groups.

†N.S., not significant.

a maximal rate. Indeed, ADP translocation into mitochondria (Table 1) is lowered significantly in ethanol organelles.

Since unesterified fatty acids and long chain acyl CoA derivatives are known to inhibit the activity of the mitochondrial adenine nucleotide translocation system, experiments were carried out to determine whether the decreased ADP uptake was due to an increased influence of these two naturally occurring inhibitors. We have eliminated these lipophilic compounds as being important in lowering the rate of ADP translocation in ethanol mitochondria for the following reasons: (1) The unesterified fatty acid concentrations are not significantly different in ethanol and control mitochondria and are in amounts too low to inhibit the adenine nucleotide translocase [10]. (2) The long chain acyl CoA derivatives are equal in concentration in ethanol and control mitochondria (0.46 ± 0.03 μmol/mg mitochondrial protein). (3) Incubation of mitochondria with carnitine to lower the concentration of long chain acyl CoA derivatives [6] did not result in an increase in ADP translocation in ethanol mitochondria. Control experiments with added palmitoyl CoA and carnitine demonstrated the ability of carnitine to reverse inhibition of ADP translocation by long chain acyl CoA derivatives.

Since the adenine nucleotide translocase normally functions to exchange cytoplasmic ADP for mitochondria matrix ATP, the possibility exists that lowered ADP translocation in ethanol mitochondria is related to decreased levels of endogenous ATP. Table 2 demonstrates a significant decrease in ATP and an increase in AMP in ethanol mitochondria, with the total adenine nucleotide content being comparable in

TABLE 3

RELATIONSHIP BETWEEN ENDOGENOUS ATP CONTENT AND ADP TRANSLOCATION IN ETHANOL MITOCHONDRIA*

Incubation conditions	ATP content (nmol/mg mitochondrial protein)	ADP translocation† (nmol/mg mitochondrial protein)
No additions	2.30 ± 0.40	1.80 ± 0.24
Glutamate-malate-P_i	5.42 ± 0.79	3.33 ± 0.39

*Ethanol mitochondria were incubated with 2 mM glutamate-2 mM malate-0.5 mM P_i at 10°C for 1 min. The ATP content and ADP translocation activity of incubated aliquots were compared with those of the same mitochondria that were not incubated. Five preparations were utilized.

†Amount of ADP translocated into mitochondria in 2 min at 0°C.

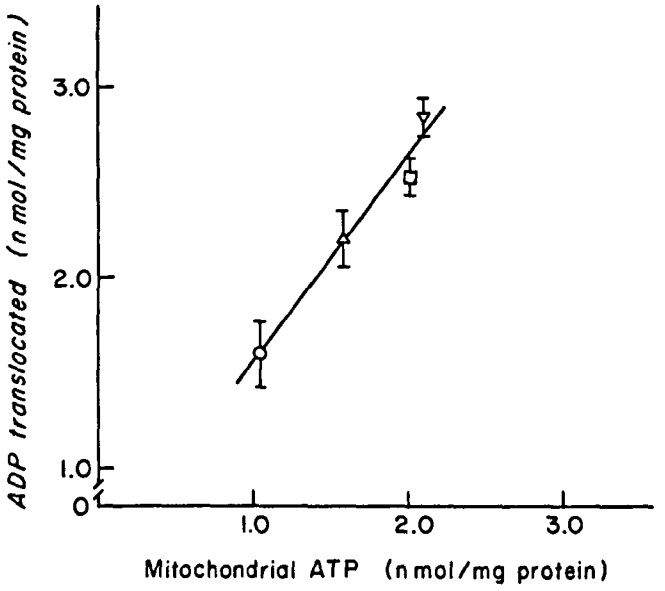

FIG. 1. The effects of defatted BSA on ATP content and ADP translocation in mitochondria. Mitochondria were incubated with defatted BSA (1 mg/mg mitochondrial protein) for 4 min at 0°C. After incubation the mitochondria were washed by centrifugation before adenine nucleotide levels were measured. ADP translocation activities were carried out on incubated but unwashed, preparations. Atractyloside-sensitive ADP translocation was measured for 2 min. n=7 for each group. Experimental protocol:

	Animal source	Incubation
○	Ethanol-fed	No additions
△	Ethanol-fed	Defatted BSA
□	Liquid diet control	No additions
▽	Liquid diet control	Defatted BSA

ethanol and control preparations. Shifting the ATP content in ethanol mitochondria to higher values by incubating with oxidizable substrate (glutamate-malate-P_i) resulted in dramatically increased ADP translocation activity (see Table 3). In a similar experiment in which both control and ethanol mitochondria were incubated with defatted BSA, both the endogenous ATP content and ADP translocation activity increased in control and ethanol mitochondria (Fig. 1). Figure 1 demonstrates that there is a direct relationship between the concentration of endogenous ATP and the amount of ADP translocated into the mitochondria in 2 minutes.

Ethanol-induced alterations in the fatty acid composition of phospholipids from liver mitochondria accompany the changes in functional properties of the organelle. The more notable changes are decreased arachidonic acid/linoleic acid ratios in phosphatidyl choline (23% decrease, $p<0.05$) and phosphatidyl ethanolamine (32% decrease, $p<0.1$). Moreover, the linoleic acid content of cardiolipin from ethanol mitochondria was 39.5 ± 5.3 mol % as compared to 55.5 ± 2.0 mol % in control mitochondria (29% decrease, $p<0.01$).

DISCUSSION

This study demonstrates that the rates of ATP synthesis and ADP translocation are decreased significantly in liver mitochondria isolated from ethanol-fed animals. The study supports the supposition that the rate of ADP uptake into ethanol mitochondria is limited by lowered endogenous ATP levels (see Tables 2 and 3, and Fig. 1). The demonstration in Table 2 that it is the distribution of adenine nucleotide that is altered, rather than the amount available as substrate for the ATP synthetase complex [8], is a key observation. It suggests that the rate of ADP translocation and the state 3 respiratory rate are controlled by the rate of ATP synthesis, and not vice versa.

The ATP synthetase complex may not be functioning at a rate rapid enough to maintain normal levels of ATP. The lowered content of exchangeable ATP depresses the rate at which ADP can be translocated into the mitochondrion. This idea is supported by previous observations demonstrating that the ATP synthetase has lowered activity in ethanol mitochondria [5, 9, 10, 11]. The alterations in the fatty acid composition may influence the activity of the ATP synthetase by changing its microenvironment in the inner mitochondrial membrane. The dramatic drop in the linoleic acid content of cardiolipin is of major interest since we have observed previously that this particular phospholipid stimulates the ATPase activity of the ATP synthetase complex to higher V_{max} values than any other phospholipid [2].

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