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The Effect of Carnitine on the Oxidation of Palmitate in Alcohol Fed Rat Liver Mitochondria

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1. The oxidation of palmitate in rat liver intact mitochondria of the alcohol fed group was found to be lower than that of the control group. However, the oxidation of acetate in intact mitochondria of the alcohol fed group was found to be almost the same as that of the control group. As for the oxidation of palmitate in sonicated mitochondria, no difference was found between the alcohol fed group and control group.

2. The stimulatory effect of carnitine on the oxidation of both palmitate and acetate in intact mitochondria, and on the oxidation of palmitate in sonicated mitochondria were diminished in the alcohol fed group.

3. The administration of alcohol to rat was found to have some pathological effect on carnitine barrier site of mitochondrial membrane.

Electron microscopic studies on the liver of alcohol fed rat have shown striking changes in the size and shape of mitochondria (1). The oxidation of fatty acid (2) and oxidative phosphorylation coupled to the oxidation of several substrates have been reported to be impaired in the mitochondria from alcohol fed rat as compared with those from normal one (3, 4). The evidence reported in this paper shows that the stimulatory effect of carnitine on the oxidation of palmitate decreases in the liver mitochondria from alcohol fed rat. This decrease appears to be important to clarify the mechanism of the impaired function of alcohol fed rat liver mitochondria.

EXPERIMENTAL

1. Animals and Preparation of Mitochondria

Twenty Wistar strain male rats (body weight of around 100 g) were fed on a synthetic diet (4) with free supply of water. In addition to the diet, ten of them were forcibly fed with 2.25 ml of an ethanol solution (30 % v/v) by gastric tube twice daily, and the other ten rats were forcibly fed with a glucose solution having the equivalent calory to the alcohol solution. The former were designated as the alcohol fed group, and the latter as the control group. After the maintenance for 15 days under these conditions, rats were killed by decapitation, and livers were quickly removed. The alcohl fed rats were killed twelve hours after the final alcohol administration. Liver mitochondria were prepared according to the method of Chance and Hagihara (5), and further washed twice to remove the cytoplasmic contaminants. Protein was determined by Biuret method (6) after dissolving the mitochondria with deoxycholate. The concentration of mitochondrial protein was adjusted to 60 mg per ml with a buffer medium containing 0.21 M mannitol, 0.07 M sucrose, 0.1 mM EDTA, and 10 mM Tris-HCl. This preparation was referred to as intact mitochondria. A part of the intact mitochondrial suspension was treated for 2 minutes by a sonic oscillation with a 5 A Super Sonic Vibrator (Model UR-150P, Tominaga Works Ltd.). The pre-

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paration was referred to as sonicated mitochondria.

2. *Measurement of Oxygen Uptake*

The oxygen uptake was measured pollarographically with a Clark-type oxygen electrode supplied by Beckman Co., Fullerton, Calif., The precise procedure is described in Table 1.

3. *Assay of Palmitate and Acetate Oxidation*

Intact or sonicated mitochondria (1.2 mg protein per ml) were incubated in the reaction medium containing 0.3 M mannitol, 10 mM KCl, 10 mM KH_2PO_4, pH 7.4, 2.5 mM $MgCl_2$, 0.25 mM EDTA, 0.4 mM ADP, 100 μM palmitate-U-^{12}C (5.4×10^8cpm/mμmoles, the Radiochemical Center, Amersham) with or without the addition of 0.6 mM DL-carnitine. The total volume of the reaction system was adjusted to 5 ml in a 25 ml flask which had a rubber cap with a hanging glass center well. In another series of experiments, 100 μM acetate-1-^{14}C (6.4×10^8cpm/mμmoles, Daiichi Pure Chemical Co., Ltd., Tokyo) was used in the place of palmitate-U-^{14}C. The flask was gassed with a mixture of O_2 and CO_2 (95 : 5), and was incubated at 37° for 30 min with constant shaking. Then, 0.4 ml hydroxide of hyamine 10-X (Packard Instrument Co., Ing., Illinois) was injected into the hanging well through the rubber cap. And 0.3 ml of 50% citric acid was also injected through the rubber cap into the reaction mixture to stop the reaction. The flask was incubated at 25° for additional 60 min with constant shaking to permit complete absorption of carbon dioxide by hyamine (7). An 0.1 ml aliquot of the hyamine solution was transfered to a scintillation medium to determine the radioactivity with a Packard Tricarb liquid scintillation spectrometer (8).

Results

1. *Mitochondrial Respiratory Rate*

The respiratory control ratio (RCI) of intact mitochondria from the alcohol fed group was lower than that from the control group, as shown in Table 1. This is apparently due to the fact that with intact mitochondria from the alcohol fed group the respiration rate at State 4 was accelerated as compared with the control group, but the rate at State 3 remained unchanged. However, when sonicated mitochondria were employed, the respiration rates at State 3 and 4 were almost identical, and the respiratory control disappeared. Practically no significant difference was observed between the control and experimental groups.

TABLE 1

State 4 and State 3 respiration rate, and RCI of control group and alcohol fed group mitochondria

Group	State 4 Respiration rate (a)	State 3 Respiration rate (b)	RCI (b)/(a)
	mμatoms O/min/ml	*mμatoms O/min/ml*	
Intact control	24	132	5.5
Intact alcohol fed	35	133	3.8
Sonicated control	80	97	
Sonicated alcohol fed	82	95	

The reaction mixture contained 0.3 M mannitol, 10 mM KH_2PO_4, pH 7.4, 10 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM EDTA and 2 mM succinate as substrate. The reaction was started by the addition of mitochondria (2 mg protein per ml) into the reaction mixture. Following two minutes meaurement (State 4 respiration), 0.2 mM ADP was introduced to the system (State 3 respiration). The final volume of each reaction mixture was 10 ml. The reaction temperature was 25°.

2. *The Oxidation of Palmitate to Carbon Dioxide in Control and Alcohol Fed Group Mitochondria with or without Carnitine*

As shown in Fig. 1, the oxidation of palmitate to carbon dioxide considerably decreased in the alcohol fed group as compared with the control group. In the control group,

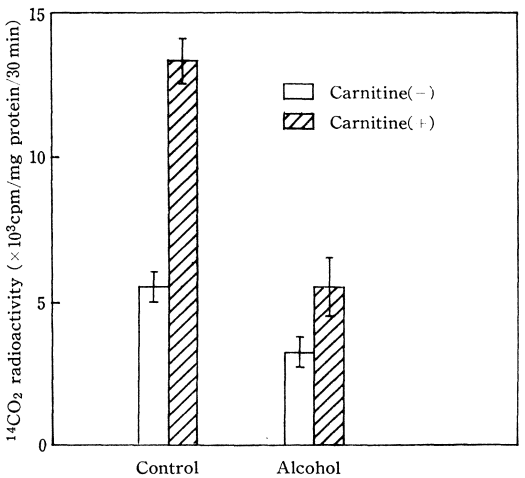

FIG. 1 *The Effect of carnitine on the oxidation of palmitate in intact rat liver mitochondria obtained from the control or alcohol fed group*

The experimental conditions were described in the text. Each column shows the mean value of ten experimental animals with standard deviation.

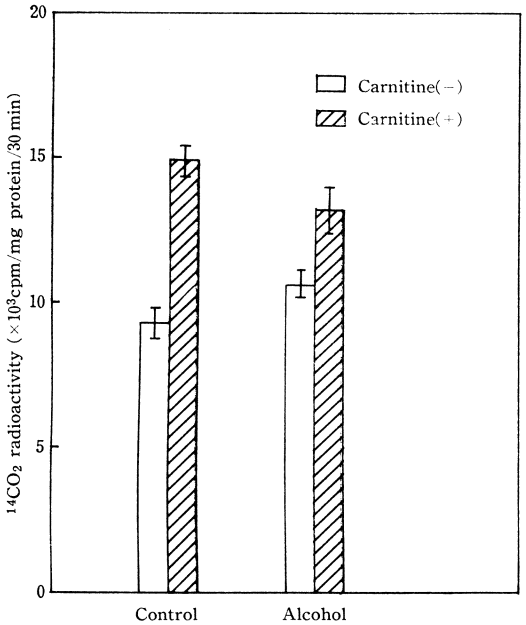

FIG. 2 *The effect of carnitine on the oxidation of acetate in intact rat liver mitochondria obtained from the control or alcohol fed group*

The experimental conditions were described in the text, except 100 μM sodium acetate-1-^{14}C(6.4×10³cpm/mμmoles, Daiichi Pure Chemical Co., Tokyo) in the place of palmitate-U-^{14}C.

TABLE 2
The stimulatory effect of carnitine on palmitate and acetate oxidation in control and alcohol fed group mitochondria

Conditions		Carnitine(+)/Carnitine(−) Ratio
Mitochondria	Substrate added	
Intact control	Palmitate	2.4
Intact alcohol fed	Palmitate	1.7
Intact control	Acetate	1.6
Intact alcohol fed	Acetate	1.2
Sonicated control	Palmitate	2.2
Sonicated alcohol fed	Palmitate	1.2

Carnitine(+)/Carnitine(−) Ratio was calculated from the values of $^{14}CO_2$ radioactivity (×10³cpm/mg protein/30 min) with carnitine/$^{14}CO_2$ radioactivity (×10³cpm/mg protein/30 min) without carnitine.

the addition of 0.6 mM DL-carnitine to the reaction system stimulated the oxidation of palmitate 2.4 times, whereas in the alcohol fed group, the stimulatory effect of carnitine was 1.7 times.

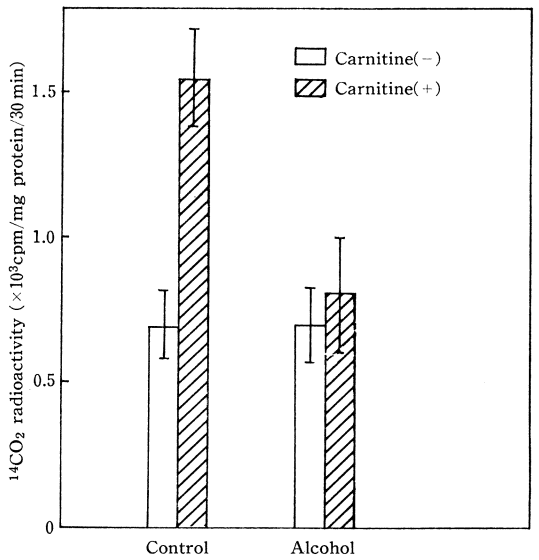

FIG. 3 *The effect of carnitine on the oxidation of palmitate in sonicated rat liver mitochondria obtained from the control or alcohol fed group*

The experimental conditions were the same as described in Fig. 1, except that sonicated mitochondria obtained from the control or alcohol fed group were employed.

3. The Oxidation of Acetate to Carbon Dioxide in Control and Alcohol Fed Group Mitochondria with or without Carnitine

As shown in Fig. 2, in the absence of carnitine, acetate was oxidized at almost the same rates in both control and experimental groups. But the stimulatory effect of carnitine on the acetate oxidation was slightly depressed in the alcohol fed group as shown in Table 2.

4. The Oxidation of Palmitate to Carbon Dioxide in Sonicated Control and Alcohol Fed Group Mitochondria with or without Carnitine

When mitochondria were disrupted sonically, the activity of palmitate oxidation decreased to approximately 12 %. In the absence of carnitine the same rate of palmitate oxidation was observed in the normal and alcohol fed groups, as shown in Fig. 3.

Nevertheless, the stimulatory effect of carnitine was apparently decreased in the alcohol fed group in consistent with the results obtained with the intact mitochondria.

This is also evident in the ratio of carnitine (+)/carnitine(−), as shown in Table 2.

Discussion

In the previous paper (4), we reported that in the serum of the alcohol fed rat, the concentrations of alcohol, acetaldehyde and free fatty acids were increased, and that this increase was supposed to disturb the mitochondrial oxidative phosphorylation. In this communication, we studied the effect of carnitine on the fatty acid oxidation in alcohol fed rat liver mitochondria.

It is well known that carnitine stimulates the oxidation of fatty acid (9). As shown in the previous paper (4) and in Table 1, RCI was lowered in the intact alcohol fed group mitochondria comparing with the intact control group mitochondria, because the State 4 respiration rate was somewhat accelerated in the alcohol fed group. However the State 3 respiration was almost the same as that of the intact control group. With sonicated mitochondria, the State 4 and State 3 respiration rate were almost the same in both the groups.

The decreased effect of carnitine on the palmitate oxidation in liver mitochondria of the alcohol fed group is of interest and this effect is suggestive of mechanism of pathological role of alcohol on the palmitate oxidation and on the mitochondrial membrane state. In intact mitochondria, as shown in Fig. 1, the palmitate oxidation decreased in the alcohol fed group. This difference became more prominent by carnitine addition. The stimulatory effect of carnitine was 2.4 times in the control group, as against 1.7 times in the alcohol fed group. This stimulatory ratio of carnitine was more clearly suppressed in the sonicated mitochondria of the alcohol fed group. As shown in Fig. 3, the palmitate oxidation to carbon dioxide in the absence of carnitine by sonicated mitochondria was almost the same in the both groups. In contrast, the stimulatory effect of carnitine was almost negligible in the alcohol fed group, but in the control group the stimulatory effect was prominent. From these results it is evident that the stimulatory effect of carnitine was considerably lowered in the alcohol fed group. These results suggest that in the alcohol fed group, carnitine barrier site of mitochondrial membrane is physiologically disarranged. This must be one of pathogenic factors responsible for the decrease in the oxidation of palmitic acid. It is interesting to find that carnitine also revealed the stimulatory effect on the acetate oxidation. This fact is supposed to be suggesting that carnitine also has some connection with short chain fatty acid activation. After complete understanding of the mechanism of disturbed carnitine function, the disturbance of lipid metabolism in relation to alcohol drinking could be expected to be clarified.

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