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PROTECTIVE ACTION OF CARNITINE ON LIVER LIPID METABOLISM AFTER ETHANOL ADMINISTRATION TO RATS*

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Abstract—Ethanol administration to rats causes a significant increase in colonic temperature 18 hr after drug administration. Carnitine administration to such animals did not significantly decrease that temperature. Carnitine gave some protection against increases in SGOT levels in animals receiving ethanol. This protective action was independent of its effect on liver lipids. Carnitine significantly lowered blood triglycerides, but this action occurred with both normal rats and rats receiving alcohol. Higher amounts of carnitine (0·5 mg/g of body weight rat) produced a significant decrease in the liver triglycerides. This effect was more marked in animals receiving ethanol than in control rats. Carnitine also showed a significant lipotropic action which was reflected in a diminution of the total lipid content in liver. Again, the effect was more marked in animals receiving ethanol than in controls.

Many investigators have shown that on the administration of acute doses of ethanol to animals, there is an almost immediate infiltration of the liver with fat [1–4]. The fatty acids which comprise this fat material are derived either from adipose tissue, from the diet or through *de novo* fatty acid synthesis [5–7].

Kalant [8] has suggested that the consumption of ethanol by rodents and other animals is a stressful condition. Such stress may be responsible for the release of free fatty acids from the adipose tissue and glucose from glycogen [9, 10]. Because these effects on fat and carbohydrate metabolism occur in fed animals, several investigators have suggested that ethanol produces these biochemical manifestations not through dietary deficiencies but through its pharmacologic properties as a hepatotoxic substance [11].

Rubin *et al.* [11] have shown that subsequent to the administration of ethanol to rats, the respiration of mitochondria harvested from these animals is low. In addition, these authors found that protein synthesis *in vitro* was inhibited as well as fatty acid oxidation. Other authors have shown that subsequent to ethanol administration there is an accumulation of α-glycerophosphate, malic and lactic acids, and indeed the ratio of NADH/NAD is increased [12, 13]. These observations suggest that the oxidative metabolism in the liver cell was presumably inhibited in some way, thereby causing the extramitochondrial accumulation of these shuttle substrates. Lehninger [14] showed that intact mitochondria were impermeable to NADH; on the other hand, other authors [15, 16] showed that the shuttle substrates, α-glycerophosphate and malic acid (formed through NADH reduction of 3-phosphoglyceraldehyde and oxaloacetic acid), can enter mitochondria. Because these shuttle substrates accumulated extramitochondrially after alcohol administration, the question arises as to the mechanism of their accumulation. While the extramitochondrial accumulation of these substrates may possibly explain why the mitochondrial respiration is low during alcohol intoxication, it does not account for the decrease in fatty acid oxidation observed. Perhaps many of these biochemical alterations contributing to the overall hepatotoxic phenomena induced by ethanol may well be related to a common pharmacologic property of ethanol on liver mitochondrial structure and function.

An alteration in mitochondrial structures after ethanol administration [9] would be expected to lead to changes in permeability [17], an accumulation of extramitochondrial substrates [12, 13], and decreased respiration [11] with concomitant decreased ATP synthesis [17]. Since the integrity of the cell is dependent on ATP synthesis, many authors [18, 19] have shown that reduction in ATP synthesis *in vivo* leads to cellular damage or death. Cellular damage *in vivo* has been assayed by measuring the movement of certain enzymes such as CPK, transaminase and aldolase from muscle and/or liver into blood [20–22]. In fact, certain authors [23, 24] have shown that after ethanol administration the SGOT level is high, indicative of some level of cellular damage.

In the present investigation we have studied the capacity of carnitine to restore metabolic balance from that previously altered by ethanol administration. Since carnitine normally facilitates fatty acid transport and oxidation into mitochondria [25–27], perhaps a very high liver acyl carnitine level *in vivo* was needed to enable oxidation of part of the ethanol-induced increase in hepatic triglycerides to take place, thereby simultaneously decreasing the liver lipid content and improving the energetics of the cell.

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METHODS

Male Sprague–Dawley rats (150–200 g) were treated as follows. *Group A.* (1) Starved: these rats were starved for 2 days before being sacrificed. (2) Control-fed: these rats received adequate food and water before being sacrificed. (3) Alcohol-injected: these rats received two injections of ethanol (4 g/kg of body weight) intraperitoneally as a 20% solution in saline. The injections were 12 hr apart and the rats were sacrificed 24 hr after the first injection. (4) Alcohol + carnitine: these rats were treated in the same manner as those in group A–3, except that carnitine (0·1 mg/kg of body weight) was added to the injected solution.

Group B. (1) Control-glucose: these rats were starved for 2 hr and then received glucose (10·5 g/kg of body weight), i.e. isocaloric with the alcohol given to group A-3 rats as a 35% solution by gastric intubation. (2) Glucose-carnitine: these rats were treated, like those in group B-1, except that carnitine (0·1 mg/g or 0·5 mg/g) was added to the solution. (3) Alcohol: these rats received alcohol (6 g/kg) by gastric intubation of a 40% solution (w/v) in saline. (4) Alcohol-carnitine: these rats were treated like those in group B-3, except that carnitine (0·1 mg/g body weight or 0·5 mg/g) was added to the solution.

Body (colonic) temperature was monitored after ethanol administration with a Yellowsprings Digital Temperature Probe. The probe was inserted into the colon and maintained at a fixed depth for 10–15 sec or until temperature readings stabilized. Blood was collected after decapitation; the liver and heart were removed and washed in cold physiological saline. The blood was centrifuged and the serum collected for assay of transaminase activity (SGOT) and analysis of blood triglycerides.

SGOT determination. The transaminase activity of the serum was assayed by the LKB 8600 Reaction Rate Analyser, which measures the change in absorbance of NAD at 340 nm.

Blood triglycerides. An aliquot of serum (0·2 ml) was hydrolyzed by addition of ethanol KOH (0·5 ml of a 0·5 N solution). The mixture was incubated for 25 min at 60–70° and then cooled; $MgSO_4$ (1 ml of a 3·7 g/100 ml solution) was added and the solution centrifuged. The supernatant was analyzed for glycerol by the LKB 8600 Reaction Rate Analyzer.

Hepatic triglycerides. Total lipids were extracted from 2 g liver according to the method of Folch *et al.* [28] and quantified by the weight of the dried liver. An aliquot of the total lipid extract containing approximately 20 mg fat was applied to a 0·5 mm thick Silica gel chromatoplate and developed in hexane–diethyl ether–acetic acid (83:16:1). After elution, the triglycerides were quantified according to the method of Snyder and Stephens [29].

RESULTS

Temperature. Alcohol administration to rats causes a significant increase ($P < 0·01$) in rectal temperature 18 hr after feeding. The rectal temperature of nine normal rats was 37·3° ± 0·055, while that of 10 alcohol-treated rats was 37·6° ± 0·037. Carnitine (0·5 mg/g) did not significantly lower this temperature (37·5° ± 0·11); similarly, the effect of carnitine on normal rats was also not significantly higher than in the control animals.

SGOT. As shown in Table 1(A), carnitine administration to rats appeared to lower the SGOT values in all experiments. However, only in those rats where ethanol was administered intraperitoneally was the decrease significant ($P < 0·05$). In this experiment, carnitine decreased the SGOT values by 24 per cent compared with rats which received alcohol alone.

In another experiment carnitine was added to the solution used in gastric intubation. Carnitine again caused a 19 per cent decrease in the SGOT values. These results were, however, less significant ($0·1 > P > 0·05$).

In the control experiment where rats received glucose and carnitine, carnitine caused an 11 per cent decrease in SGOT, but these results were not significant. Fasting caused no change in SGOT levels but there was an increase in hepatic lipids. Therefore, it would appear that the increase in SGOT caused by alcohol is not related to an increase in hepatic lipids. Similarly, the protection given by carnitine on ethanol-induced increased SGOT is not related to lipotropic activity.

Blood triglycerides. Carnitine (0·1 mg/g) significantly lowered blood triglycerides in all experiments shown in Table 1(B). Carnitine did not restore control levels but did produce significant lowering (23 per cent) of the blood triglycerides.

In the control experiments, carnitine caused a similar lowering of triglyceride levels (22 per cent). Thus, it would appear that the protective effect of carnitine is a normal physiological effect independent of the effect of alcohol.

Although the decrease in blood triglycerides paralleled the decrease in liver lipids, these effects do not appear to be directly related. In the liver (below), higher amounts of carnitine gave the maximal effect, whereas in the blood lower amounts of carnitine showed the maximal effect. Thus, carnitine may be acting either on peripheral tissues (adipose tissue, muscle) to increase fatty acid oxidation or perhaps to increase uptake of blood triglycerides.

Liver triglycerides. As shown in Table 1(C), alcohol causes significant increases in liver triglycerides which are not affected by lower amounts of carnitine (0·1 mg/g). Higher amounts of carnitine (0·5 mg/g of body weight) give significant ($P < 0·05$) lowering and restore the liver triglyceride levels to normal. This represents a 50 per cent decrease in hepatic triglycerides.

Higher amounts of carnitine also decreased hepatic triglycerides in the glucose control experiments. This decrease, however, was less marked (22 per cent) and less significant ($0·1 > P > 0·05$). Thus, higher amounts of carnitine gave increased protection to animals receiving alcohol by decreasing hepatic triglycerides.

Total hepatic lipid. Carnitine caused significant lowering of hepatic lipids in all experiments summarized in Table 1(D). In the rats which received ethanol by intraperitoneal injection, carnitine caused a significant ($P < 0·05$) decrease (18 per cent) of hepatic lipids. Tube-fed rats receiving lower amounts of carnitine (0·1 mg/g) also had a similar significant ($P < 0·01$) decrease (19 per cent).

Table 1. Influence of acute ethanol and carnitine administration to rats on SGOT and lipid metabolism*

	Starved	Control		Carnitine		Alcohol		Alcohol + carnitine
A. SGOT† (μU/ml serum)	Mean (10) 217 S.E.M. 18·8	(10) 226 29·1		(C_1 = 0·1 mg/kg C_2 = 0·5 mg/kg)		(7) 505 31	$t_{4,5}$ = 2·196 P = 0·05 Δ4,5 = 0·24	(8) 382 24
SGOT‡		(9) 263 5·3				(10) 358 26·8	$t_{4,5}$ = 1·971 P = 0·1 Δ4,5 = 0·19	(8) 289 23·2
		(6) 202 17·1	$t_{2,3}$ = 1·173 P = 0·3 Δ2,3 = 0·11	(6) 179 C_1 9·6 (6) 180 C_2 10·4				
B. Serum‡ (mg triglyceride/ 100 ml serum)	Mean S.E.M.	(10) 73 2·1 87 5·3	$t_{2,3}$ = 2·836 P = 0·02 Δ2,3 = 0·22 $t_{2,3}$ = 2·088 P = 0·05	(6) 68 C_1 4·1 (6) 71 C_2 4·1		(10) 144 9·9	$t_{4,5}$ = 2.946 P = 0·01 Δ4,5 = 0·23	(9) 111 5·2
C. Liver‡ (mg triglyceride/ 2 g liver)	Mean S.E.M.	(6) 45 6	$t_{2,3}$ = 1·630 P = 0·2	(6) 30 C_1 3·75 34·5 C_2 1·5	$t_{2,4}$ = 2·985 P = 0·01	(9) 68 9	$t_{4,5}$ = 2·671 P = 0·05 Δ4,5 = 0·50	(8) 79 C_1 9 39 C_2 3
D. Liver† (total mg triglyceride/ 2 g liver)	Mean (9) 73 S.E.M. 7·7	(10) 61 3·8	$t_{1,5}$ = 2·75 P = 0·02			(7) 92 6·2	$t_{4,5}$ = 2·394 Δ4,5 = 0·18	(10) 75 3·4
		(6) 66 4·6	$t_{2,3}$ = 2·679 P = 0·05 Δ2,3 = 0·22	(6) 61 C_1 4·5 51 C_2 3·2		(10) 77 2·4	$t_{4,5}$ = 3·000 P = 0·01 Δ4,5 = 0·19	(9) 62 4·4

* Number of samples is given in parentheses.
† Injected animal.
‡ Tube-fed animal.

In control animals, lower amounts of carnitine did not decrease lipid content, but higher amounts of carnitine (0·5 mg/g) did give a significant ($P < 0·05$) lowering (22 per cent). Thus, carnitine again caused a decrease in total liver lipids in animals receiving acute alcohol.

DISCUSSION

Carnitine caused a lowering of SGOT values in all experiments discussed. The significant protection in the ethanol-injected animals perhaps reflects the greater toxic effect of alcohol on liver metabolism when administered parenterally. This protective action does not appear to be related to body temperature nor to a decrease in liver lipids, since with the latter the SGOT values are not proportional to the liver lipid values. Also, fasting animals showed an increase in liver lipid but no increase in SGOT levels. Thus, this protective action of carnitine is a separate but simultaneous effect.

Carnitine may thus act indirectly by somehow stimulating mitochondrial metabolism. Alcohol appears to reduce brain mitochondrial membrane permeability after chronic administration [17]. There is also evidence that, after alcohol treatment, carnitine-specific entry sites on the liver mitochondrial membrane may be altered [30]. By facilitating the transfer of substrates across such altered membranes, carnitine may be stimulating oxidative processes within such mitochondria. An increase in metabolic activity in the liver cell could then initiate repair of many biochemical systems which were deranged by the toxic effects of the alcohol. Hyams and Isselbacher [31] have reported that large doses of ATP do protect animals against acute ethanol-induced fatty liver, whereas moderate amounts were ineffective [32].

Rubin *et al.* [11] have shown that carnitine *in vitro* does not alter the rate of fatty acid oxidation by liver mitochondria from normal or chronic ethanol-fed rats. Katsumata [30], in a similar series of experiments, concluded that ethanol administration to rats caused "a pathological effect on carnitine barrier site" of the liver mitochondrial membrane, causing it to be "physiologically disarranged". These results suggest that carnitine *in vitro* is incapable of reversing the ethanol-induced effect on mitochondrial impermeability. Results from the present experiments show that carnitine *in vivo* can somehow partly overcome this induced derangement. If elevated SGOT levels are indicative of cellular damage, then the reversal effect of carnitine on SGOT levels and on blood and liver triglycerides suggests that carnitine in some way improved the energetics of the cell. Gordon and Lough [9] showed that by day 7 of withdrawal after chronic ethanol feeding, liver mitochondrial structure and function had essentially returned to normal.

The mechanism by which this protective action of carnitine on ethanol-induced derangement takes place *in vivo* is not understood nor is the reason known why a similar reversal does not occur *in vitro*.

Lieber and De Carli [33] have shown that choline caused a decrease in hepatic triglycerides after chronic but not after acute ethanol feeding to rats. Kondrup and Grunnet [34] found that after acute and/or chronic alcohol administration to rats, there was an increase of liver carnitine and acetyl carnitine but a decrease in acyl carnitine. They suggested that this probably was an effect of the alcohol that inhibited or prevented the influx of free fatty acids to the liver. Lieber *et al.* [35] also are of the opinion that ethanol in some manner interfered with the oxidation of long-chain triglycerides.

Since results from the present experiments have shown that carnitine does cause a significant lowering of serum triglycerides and SGOT after acute ethanol administration, it would appear that perhaps the beneficial effect of extra carnitine after acute ethanol administration is to overcome this induced inhibition on long-chain fatty acid metabolism by increasing the acyl carnitine influx into mitochondria. This would mean that the accumulation of NADH [12, 13] and other substrates [15, 16] was not the limiting factor but rather that the induced impermeability in the membrane, which reduced their influx, was probably the most important effect of ethanol as a hepatotoxic substance.

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