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Effects of Ethanol Feeding on the Activity and Regulation of Hepatic Carnitine Palmitoyltransferase I

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The effects of ethanol administration on activity and regulation of carnitine palmitoyltransferase I (CPT-I) were studied in hepatocytes isolated from rats fed a liquid, high-fat diet containing 36% of total calories as ethanol or an isocaloric amount of sucrose. Cells were isolated at several time points in the course of a 5-week experimental period. Ethanol consumption markedly decreased CPT-I activity and increased enzyme sensitivity to inhibition by exogenously added malonyl-CoA. Changes in enzyme activity occurred sooner than those in enzyme sensitivity. Fatty acid oxidation to CO_2 and ketone bodies was depressed in hepatocytes from ethanol-fed animals during the first part of the treatment. At the end of the 35-day period, there were no longer differences in the rate of ketogenesis between the two groups. At that time, however, the rate of CO_2 formation was still impaired in the ethanol-fed animals. Furthermore, addition of ethanol or acetaldehyde to the incubation medium strongly depressed CPT-I activity and rates of fatty acid oxidation in hepatocytes from ethanol-treated rats, whereas these effects were much less pronounced in cells from control animals. The response of CPT-I activity to insulin, glucagon, vasopressin, and phorbol ester was blunted in cells derived from ethanol-fed rats. These changes in the regulation of CPT-I activity corresponded with those observed in the rate of fatty acid oxidation. It is concluded that CPT-I may play a role in the generation of the ethanol-induced fatty liver.

It is well established that both acute and prolonged ethanol administration cause accumulation of triacylglycerols in liver. This has been ascribed to a stimulatory effect of ethanol on the uptake, synthesis, and esterification of fatty acids in the liver and to an inhibitory effect of this drug on hepatic fatty acid oxidation. Changes in the rate of secretion of triacylglycerols as very low density lipoproteins (VLDL)[3] and enhanced mobilization of fatty acids from adipose tissue are also thought to contribute to this disorder (for review see Refs. (1–3)). The present study focuses on the effects of ethanol feeding on hepatic fatty acid oxidation, while the accompanying paper discusses alterations in hepatic fatty acid and glycerolipid synthesis (4).

Earlier studies have shown that ethanol inhibits hepatic fatty acid oxidation on the short term (1, 5–7) and that long-term ethanol consumption decreases oxygen uptake and fatty acid oxidation by hepatic mitochondria (8). Carnitine palmitoyltransfer-

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[3] Abbreviations used: CPT, carnitine palmitoyltransferase (EC 2.3.1.21); CPT-I and CPT-II, CPT activity located in the outer and the inner surfaces of the mitochondrial inner membrane, respectively; PMA, 4β-phorbol 12β-myristate 13 α-acetate; TDGA, tetradecylglycidic acid; DMSO, dimethyl sulfoxide; VLDL, very low density lipoproteins.

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ase I (CPT-I), the overt form of carnitine palmitoyltransferase, is generally considered to catalyze the rate-limiting step in the transport of long-chain fatty acids into the mitochondrial matrix, both in liver and in extrahepatic tissues (reviewed in (9, 10)). In physiopathological states characterized by variations in the rate of hepatic fatty acid oxidation, the flux through this step is changed in parallel (9–15). These long-term alterations in CPT-I activity are often accompanied by changes in enzyme sensitivity to malonyl-CoA, a physiological intracellular inhibitor of CPT-I activity (11–15). The short-term regulatory properties of the enzyme have not been studied as extensively as the long-term adaptive changes due to a lack of a reliable assay to preserve short-term modulation of CPT activity. Recently, we have designed a procedure for rapid measurement of cellular CPT-I activity which allows assessment of short-term changes in enzyme activity (16).

In the present paper we report the effects of ethanol feeding on the rates of hepatic fatty acid oxidation and on the activity and regulation of liver CPT-I in the course of a 35-day experimental period. The data suggest a crucial role for acetaldehyde—the first product of hepatic ethanol oxidation—in the inhibition of CPT-I activity and consequently in the rate of fatty acid oxidation after prolonged ethanol administration. In addition, the short-term regulatory properties of this enzyme are markedly altered by ethanol treatment.

MATERIALS AND METHODS

Materials. L-[*Me*-^{14}C]Carnitine was kindly provided by Dr. H. R. Scholte, Erasmus University, Rotterdam (The Netherlands); [1-^{14}C]oleate was from New England Nuclear, Dreieichenhain (FRG); phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma (St. Louis, MO); vasopressin was from Boehringer, Mannheim (FRG); tetradecylglycidic acid (TDGA) was a gift from Dr. J. M. Lowenstein, Brandeis University (Waltheim, MA); insulin and glucagon were donated by Lilly Research Laboratories (Indianapolis, IN); 4-methylpyrazole was from Aldrich (Milwaukee, WI); the origin of all other chemicals has been described in (16, 17).

Animals and their treatment. Male Wistar rats (195 ± 8 g initial body weight) were used in this study. They were housed and treated as described in the preceding paper (4).

Isolation and incubation of hepatocytes. Parenchymal liver cells were isolated, purified, and incubated as described in the preceding paper (4). Since stock solutions of PMA, TDGA, and 4-methylpyrazole were prepared in dimethyl sulfoxide (DMSO), control incubations had the corresponding DMSO content. No significant influence of DMSO on CPT-I activity and on the rate of fatty acid oxidation was observed at the concentration used (0.1%, v/v).

Measurement of CPT-I activity. In order to preserve short-term changes of CPT-I activity, rapid measurement of enzyme activity was assessed in permeabilized hepatocytes by monitoring the incorporation of L-[*Me*-^{14}C]carnitine and palmitoyl-CoA into palmitoylcarnitine. Further details of this novel assay are described elsewhere (16, 17).

Measurement of the rate of fatty acid oxidation. Isotope-containing incubations were run in parallel in order to monitor rates of fatty acid oxidation. Reactions were initiated by the addition of 0.4 mM albumin-bound [1-^{14}C]oleate (0.05 Ci/mol) and carried on for 30 min. Further details are given in (16, 17). Ketone bodies were determined as nonvolatile, acid-soluble products in the remaining flask content (18). Total oxidation products were calculated as the sum of CO_2 plus ketone bodies.

Other analytical procedures. Cell protein was measured by the method of Lowry *et al.* (19) using bovine serum albumin as a standard.

Statistical analysis. Results shown represent means ± SD of at least two different cell preparations with incubations carried out in triplicate. Statistical analysis was performed using the paired *t* test.

RESULTS

Effects of ethanol feeding on CPT-I activity and on fatty acid oxidation. The effects of ethanol administration on CPT-I activity and on fatty acid oxidation were studied in hepatocytes isolated from rats fed a liquid, high-fat diet containing 36% of total calories as ethanol (ethanol group) or an isocaloric amount of sucrose (control group) for 35 days. During this period ethanol consumption induced a marked increase of triacylglycerol levels in both liver and serum VLDL (4). CPT-I activity was determined in hepatocytes isolated from both groups of animals after 1, 14, and 35 days on the experimental diets. As can be inferred from Fig. 1A, ethanol feeding rap-

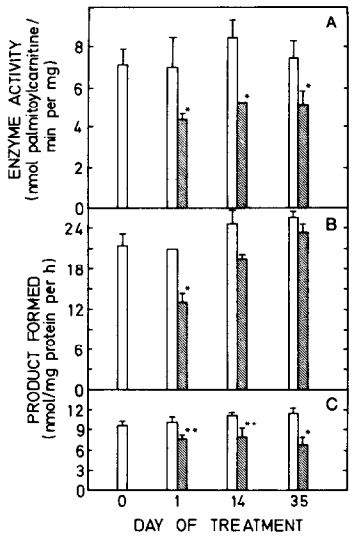

FIG. 1. Activity of carnitine palmitoyltransferase I and rate of fatty acid oxidation in hepatocytes isolated at different time points in the course of a 35-day dietary treatment period in ethanol-treated (shaded bars) and control (open bars) rats. (A) Carnitine palmitoyltransferase I activity; (B) fatty acid oxidation to ketone bodies; (C) fatty acid oxidation to CO_2. Error bars indicate ±SD. Significantly different from control: $^*P < 0.01$; $^{**}P < 0.05$.

idly decreased hepatic CPT-I activity. After 1 day of ethanol consumption the enzyme activity was reduced to about 65% of that measured in the control group. This difference in enzyme activity can still be observed at the end of the 5-week experimental period.

It is generally agreed that acute doses of ethanol inhibit fatty acid oxidation (5–7). This effect has been proposed to make a major contribution to the generation of a fatty liver after acute ethanol treatment (reviewed in (1)). The effects of continuous ethanol feeding on the rate of fatty acid oxidation, on the other hand, are less well established. As shown in Fig. 1C, ethanol administration produced a rapid decrease in the rate of CO_2 production from added oleate. This effect is still apparent in the ethanol-fed group after 5 weeks of dietary treatment. After 1 day of ethanol consumption ketone body formation was also depressed in hepatocytes from ethanol-fed animals as compared to control cells (Fig. 1B). However, upon continuation of ethanol feeding the rate of ketogenesis is gradually restored to control values at the end of the 35-day treatment period.

Effects of ethanol feeding on enzyme sensitivity to inhibitors. Ethanol administration also led to alterations in enzyme sensitivity to malonyl-CoA, a physiological intracellular inhibitor of CPT-I activity (9). At Day 1 and Day 14 of dietary treatment no significant effect of ethanol feeding on the sensitivity of hepatic CPT-I to added malonyl-CoA was observed (Fig. 2A). However, enzyme activity was markedly more sensitive to inhibition by malonyl-CoA in the ethanol-fed group than in the controls after 35 days of dietary treatment. Hence, differences in enzyme activity seem to precede alterations in enzyme sensitivity to malonyl-CoA.

Several synthetic inhibitors of CPT-I activity are available at present (20). The use of these compounds provides a tool for the study of the regulatory properties of this complex enzyme system. When transformed into its CoA ester, TDGA is a po-

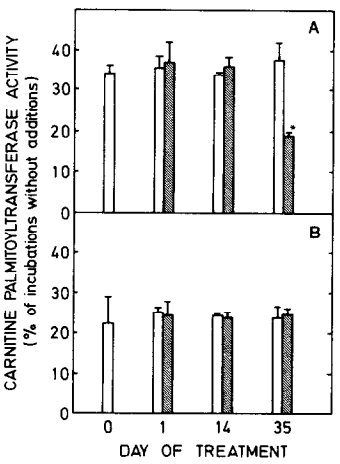

FIG. 2. Hepatic carnitine palmitoyltransferase I sensitivity to inhibitors as determined in hepatocytes isolated at different time points during a 5-week experimental period in ethanol-treated (shaded bars) and control (open bars) animals. (A) Enzyme activity was determined in the absence or presence of exogenously added malonyl-CoA (50 μM); (B) prior to measuring enzyme activity, cells were preincubated for 30 min with or without 10 μM TDGA. Error bars indicate ±SD. Significantly different from control: $^*P < 0.01$.

tent inhibitor which interacts specifically and directly with CPT-I (20). In agreement with previous data (21), addition of TDGA to hepatocytes strongly inhibited CPT-I activity (Fig. 2B) as well as the rate of fatty acid oxidation (not shown). No difference in the sensitivity of these two parameters to inhibition by TDGA was observed between hepatocytes isolated from ethanol-fed rats and those from control animals. Thus, chronic ethanol administration modifies CPT-I sensitivity to the physiological inhibitor malonyl-CoA but not to the synthetic inhibitor TDGA.

Short-term effects of ethanol and acetaldehyde on CPT-I activity and on fatty acid oxidation. The alterations in CPT-I activity and fatty acid oxidation as described above for rats fed the ethanol-containing diet may be accompanied by changes in the response of these two parameters to acute doses of ethanol. Hence, CPT-I activity and rates of fatty acid oxidation were also determined in cells incubated in the presence of 20 mM ethanol. Hepatocytes isolated from ethanol-fed rats on the first day after the initiation of the ethanol administration behave in the same manner as cells obtained from the control animals (Table I). In both types of hepatocytes, addition of ethanol to the incubation medium markedly decreased CPT-I activity and rates of fatty acid oxidation, mainly CO_2 formation. However, at Day 35 of the dietary treatment period, CPT-I activity and fatty acid oxidation in hepatocytes from ethanol-fed rats were much more sensitive to addition of ethanol to the incubation medium than were these parameters measured in cells isolated from control rats (Table I). Thus, prolonged ethanol administration to rats magnifies the ethanol-induced inhibition of CPT-I activity, as well as that of CO_2 formation and ketone body production from exogenous fatty acids. This may spare fatty acids for esterification and may thus contribute to the accumulation of triacylglycerols in the liver after chronic ethanol consumption.

Oxidation of ethanol in the liver produces acetaldehyde, which is primarily oxidized within mitochondria (22–24). Acetaldehyde is an extremely toxic compound with numerous effects on mitochondrial functions (25–27). Therefore, we examined the effects of acetaldehyde on CPT-I activity and on fatty acid oxidation in cells isolated from ethanol-fed and control rats at different times during a 5-week experimental period. Table I shows that addition of acetaldehyde to the incubation medium inhibited CPT-I activity as well as fatty acid oxidation after 1 day of initiation of the dietary treatment. Continuation of the dietary treatment led to an increased susceptibility of both CPT-I activity and fatty acid oxidation to inhibition by acetaldehyde in cells obtained from ethanol-fed animals, but not from controls (Table I). This toxic effect of acetaldehyde was indeed magnified when the conversion of acetaldehyde to ethanol was blocked by 4-methylpyrazole, a well-known inhibitor of alcohol dehydrogenase (28). Addition of 4-methylpyrazole alone to the incubation medium had no significant effect on CPT-I activity and fatty acid oxidation at the concentration used (0.5 mM). Furthermore, addition of 4-methylpyrazole to the incubation medium completely prevented the ethanol-induced inhibition of CPT-I activity and fatty acid oxidation (data not shown). Taken together, these data suggest that acetaldehyde may play an important role in the ethanol-induced inhibition of the fatty acid oxidative process.

Effects of ethanol feeding on short-term hormonal regulation of CPT-I activity and fatty acid oxidation. Short-term regulation of CPT-I activity and fatty acid oxidation by hormones was studied in hepatocytes isolated from ethanol-treated and control animals in the course of the 35-day dietary treatment period.

Insulin and glucagon displayed opposite effects on CPT-I activity. The former inhibited whereas the latter increased enzyme activity (Table II). At Day 1 of the dietary treatment, hepatocytes isolated from ethanol-fed rats showed the same sensitivity to these two hormones as did cells from control animals. However, continuation of ethanol administration led to loss of CPT-I sensitivity to short-term incubation with insulin and glucagon (Table II). These changes in the regulatory prop-

TABLE I

SHORT-TERM EFFECTS OF ETHANOL AND ACETALDEHYDE ON CARNITINE PALMITOYLTRANSFERASE-I ACTIVITY AND FATTY ACID OXIDATION IN ISOLATED RAT HEPATOCYTES

Addition	Type of diet (day)	CPT-I activity	Oleate oxidation		
			CO_2	Ketone bodies	Total oxidation products
		(% of incubations without additions)			
Ethanol (20 mM)	C-1	75.9 ± 7.8	60.4 ± 12.8	84.1 ± 10.9	76.4 ± 11.9
	E-1	69.4 ± 3.0	43.0 ± 6.6	89.1 ± 13.4	72.1 ± 6.2
	C-14	78.4 ± 2.2	65.2 ± 4.6	84.5 ± 14.1	78.8 ± 12.0
	E-14	64.4 ± 3.1*	38.3 ± 10.2**	76.8 ± 10.6	67.1 ± 8.7
	C-35	90.9 ± 2.9	65.0 ± 8.3	96.7 ± 5.7	86.8 ± 6.3
	E-35	57.4 ± 5.8**	31.9 ± 5.8**	74.0 ± 2.5**	64.8 ± 1.7**
Acetaldehyde (10 mM)	C-1	83.0 ± 7.4	63.7 ± 5.4	91.2 ± 8.6	82.2 ± 2.2
	E-1	84.9 ± 6.2	57.3 ± 9.9	84.7 ± 13.7	71.0 ± 5.2
	C-14	90.7 ± 8.3	59.3 ± 13.2	94.5 ± 8.6	83.5 ± 8.4
	E-14	69.4 ± 7.7*	46.1 ± 10.7	81.0 ± 10.8	71.0 ± 6.9
	C-35	87.4 ± 5.9	87.1 ± 9.8	94.6 ± 3.9	91.8 ± 0.9
	E-35	64.1 ± 5.7**	33.6 ± 14.6**	77.8 ± 2.4*	68.4 ± 0.4**
Acetaldehyde (10 mM) plus 4-methylpyrazole (0.5 mM)	C-1	83.1 ± 14.3	31.0 ± 2.7	83.4 ± 3.2	66.7 ± 3.8
	E-1	80.7 ± 5.7	33.4 ± 5.6	84.8 ± 7.4	65.8 ± 2.4
	C-14	81.4 ± 1.7	38.8 ± 2.5	86.2 ± 9.3	70.5 ± 3.2
	E-14	59.4 ± 5.8**	32.6 ± 4.1	81.0 ± 6.2	67.8 ± 10.0
	C-35	80.7 ± 8.1	54.2 ± 12.6	90.4 ± 4.6	78.9 ± 6.6
	E-35	46.9 ± 9.5**	18.0 ± 7.3**	68.9 ± 4.6**	57.8 ± 6.5**

Note. In the course of the 35-day dietary treatment period hepatocytes were isolated at the indicated days from ethanol-fed (E) or control (C) animals. Following a 20-min preincubation period of cells with the indicated additions, assays for CPT-I activity and oleate oxidation were started. Versus the respective control: $*P < 0.05$; $**P < 0.01$.

erties of CPT-I activity corresponded to parallel variations in the rate of fatty acid oxidation (Table II). Similarly, the response of CPT-I activity and fatty acid oxidation to inhibition by vasopressin was progressively blunted in hepatocytes isolated from ethanol-fed animals along the experimental period (Table II). Therefore, CPT-I activity becomes progressively insensitive to short-term hormonal regulation after chronic ethanol feeding. Tumor promoting phorbol esters such as PMA are able to mimic certain aspects of vasopressin action (29). Therefore, we studied the effects of PMA on CPT-I activity and on fatty acid oxidation during the dietary treatment period. As shown above for vasopressin, long-term ethanol consumption led to a progressive loss of response of both CPT-I activity and fatty acid oxidation to inhibition by PMA (Table II).

DISCUSSION

In the present paper we have presented the effects of ethanol feeding for 1, 14, or 35 days on the activity and regulation of CPT-I in isolated rat hepatocytes made permeable with digitonin. Parallel incubations were run to monitor rates of fatty acid oxidation. The results of this study show a good correlation between changes in hepatic CPT-I activity and those in the rate of fatty acid oxidation, supporting the idea that this enzyme represents an important control point of the fatty acid oxidative process (reviewed in (9, 30)).

As can be seen in Fig. 1A, ethanol administration leads to a decrease of CPT-I

TABLE II

EFFECTS OF ETHANOL ON SHORT-TERM HORMONAL CONTROL OF CARNITINE PALMITOYLTRANSFERASE-I ACTIVITY AND OF FATTY ACID OXIDATION IN ISOLATED HEPATOCYTES

Additions	Day	CPT-I		Total oxidation products	
		Control	Ethanol fed	Control	Ethanol fed
		(% of incubations without additions)			
Insulin (85 nM)	0	85.0 ± 7.8		84.6 ± 1.0	
	1	88.7 ± 1.0	90.4 ± 2.1	87.6 ± 1.2	88.1 ± 4.6
	14	88.1 ± 4.5	93.8 ± 1.6	82.3 ± 1.9	89.2 ± 2.0
	35	86.4 ± 3.2	95.3 ± 2.4*	83.6 ± 3.8	95.7 ± 0.6*
Glucagon (10 nM)	0	120.7 ± 2.5		129.7 ± 3.3	
	1	130.2 ± 19.2	136.8 ± 1.8	122.8 ± 10.7	128.1 ± 11.2
	14	132.1 ± 6.3	110.0 ± 10.6*	122.0 ± 8.7	109.9 ± 0.4*
	35	136.4 ± 4.1	112.3 ± 6.3*	134.7 ± 0.5	115.5 ± 7.8*
Vasopressin (100 nM)	0	81.9 ± 6.7		87.8 ± 2.3	
	1	88.3 ± 8.3	91.0 ± 2.3	90.4 ± 7.9	90.1 ± 1.5
	14	84.0 ± 3.3	88.9 ± 2.6	82.7 ± 2.7	93.0 ± 8.4
	35	88.3 ± 1.8	100.4 ± 4.1*	86.1 ± 4.1	96.8 ± 1.5*
PMA (1 μM)	0	56.8 ± 9.7		52.0 ± 9.3	
	1	56.2 ± 13.8	77.9 ± 16.7	62.1 ± 0.6	66.6 ± 8.4
	14	64.2 ± 3.1	75.8 ± 10.6	59.3 ± 4.0	70.4 ± 9.3
	35	58.1 ± 1.9	90.6 ± 4.8**	56.4 ± 4.5	86.8 ± 7.3**

Note. In the course of the 35-day dietary treatment period hepatocytes were isolated at the indicated days from ethanol-fed or control animals. Following a 30-min preincubation period of cells with the indicated additions, assays for CPT-I activity and oleate oxidation were started. Versus the respective control: *$P < 0.05$; **$P < 0.01$.

activity when measured with the permeabilized cell assay. This is in agreement with results in which isolated liver mitochondria were used (15, 31). Others, on the other hand, did not observe a reduction of hepatic CPT activity after chronic ethanol feeding to rats (8). These authors used freeze-thawed mitochondria for measuring enzyme activity. This may be the reason for the discrepancy since such a procedure may damage the mitochondrial inner membrane and may thereby modify the catalytic and regulatory properties of the CPT system as well as expose the inner form of the enzyme (CPT-II). As CPT-II has a much higher activity than CPT-I (9, 21), variations in CPT-I activity due to ethanol consumption will be masked when the combined activities of CPT-I and CPT-II are determined.

It is important to point out that other enzyme activities related to lipid metabolism reveal certain adaptive changes after 35 days of ethanol feeding. For example, the activities of rat liver acetyl-CoA carboxylase, fatty acid synthase, and diacylglycerol acyltransferase differ between ethanol-fed and control animals in the beginning of the dietary treatment, but after 5 weeks of ethanol administration these differences disappeared despite continuation of triacylglycerol accumulation in the livers of the ethanol group (see preceding paper (4)). Thus, inhibition of CPT-I activity all along the experimental period may make a major contribution to the generation of ethanol-induced fatty liver.

Changes in CPT-I activity under different physiopathological conditions such as fasting (11), hyper- and hypothyroidism (12, 14), and diabetes (13) are accompanied by variations in enzyme sensitivity to malonyl-CoA, the physiological enzyme inhibitor (9). Ethanol administration reduced

CPT-I activity and increased enzyme sensitivity to exogenously added malonyl-CoA (Fig. 2). Interestingly, changes in CPT-I activity appeared sooner than those in enzyme sensitivity. However, when isolated rat liver mitochondria were used, variations in enzyme activity and malonyl-CoA sensitivity by ethanol feeding were observed to occur in parallel (15). The reason for this discrepancy is unknown yet. It is noteworthy that enzyme sensitivity to the synthetic unhibitor TDGA was not altered by ethanol feeding (Fig. 2B). Hence, the alterations in CPT-I response to inhibition by malonyl-CoA seem to be rather specific.

Results shown in Fig. 1C demonstrate that CO_2 production from exogenous fatty acids is inhibited in hepatocytes isolated from ethanol-fed animals as compared to control rats already after 1 day of dietary treatment. Decreased oxidation of fatty acids in the liver has been proposed as the most likely explanation for the ethanol-induced hepatic deposition of dietary fat or fat derived from endogenous synthesis (1). This has been ascribed to effects of ethanol on β-oxidation, citric acid cycle activity, and the electron-transport chain (reviewed in (1–3)). Although the rate of fatty acid oxidation to CO_2 was depressed in the ethanol-fed rats at the indicated time points, the rate of ketogenesis which is inhibited right at the beginning of ethanol consumption is less inhibited at Day 14 of treatment and reached control values at the end of the 5-week experimental period (Fig. 1B). This could be associated with the striking functional abnormalities occurring in liver mitochondria after chronic ethanol feeding (25–27). Lefevre *et al.* (32) have shown enhanced ketogenesis in liver slices from ethanol-fed rats in the absence of ethanol. Our results show that fatty acid oxidation is depressed in hepatocytes isolated from long-term ethanol-treated animals as compared to controls. The diminution of fatty acid oxidation after 35 days of treatment, however, is mainly due to impairment of the tricarboxylic acid cycle.

Acetaldehyde, the product of the action of alcohol dehydrogenase, catalase, or the microsomal ethanol-oxidizing system on ethanol, is also able to inhibit hepatic fatty acid oxidation (23, 33). Acute doses of acetaldehyde have been shown to inhibit β-oxidation, citric acid cycle activity, and the respiratory chain in rat liver mitochondria (33). In addition, prolonged ethanol administration reduces the ability of rat liver mitochondria to metabolize acetaldehyde (34). Our results demonstrate that hepatocytes from ethanol-treated animals develop a markedly increased susceptibility to the inhibitory effects of acute doses of ethanol and acetaldehyde on CPT-I activity and the rates of fatty acid oxidation (Table I). These findings support the idea that after chronic ethanol consumption acetaldehyde becomes really injurious to hepatic mitochondria, contributing to the ethanol-induced accumulation of triacylglycerols in the liver (34, 35). More than a decade ago, Hasumura *et al.* (34) proposed the theory of a "vicious cycle" induced by long-term ethanol consumption: chronic ethanol uptake would lead to an increased acetaldehyde concentration, which will impair mitochondrial functions including the capacity of mitochondria to oxidize acetaldehyde. This in turn will elevate acetaldehyde levels even further, thereby perpetuating liver damage. Our results not only support this notion, but also suggest a central role of CPT-I in the ethanol-induced inhibition of fatty acid oxidation.

There is accruing evidence indicating that hepatic CPT-I activity is regulated on the long term by changes in the hormonal and nutritional status of the animal (11–15). However, few data are available showing short-term regulation of the enzyme activity by cellular effectors. This may indicate that short-term changes in CPT-I activity are difficult to preserve during the procedure of cell breakage and subsequent isolation of mitochondria. Using the permeabilized cell assay for measuring CPT-I activity, it is possible to demonstrate short-term hormonal modulation of CPT-I activity (16). Thus, we also investigated short-term regulation of CPT-I activity and the rate of fatty acid oxidation in hepatocytes isolated from both experimental groups. The well-known opposite effects of insulin and glucagon on fatty acid oxidation (18) were also reflected in CPT-I activ-

ity. However, the response to these hormones was blunted in cells isolated from ethanol-treated rats at the end of the 5-week experimental period (Table II). The molecular basis for these adaptive changes is presently unknown. Recently, Boon and Zammit (36) also published a procedure for determining CPT-I activity in digitonin-permeabilized hepatocytes. However, these authors did not observe short-term effects of insulin or glucagon on activity and malonyl-CoA sensitivity of hepatocellular CPT-I. Their method is quite different from the one presented in the present article. We perform both the permeabilization of the cells and the assay of enzyme activity at the same time in a 1-min procedure while Boon and Zammit follow a much more complicated and time-consuming schedule. This may be the reason for the lack of hormone effects on CPT-I activity measured with their method.

CPT-I response to vasopressin was also blunted after chronic ethanol consumption (Table II). Binding of vasopressin to its specific membrane receptor triggers phosphatidylinositol 4,5-bisphosphate hydrolysis to generate diacylglycerol and inositol 1,4,5-trisphosphate as second messengers (37). It has been suggested that the diacylglycerol limb of the phosphatidylinositol 4,5-bisphosphate breakdown is solely responsible for the regulation of hepatic fatty acid synthesis by vasopressin (38). Tumor promoting phorbol esters such as PMA are able to replace endogenous diacylglycerols in the activation of protein kinase C through promotion of the translocation of this enzyme from the soluble to the particulate fraction of the cells (29). The fact that CPT-I response to PMA is quite similar—at least from a qualitative point of view—to that of vasopressin during ethanol administration suggests that (i) the diacylglycerol limb of the phosphatidylinositol 4,5-bisphosphate hydrolysis may control fatty acid transport into the mitochondria and/or (ii) the disappearance of enzyme response to vasopressin could be, at least partially, a postreceptor defect. Further research is required to clarify the molecular basis of these effects.

In conclusion, the present article provides evidence that ethanol feeding profoundly alters the activity and short-term regulation of hepatic CPT-I, the key enzyme in the transport of fatty acids into the mitochondria. The impairment of both the oxidation of fatty acids and the activity of CPT-I after prolonged ethanol consumption, as well as the enhanced susceptibility of these two parameters to inhibition by acute doses of ethanol and acetaldehyde, may play a crucial role in the generation of ethanol-induced fatty liver.

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