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EFFECT OF ETHANOL INTAKE ON LIPOPROTEIN LIPASE ACTIVITY IN RAT HEART

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Abstract—We examined the effects of the *in vivo* administration of ethanol on lipolytic activities assayed in rat post-heparin heart effluents, that hydrolyse tri-, di- and monoacylglycerol. Properties of triacylglycerol lipase (TAGL) are typical of lipoprotein lipase (LPL) whereas diacylglycerol (DAGL) and monoacylglycerol (MAGL) lipase activities hydrolyse sequentially the products of LPL action. After 15 days of ethanol intake, TAGL, DAGL and MAGL activities in post-heparin heart effluents were decreased respectively by 25, 38 and 22%; after 30 days, the decreases amounted to 81, 79 and 71%. After 30 days, but not after 15 days, ethanol increased the levels of triacylglycerol in plasma. Ethanol intake concomitantly decreased TAGL and DAGL activities in post-heparin effluents and in heart tissue extracts, whereas MAGL activity was decreased only in the latter extracts. We conclude that ethanol intake causes a marked impairment in heart LPL and in two closely-related heparin-releasable activities, seemingly by altering the production of a catalytically active enzyme. A distinct heparin-unreleasable MAGL appears to exist in heart, that could be ethanol-insensitive. Overall, the results suggest that a LPL-related alteration in fatty acid supply could contribute to the toxicity of ethanol in heart.

Fatty acids circulating in plasma either as free acid or as triacylglycerol represent major substrates for myocardial energy metabolism [1, 2]. The removal of acyl chains from circulating triacylglycerol is regulated via the activity of lipoprotein lipase (EC 3.1.1.34, LPL), a triacylglycerol acylhydrolase synthesized in myocardial parenchymal cells [3, 4]. The functional fraction of this secretory emzyme exerts its catalytic activity as the luminal surface of the vascular endothelium, from which it can be released by heparin [5]. LPL preferentially splits the ester bond at the position *sn*-1 of its triacylglycerol substrate but seems to be also active toward other fatty esters [6]. This apparent broad specificity is consistent with the assay in post-heparin heart effluents of three activities that are located at the same endothelial site, where they catalyse sequential reactions in the hydrolysis of triacylglycerol [7]. Triacylglycerol lipase (TAGL) exhibits all of the highly regulatory properties of LPL, whereas diacylglycerol (DAGL) and monoacylclycerol (MAGL) lipase activities exhibit a lesser functional dependence on nutritional stimuli [8]. The three activities appear to have similar properties in male and female animals.

Chronic alcoholism is associated with numerous disorders in many organs. Alterations resulting from ethanol metabolism are invoked as a possible cause for chronic alcohol-associated diseases via the initial and highly reactive metabolite of ethanol, acetaldehyde, or other oxidative products [9]. Alternatively, ethanol can interact directly with the cell membrane [10], resulting in alterations of membrane functions, namely changes in activities of membrane-bound enzymes such as (Na,K)-ATPase [11], adenylate cyclase [12] or monoacylglycerol lipase [13].

In the present experiments, we have searched for an influence of the *in vivo* administration of ethanol on the TAGL, DAGL and MAGL activities released from isolated heart preparations upon heparin infusion. We have also investigated an alcohol effect on the residual lipolytic activities assayed in heart tissue homogenates.

MATERIALS AND METHODS

Isolated rat heart preparation. Female Sprague–Dawley rats (IFFA-CREDO, 69120 L'Arbresle, France) weighing approximately 160 g were maintained at 22° on a 1200:1200 light–dark cycle. They were fed food pellets (AO4, UAR, 91360 Epinay sur Orge, France) containing, by weight, 5% lipid, 49.5% carbohydrate and 25% protein; rats were allowed to acclimate to their environmental conditions for at least 2 weeks before beginning ethanol administration. All animals were food- and water-deprived for 24 hr at the start of the experiments, in order to improve the subsequent ingestion of ethanol-supplemented drinking water [14]. At the end of the deprivation period, the rats were divided in two groups. In group A, rats had free access to the standard diet and to a drinking solution of ethanol in water at 10% (v/v) for 5 days, then at 20% for 10 or 25 additional days, as indicated. The volumes of drinking solution ingested were measured daily; on average, they mounted to 27 ± 5 vs 32 ± 4 mL/day in ethanol-fed and control animals, respectively. On this basis, we estimated the average ethanol intake to be 12 ± 3 g/kg body weight per day and per animal at the 20% regimen. Control groups were treated identically except that ethanol was not added to the drinking water. A current

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practice is that in experiments where rats receive ethanol mixed with a totally liquid diet, the control animals are supplemented isocalorically with carbohydrates. With our standard food regimen and during the relatively short periods (15–30 days) of ethanol feeding, body weight gains were similar in the two rat groups. The mean food intakes, estimated to be equivalent to 48 ± 7.2 and 55 ± 8.9 kcal per day and per animal in control and treated rats, respectively, did not significantly differ ($P > 0.05$). As observed by others [15], pair feeding the animals turned out to be inappropriate because ethanol-fed rats tended to consume their diet steadily throughout the 24 hr period while animals fed the control diet consumed it during the first hours after administration. Such changes in feeding schedule might alter LPL activity, that is known to be diminished postprandially in heart and muscle compared with adipose tissue [16].

During alcoholic treatment, mean (± SD) plasma levels of ethanol measured at the end of the feeding period (between 9 and 11 a.m.) was 9.6 ± 6.9 mM (N = 12). At the end of the experimental period, rats were killed by decapitation under light anesthesia (pentobarbital 25 mg/kg body weight, intraperitonally) after a 12 hr fast during which animals were given only ethanol-free drinking water. This deprivation period before killing aimed at again preventing any change in heart LPL activity, which could be due to nutritional factors.

After killing, the hearts were immediately removed and perfused (5 mL/min) without recycling via the aorta in the Langendorff mode [17] at a constant temperature of 37° and a constant pressure of 80 mm Hg, in order to rinse the vascular bed. The perfusion medium was a Krebs–Henseleit buffer at pH 7.4 (KHB) containing 1% (w/v) serum albumin. After 4 min, a recirculating perfusion (flow rate, 10 mL/min) was continued for another 4 min with 5 mL of buffer KHB containing 16 units/mL heparin (160 units/mL, Schuchart, Buchs, Switzerland). The heparin effluent was collected, centrifuged at 4° for 3 min at 2000 *g* and immediately assayed for lipase activities. During all the perfusion procedure, the release of lactic dehydrogenase [18] in the effluents did not exceed 1% of the total heart tissue activity. In some experiments, the heparin-perfused hearts were chilled, trimmed of adherent fat, weighed and homogenized in buffer KHB (4 mL/g). Homogenates were centrifuged (9000 *g* for 20 min at 4°), and the supernatants were used as the enzyme sources. No ethanol was detectable in the heart effluents or in the heart tissue preparations used for the enzyme assays.

Assay of enzymatic activities. Tri-[9,10-^{3}H]-oleoylglycerol (1.0 Ci/mmol) and [9,10-^{3}H]oleic acid (10 Ci/mmol) were purchased from Amersham International (Amersham, U.K.). Di-[9,10-^{3}H]-oleoylglycerol and mono-[9,10-^{3}H]oleoylglycerol were synthesized and purified in the laboratory by conventional methods. Radiolabelled fatty esters used as the substrates were obtained >98% radiochemically pure as checked by scanning in a Berthold TLC-Trace master model LB 282 (BAI, Elancourt, France). Dioleoylglycerol had a composition of about 40% 1,2- and 60% 1,3-diacyl-*sn*-glycerols; monooleoylglycerol was ~90% 1(3) monoleoyl-*sn*-glycerol. Prior to assay, the substrate mixtures were sonicated (approx. 30 W) at room temperature [7].

TAGL activity was assayed for 15 min in Fain's medium [19] containing 20 mM Hepes instead of sodium phosphate, to which was added 1% (w/v) defatted albumin (fraction V, Boehringer, Mannheim, F.R.G.) at pH 8.0 (buffer A). To this medium were added emulsified tri-[^{3}H]oleoylglycerol (1 mM) as the substrate and 0.1 mL of heart perfusate (~ 0.05 mg protein) or 0.05 mL of heart tissue extract (~ 0.3 mg protein) as the enzyme sources, in a final volume of 1 mL at 37°. TAGL assays were concomitantly performed with and without addition of fasted rat serum (10% by vol) inactivated for 20 min at 55° as source of cofactor apolipoprotein C-II [6]. After 20 min of incubation, the amount of [^{3}H]oleic acid released was extracted by a liquid–liquid partition system and quantified as previously described [20]. Differences between the rates of fatty acid release in serum-activated and serum-free media expressed serum-dependent TAGL, referred to as LPL activity. DAGL and MAGL lipase activities were assayed for 15 min at 37° in buffer A containing emulsified di-[^{3}H]oleoglycerol or mono-[^{3}H]oleoylglycerol, respectively, as the substrates (1 mM) at pH 7.4; for these assays no serum was added as cofactor. After incubation, the assay media were treated as for the TAGL assay. All assays were linear with time and were carried out at least in duplicate. One milliunit (mUnit) of lipase activity corresponds to the release of one nmole of acid per min.

Other experimental procedures. Protein concentration was determined by the method of Lowry *et al.* [21]. Triacylglycerol, ethanol and insulin concentrations were determined by enzymatic procedures (respectively Diagnostica-Merck, Darmstadt, F.R.G.; Sigma Diagnostics, St Louis, MO, U.S.A. and CIS international, Saclay, France).

Expression of results. Results were expressed as mean ± SD. Values obtained for the ethanol-fed and control groups were compared by the Dunnett's *t*-test, using the statistical analysis program Statview 512 (Brain Power Inc., Calabasas, CA, U.S.A.). P values <0.05 were considered as significant.

RESULTS

Ethanol-fed rats initially exhibited a slight (<10%) decrease in body weight gain but at the end of the experiments, there was no significant difference in body weight between ethanol-fed and control animals, whether ethanol had been administered for 15 or 30 days. The ratio of heart weight to body weight was unchanged in rats treated with ethanol for 15 days but was slightly increased after 30 days (0.42 vs 0.46% in control rats, $P < 0.01$; $N = 6$). Compared to control values, ethanol administered for 30 days produced an increase in plasma triacylglycerol levels from 0.46 ± 0.09 to 0.65 ± 0.11 g/L ($P < 0.001$, $N = 6$) but no increase was observed after 15 days (Fig. 1). Control values for TAGL, DAGL and MAGL activities were 56 ± 8.9, 46 ± 7.3 and 36 ± 1.7 mUnits/mL of post-heparin heart effluent, respectively; the intake of

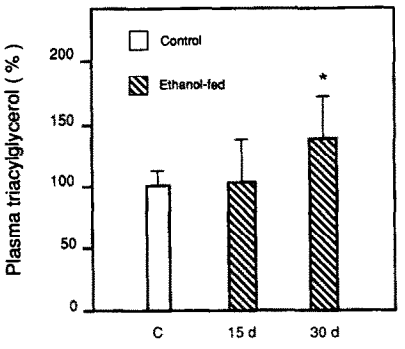

Fig. 1. Mean (± SE) changes in plasma triacylglycerol concentration after ethanol intake for the number of days indicated under each column are expressed as per cent of a control (C) value of 0.46 ± 0.09 mg/mL. Six rats contributed to each group. *, $P < 0.01$ vs C value.

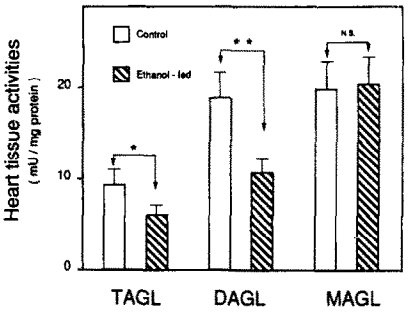

Fig. 3. Mean (± SE) changes in lipolytic activities assayed in heart tissue extracts after 30 days of ethanol intake. Abbreviations as in the legend of Fig. 2. Six rats contributed to each group. *, $P < 0.01$; **, $P < 0.001$ vs C values; N.S., not significant.

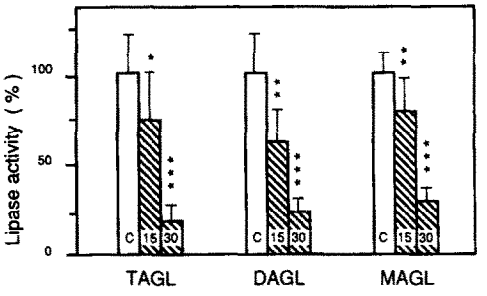

Fig. 2. Mean (± SE) changes in triacylglycerol (TAGL), diacylglycerol (DAGL) and monoacylglycerol (MAGL) activities measured in post-heparin heart effluents after ethanol intake for the number of days indicated in each column. Values are expressed as per cent of control (C) values. Six rats contributed to each group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ vs C value.

ethanol decreased the values respectively by 25, 38 and 22% after 15 days and by 81, 79 and 71% after 30 days (Fig. 2).

It could be excluded that the activity decreases represented a physiological response to a nutritional stimulus: the ethanol-fed rats were well in fasted state at the time of killing, as ascertained by measuring their mean plasma insulin concentration, which was similar to that of control animals (87 ± 11 vs 83 ± 10 pM, respectively; $P > 0.1$, $N = 6$). To explore the possibility that ethanol could diminish the amount of heparin-releasable activities by acting directly on the enzyme at its endothelial site, ethanol was infused (10 mL/min) for 5 min to the perfusion system at concentration up to 0.1 M in buffer A. The ethanol perfusion neither released by itself any one of the three activities, nor reduced their levels as assayed in the heart effluents following the perfusion of heparin (16 units/mL) immediately after completion of the ethanol infusion (data not shown).

Another potential mechanism for producing changes in heparin-released activities was ethanol-induced alterations of synthesis and/or processing of the enzyme in heart tissue. We therefore determined the amounts of lipolytic activities in heart tissue extracts prepared immediately after heparin infusion in rats exposed to ethanol for 30 days. Compared to control levels, TAGL and DAGL activities in the homogenates were found to be decreased by respectively 65 and 58%, whereas MAGL activity was unchanged (Fig. 3); the results were similar whether the values were expressed per mg protein in the extracts or per g heart tissue. Finally, it was ascertained that ethanol added directly to the assay medium at concentration up to 0.1 M had no effect on TAGL, DAGL or MAGL activities.

DISCUSSION

Our knowledge of the effects of alcohol on LPL is very fragmentary. In human subjects, LPL activity assayed toward triacylglycerol in adipose tissue [22] or in post-heparin plasma [23–25] has been reported to be unchanged after acute administration of ethanol; in one study after chronic intake [26], LPL activity has been found to be increased in post-heparin plasma. In rat heart, no alcohol-induced changes have been reported in LPL activity as assayed either in tissue homogenates after acute administration [27] or in acetone powders after chronic intake [28].

For the first time, the influence of ethanol on heart LPL has been explored using a heart preparation which may be regarded as an integrated model approaching physiological conditions more closely than do tissue extracts. Moreover, the ethanol effect was measured comparatively on three closely-related activities of the LPL system. The results unequivocally indicate that the administration of ethanol impairs the efficiency of the whole enzyme complex since the three activities assayed in the post-heparin heart effluents were lower in ethanol-fed than in control rats. Clearly, these decreases were not merely caused in the assay systems by a nucleophilic competition of ethanol toward the formation of the enzyme–substrate complex, as was described for alcohols added to hydrolytic reaction mixtures [29]. We have verified that: (1) the lipolytic

assays were performed in the absence of detectable amounts of ethanol, and (2) exogenous ethanol added to the assay medium at concentration up to 0.1 M had no effect on any of the three activities.

It is noteworthy that, after 30 days of ethanol intake, the activity decreases were associated with an increase in triacylglycerol concentration in blood. This latter change is one of the most frequent metabolic side effects induced by alcohol, mainly via the increased hepatic production of triacylglycerol-rich very low-density lipoproteins [30]. Since the heart LPL levels are physiologically low when triacylglycerol-rich lipoproteins increase postprandially [16], the ethanol-induced enzymic changes could have been related to the triacylglycerol increase. However, the enzymic decreases were clearly measurable after 15 days of ethanol administration, at which time no triacylglycerol change occurred in blood, suggesting that there is no direct and causal relationship between the two metabolic events.

Alternatively, the data indicate that ethanol intake decreased in a comparable manner TAGL and DAGL activities in post-heparin effluents and in heart tissue, suggesting that ethanol primarily impairs the production of LPL in heart. There is little knowledge of the mechanisms that govern the production of catalytically active LPL in cardiomyocytes. From current studies carried out in adipocytes, LPL is thought to be localized in an inactive form in the endoplasmic reticulum, and then activated via the acquisition of *N*-linked oligosaccharide chains in the Golgi apparatus [31, 32]. The overall mechanism of the transendothelial enzyme export remains largely unknown. Hence, the question of how ethanol may alter the production of these lipolytic activities in the heart cannot be at present answered. Interestingly, experimental ethanol feeding has been shown to produce in liver acetaldehyde-induced impairment of the protein secretion as well as alterations in enzyme activities, possibly due to binding with critical functional groups [9]. Whether a comparable mechanism may operate in heart is at present uncertain, although decreased protein synthesis has been described in alcoholic cardiomyopathy [33].

In contrast to TAGL and DAGL, MAGL appears to respond to ethanol differently as assayed in post-heparin effluents, where it is decreased, and in tissue extracts, where it is unchanged. This difference is consonant with other physical and kinetic features that tend to differentiate MAGL form TAGL and DAGL in heart [7, 8, 34, 35]. MAGL activity assayed in heart tissue extracts might correspond, at least in part, to the lipase characterized long ago in heart by Yamamoto and Drummond [36] that hydrolyses specifically monoacylglycerol and is not releasable by heparin. In turn, MAGL activity in post-heparin effluents seems to represent an intrinsic activity of LPL, that amounts to approx. one-third of its TAGL activity [7, 37]. Thus, the occurrence in heart of two different MAGL enzymes is likely to explain the observed difference in response to ethanol.

This study examined heart lipolytic activities in rats treated for 15 and 30 days. It is certainly possible that activity levels could be modified to different degrees after more prolonged ethanol administration. In any case, we have observed that the activity decreases persist at least after 2 months of ethanol administration, although to a slightly lesser extent than after 1 month (data not shown).

Chronic alcoholism causes myocardial dysfunction [9, 38, 39]. The present results, obtained with blood levels of ethanol well within those compatible with life (generally < 0.1 M) suggest that alteration in the fatty acid supply due to impairment of the LPL system could be, among others a possible factor of the cardiac toxicity of alcohol.

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