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CYCLIC AMP-LINKED MECHANISMS IN ETHANOL-INDUCED DERANGEMENTS OF METABOLISM IN RAT LIVER AND ADIPOSE TISSUE

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Abstract—An acute ethanol load was achieved by gastric administration of 5 g ethanol per kg body wt to fasted rats. The concentrations of cyclic AMP during the following 24 hr were measured in the liver and adipose tissue and correlated with simultaneously measured concentrations of blood glucose, plasma free fatty acids (FFA), hepatic triglycerides (TG), hepatic glycogen and blood ethanol. Ethanol induced a significant increase in hepatic cAMP reaching a maximum at 16 hr after administration. Hepatic glycogen decreased considerably after 6 hr and blood glucose decreased slightly. Plasma FFA levels decreased, with a minimum at 4 hr. Hepatic TG levels increased steadily from 6 to 24 hr after the ethanol administration. An unexpected small decrease was observed in adipose tissue cAMP. In neither hepatic nor adipose tissue did the cAMP concentration correlate with the blood ethanol concentration. It was concluded that the changes in cAMP were more likely to be due to the regulation of blood glucose than to an unspecific stress response. Experiments with isolated perfused rat livers demonstrated that ethanol has no direct effect on the metabolism of hepatic cAMP.

It is well established that in rats a single large dose of ethanol causes acute reversible fatty liver characterized by an accumulation of triglycerides [1]. The pathogenesis of this phenomenon is still subject to debate [2, 3]. Experimental results on hepatic fatty acid synthesis *de novo* and its significance in the accumulation of triglycerides have been controversial. Previous reports indicated that fatty acid synthesis is increased by ethanol [4], but negative results have been obtained with methods employing ^3H incorporation into fatty acids from tritiated water [5–7] and the opinion emerges that changes in hepatic fatty acid synthesis play a minor role in the development of hepatic steatosis.

The role of cyclic 3′,5′-AMP in the regulation of lipid and carbohydrate mobilization and storage is well documented [8, 9]. Only recently it has also been reported that cAMP may regulate hepatic lipogenesis [10–12]. On the other hand, ethanol interferes with hepatic gluconeogenesis [13], and under certain nutritional conditions this leads to ethanol-induced hypoglycemia. There are thus several possible mutual reactions between lipid and carbohydrate metabolism, which may be affected by ethanol.

Reports of the effects of ethanol on cAMP-linked mechanisms *in vivo* are scarce [14]. Ethanol *in vitro* has a stimulatory effect on adenylate cyclase in a variety of tissues [15–18], but in many cases high concentrations of ethanol were needed to demonstrate any effects. We therefore undertook the present work in which the concentrations of cAMP were measured and correlated with some parameters of carbohydrate and lipid metabolism in liver and adipose tissue after acute ethanol loading. This approach may also help to distinguish between a causal or compensatory role for the hormonal, cAMP-mediated mechanisms in the metabolic effects of ethanol.

EXPERIMENTAL

Male Long–Evans rats weighing 180–310 g were used. The rats fasted for 24 hr before the experiment. Ethanol was administered through a stomach tube at a dose of 5 g per kg body wt as a 20% (w/v) solution in water. Control animals received the same amount of water and a third group received an isocaloric dose of glucose (8·7 g per kg body wt).

The rats were decapitated without anaesthesia 0·5, 2, 4, 6, 10, 16, and 24 hr after the gastric intubation, or without gastric intubation. Blood samples for blood glucose and plasma free fatty acid (FFA) determinations were taken into heparinized ice-cold glass tubes immediately after decapitation. Liver samples for cAMP, triglyceride (TG) and glycogen determinations, and samples from the epididymal fat pads for cAMP determinations were taken by the freeze-clamp technique [19]. The time course of the blood ethanol concentration was studied in separate experiments, in which blood samples were taken from the tip of the tail.

cAMP was assayed by the displacement method of Gilman [20] using the binding protein and protein kinase inhibitor of bovine heart muscle and [^3H]cAMP as the indicator ligand. The binding protein and protein kinase inhibitor were extracted as described by Gilman [20]. Cyclic [G-^3H]adenosine-3′,5′-monophosphate (ammonium salt, sp. act. 38·15 Ci/m-mole) was purchased from New England Nuclear Co., Boston, MA. Bound [^3H]cAMP was assayed in a Wallac liquid scintillation spectrometer and the radioactivity data were processed and converted to amounts of cAMP on a Honeywell 1642 computer by a program modified from that of Burger *et al* [21].

For the preparation of the adipose tissue for cAMP determinations, a piece of epididymal fat pad weighing about 200 mg was homogenized in 2 ml of 6%

trichloroacetic acid at 0°. After centrifugation the supernatant was filtered through glass wool. 2 N HCl was added to 1 ml of supernatant to a final concentration 0·1 N. This solution was then extracted four times with five volumes of water-saturated ether. The residue was dried and redissolved in 50 mM sodium acetate buffer, pH 4·0, and the cAMP was determined as described above. The adequacy of the sample processing was checked by unlabelled internal standards, and the specificity of the method was confirmed with blank values obtained by phosphodiesterase treatment of the tissue samples.

Hemoglobin-free perfusion of isolated rat livers was performed essentially according to Scholz and Bücher [22] with Krebs–Ringer bicarbonate medium in equilibrium with 95% O_2–5% CO_2. After a stabilization period of 20 min, one lobe was freeze-clamped and ligated, 80 mM ethanol was added to the perfusion medium and further liver samples were taken at one-hour intervals.

The hepatic TG concentration was determined by the method of Carlson [23], and the hepatic glycogen by the method of van Handel [24]. Plasma FFA concentration was determined according to Novak [25], and blood glucose by a modification of the method of Hugget and Nixon [26] using glucose oxidase reagent obtained from Kabi Ab, Stockholm, Sweden. Blood ethanol determinations were carried out by gas chromatography on a Porapak Q column, using isopropanol as an internal standard [27].

Statistical significance of the results was calculated using the Student's *t*-test.

RESULTS

The effect of ethanol on the hepatic cAMP concentration is illustrated in Fig. 1. In the water control group the concentrations of cAMP were in accord with the values reported previously for the Gilman method [28]. In the ethanol group there was a small decrease at 30 min and thereafter a slow increase, which reached a maximum at 16 hr. The difference between the control and ethanol groups at 16 hr was statistically significant ($P < 0{\cdot}025$). At the end of the experiment at 24 hr the concentration of cAMP had returned almost to the control values. In the group which had received an isocaloric amount of glucose the cAMP concentration was lower than the water control group throughout the experiment.

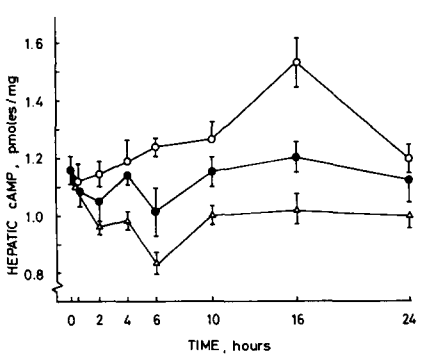

Fig. 1. Effect of ethanol load on hepatic cAMP concentration. Results are means ± S.E.M. of 4–8 separate experiments. At zero time one group of animals received ethanol (5 g per kg body wt), a second group an isocaloric dose of glucose (8·7 g per kg body wt), and a third group an equal amount of water (25 ml per kg body wt). Symbols: ethanol group, -O-O-O-; glucose group, Δ-Δ-Δ-; water control group, -●-●-●-.

To distinguish between a direct and an indirect effect on the concentration of hepatic cAMP, 80 m-moles/l. ethanol was added to the perfusate in a hemoglobin-free perfusion of an isolated liver. The cAMP concentration was 1·46 ± 0·10 (S.E.M.) pmoles/mg liver wet wt after a 20-min stabilization period. Liver samples were taken 60 and 120 min after the addition of ethanol. Ethanol had no effect on the concentration of cAMP.

A paradoxical effect was found in the concentration of adipose tissue cAMP (Fig. 2). In the ethanol-treated group there was a small decrease in cAMP, which was statistically almost significant ($P < 0{\cdot}1$) compared with the water control animals at 16 hr, but at 24 hr the cAMP concentration had returned to the initial value. Little change took place in the glucose-treated group.

To reveal the mechanisms of ethanol-induced elevation of hepatic cAMP, an attempt was made to correlate these changes with some parameters known to be modulated by cAMP-linked mechanisms and by ethanol.

In the water control group and in the ethanol group a small reversible increase in the blood glucose concentration was observed with a maximum at 30 min (Fig. 3). This increase was probably a stress-effect of gastric intubation. However, no rapid initial increase in cAMP due to the same effect can be detected by this schedule of sampling (Fig. 1). In the glucose group the maximum blood glucose concentration was observed at 2 hr. With this dose of ethanol no pronounced ethanol-induced hypoglycemia could be seen. Up to 6 hr the blood glucose was slightly above the control values and at 16 hr slightly hypoglycemic values were observed.

During the first 4 hr the hepatic glycogen in the ethanol-treated group remained the same as in the water control group (Fig. 4). At 6 hr the hepatic glycogen diminished to very low values in the ethanol-treated group and remained constantly low during the remainder of the experiment. This glycogenolysis is possibly correlated with the changes demonstrated

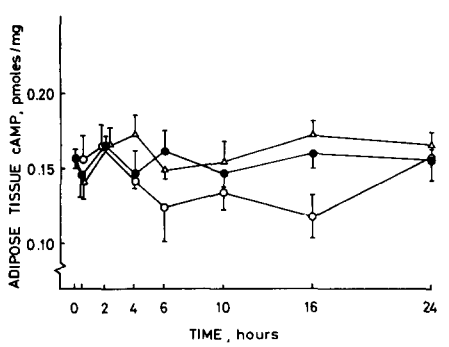

Fig. 2. Effect of ethanol load on cAMP concentration in adipose tissue. Results are means ± S.E.M. of 4–7 separate experiments. Symbols and experimental conditions as in Fig. 1.

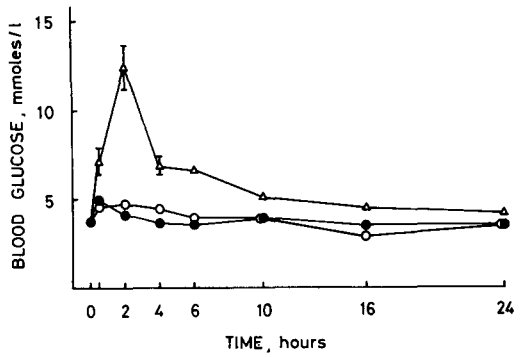

Fig. 3. Effect of ethanol load on blood glucose concentration. Results are means ± S.E.M. of 4–8 separate experiments. Symbols and experimental conditions as in Fig. 1.

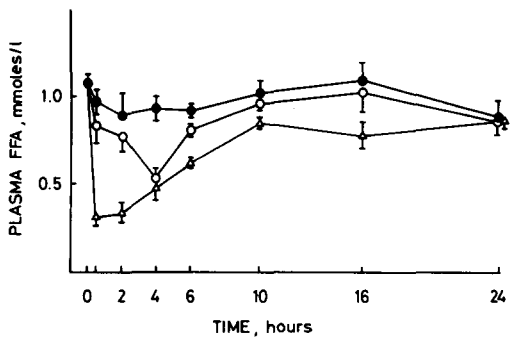

Fig. 5. Effect of ethanol load on plasma FFA concentration. Results are means ± S.E.M. of 4–8 separate experiments. Symbols and experimental conditions as in Fig. 1.

in Fig. 1. As expected, a 15-fold increase in hepatic glycogen was observed in the glucose group with a maximum at 6 hr.

The concentration of plasma FFA was reduced by ethanol, a minimum occurring 4 hr after administration (Fig. 5). The decrease at this point was statistically significant ($P < 0{\cdot}001$). At 10 hr the FFA concentration in the ethanol-treated animals reached that in the water control group. An immediate decrease in plasma FFA was observed upon the administration of glucose, which is typical for refeeding in a fasted animal [29].

Accumulation of TG in the liver started 6 hr after the administration of ethanol and continued almost linearly reaching about a 5-fold increase at 24 hr (Fig. 6). In the water control group the TG concentration remained almost constant. In the group receiving an isocaloric amount of glucose the TG concentration was somewhat lower than in the water control group throughout the experiment.

The dose of ethanol used (5 g per kg body wt) caused a maximal blood ethanol concentration of 94 ± 9 (S.E.M.) m-moles/l. at 4 hr, and the ethanol was cleared from the blood within 23–24 hr (Table 1).

DISCUSSION

The effects of ethanol on hepatic and adipose tissue cAMP are somewhat paradoxical. The small decrease in the concentration of cAMP in the adipose tissue was rather unexpected. When considering these changes it must be borne in mind that the results are calculated on a tissue-weight basis, so that if any marked lipolysis occurs under these experimental conditions, the percentage of the aqueous cytoplasmic weight should increase, resulting in an apparent increase in the concentration of cAMP per unit wet wt of the tissue. The real decrease in the cAMP concentration may therefore be considerably greater than that observed.

The present data on the hepatic cAMP concentration are subject to similar reservations. However, the apparent cAMP concentration is not significantly affected by changes in liver weight due to glycogenolysis, since the hepatic glycogen concentration of the fasted experimental animals was only about 2 mg per g liver wet wt. Dehydration could cause an apparent increase in the cAMP concentration, but even this could not explain the opposing changes in cAMP concentration in the liver and adipose tissue after ethanol administration.

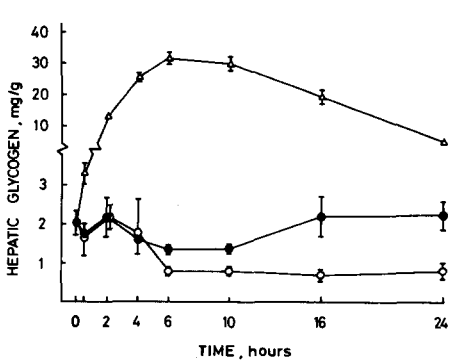

Fig. 4. Effect of ethanol load on hepatic glycogen concentration. Results are means ± S.E.M. of 4–7 separate experiments. Symbols and experimental conditions as in Fig. 1.

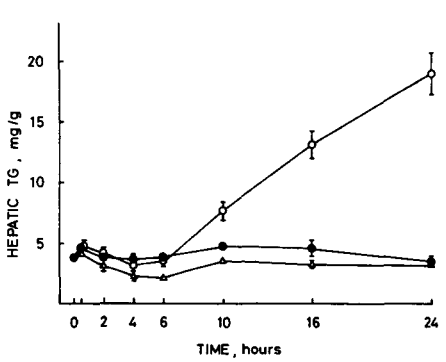

Fig. 6. Effect of ethanol load on hepatic TG concentration. Results are means ± S.E.M. of 6–8 separate experiments. Symbols and experimental conditions as in Fig. 1.

Table 1. Blood ethanol concentration after single ethanol load (5 g per kg body wt)

Time after administration of ethanol (hr)	Blood ethanol concentration (m-moles/l.)
2	76 ± 10
4	94 ± 9
8	71 ± 6
16	36 ± 5
24	None

Results are means ± S.E.M. of four separate experiments.

The time course of the changes suggests that the effect of ethanol on hepatic cAMP is mainly indirect, and results from a regulation of the carbohydrate metabolism secondary to the effects of ethanol on gluconeogenesis. The negative results of the experiments with isolated perfused livers show that the small increase in the cAMP concentration detected *in vivo* at two hours is similarly indirect in character. Reliable perfusions of isolated livers are limited in duration, so that the late effects of ethanol could not be tested with this experimental model.

The liver glycogen concentration dropped to very low values 6 hr after the administration of ethanol and concomitantly blood glucose tended to be lower than in the controls. It has been shown previously that plasma insulin in man decreases during ethanol loading [30]. Recently glucagon secretion has also been reported to increase within 2 hr during ethanol infusion in fasted pigs [14]. The present results show a more retarded response of hepatic cAMP concentration to a single ethanol load in fasted rats.

If it is assumed that an increased concentration of cAMP, a positive effector in the activation system of hormone-sensitive lipase, can also function as an indicator of the activation of the latter in the adipose tissue, peripheral lipolysis does not increase under our experimental conditions. The results are in good agreement with those of Jones *et al.* [31], which indicate that the FFA turnover in man actually decreases after a moderate dose of ethanol, when studied with [1-^{14}C]palmitate. This is, in fact, in accordance with the reports on the increase of glucagon secretion, since it has been repeatedly demonstrated that in man glucagon administration *in vivo* increases the plasma glucose concentration with no effect or with a depressing effect on the level of plasma FFA in spite of the *in vitro* lipolytic effect of glucagon [32].

The non-specific stress-effect of ethanol could be expected to be proportional to the blood ethanol concentration. In the present study cAMP levels in hepatic or adipose tissue did not correlate with ethanol concentrations in the blood. On the other hand, it has been shown that the principal metabolic change induced by ethanol, namely the oxidation–reduction change of hepatic nicotinamide nucleotides, persists until the peripheral ethanol concentration falls below 2–3 m-moles/l. [33]. This limiting value is achieved in the present experiments about 22 hr after the administration of ethanol, and the accumulation of TG advances steadily for the same time. This lends support to the view that the inhibition of fatty acid oxidation in the liver [3, 4] is a major factor in ethanol-induced triglyceride accumulation and that increased peripheral lipolysis due to a non-specific stress-effect plays an insignificant role.

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