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REDUCED GLUCAGON INDUCTION OF TYROSINE AMINOTRANSFERASE IN PERFUSED LIVERS AFTER ETHANOL TREATMENT

JØRG MØRLAND

National Institute of Forensic Toxicology and Department of Pharmacology, University of Oslo, Blindern, Oslo 3, Norway

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Abstract—Male Wistar rats (250–290 g body wt) were given ethanol for 7 weeks to provide either 22 or 35 per cent of the total calories while sucrose replaced ethanol isocalorically in two respective control groups. Ethanol treatment did not influence basal hepatic tyrosine aminotransferase (TAT) activity. When the livers were isolated and perfused in the presence of glucagon for 3 hr, the subsequent increase of TAT activity was reduced to approximately 70 per cent in livers from animals treated with ethanol. This reduction was probably caused by reduced enzyme synthesis, since no action could be found on enzyme activation, degradation or leakage. Cyclic AMP levels in the livers were not affected by previous ethanol treatment. The reduced TAT synthesis seems therefore to be independent of changes in the hormone receptor or in the accumulation of cyclic AMP in the liver after glucagon. General protein synthesis was not influenced by ethanol treatment in livers perfused with glucagon.

CHRONIC ethanol treatment reduces *in vivo* synthesis of proteins,[1] as well as the amount of rough microsomes.[2] The induction of tryptophan oxygenase (TO) and tyrosine aminotransferase (TAT) by dexamethasone during perfusion *in vitro* was markedly diminished in livers from ethanol-treated rats.[3] General protein synthesis was also significantly reduced in these livers after dexamethasone, while protein synthesis in livers not given dexamethasone was less affected.[3]

The observed effect of ethanol treatment on TO and TAT induction could be due either to a general inhibition of protein synthesis or to a more specific interference with the action of dexamethasone. We have, therefore, examined the effect of ethanol feeding on the induction of TAT in perfused livers by glucagon, a hormone which also induces TAT.[4–6] The primary cellular response to this hormone, i.e. the accumulation of cyclic AMP, which is regarded as the mediator for the induction,[7] was also studied.

MATERIALS AND METHODS

Animals. Male Wistar rats (250–290 g body wt) housed as described elsewhere[3] were used.

Experimental design. In experiment no. 1 the rats were divided into two groups. One group (E-22, ethanol-treated rats) were offered 25 ml/rat of a liquid solution containing 12·5% (v/v) ethanol and 12% (w/w) sucrose each day as the sole drinking fluid. This volume was consumed completely every day by the rats through the whole experimental period. The other group (C-22, control group) received 25 ml/rat and day (consumed completely) of a solution containing 28% sucrose as the sole drinking

fluid. Both groups received in this way 28–29 cal/day through their drinking liquid. In experiment no. 2, the ethanol group (E-35) consumed approximately 30 ml/rat and day from the 35 ml offered of a liquid solution containing 16·7% (v/v) ethanol and 15% (w/w) sucrose as the sole drinking fluid. The control group (C-35) drank an equicaloric amount of sucrose, i.e. approximately 40 ml per rat and day (the total volume offered) of 30% (w/w) sucrose. In this way both groups received 46–48 cal/day from the liquids.

The rest of the calories was provided by a synthetic solid diet[3] given *ad lib.* to all groups. The consumption of this solid food was not measured. All rats were weighed once a week. The weight gain was not significantly different among the four groups (Table 1), and was close to that reported for rats growing at optimal conditions,[8] and contrasted with the results of previous experiments.[3,9] The daily caloric intake was therefore assumed to be approximately 80 cal in all groups.[8] The relative intake of the various main nutrients (Table 1) was calculated from the known intake of liquid calories and the composition of the solid diet.

Liver perfusion. Rats treated as described above for 7 weeks, which were to be used as liver donors, were fasted and given only water for 24–28 hr. Male Wistar rats kept on ordinary laboratory chow were fasted for 24 hr and used as blood donors. Perfusions were conducted as described elsewhere[3] with no addition of ethanol to the perfusates. Glucagon (Glucagon Novo®, Novo Industri A/S, Copenhagen, commercial preparation) was dissolved in perfusion buffer, 1 mg was added at 10 min of perfusion (final concn $7{\cdot}1 \times 10^{-6}$ M) and 200 μg was added to the perfusate every 15 min throughout the rest of the perfusion. Protein hydrolysate (CFB 25, 57 mCi/m-atom carbon, The Radiochemical Centre, Amersham) 1·5 μCi (100 μl) was added after 15 min of perfusion. Cycloheximide (Sigma) was added after 3 hr (final concentration 20 μg/ml) in some experiments. Liver samples were taken for analysis at the times indicated, frozen and stored in liquid nitrogen until the assays were performed.

Liver analysis. Tyrosine aminotransferase activity, TAT, (L-tyrosine-2-oxoglutarate aminotransferase, EC 2.6.1.5) was assayed according to Rosen *et al.*[10] Enzyme units are equal to the number of μmoles of reaction product formed per hr at 37°. In mixed homogenate experiments one part of a liver homogenate was incubated alone, while the other part was incubated together with another homogenate. Protein was determined according to Lowry *et al.*[11] Cyclic AMP was determined by a slight modification[12] of the Gilman method.[13] Radioactive proteins, and TCA-soluble material were prepared and measured as described elsewhere.[3]

RESULTS AND DISCUSSION

Effect of ethanol treatment on glucagon induction of TAT. Livers from ethanol-treated rats showed reduced glucagon induction of TAT expressed either as absolute enzyme activity after perfusion for 3 hr or as increase of activity during perfusion for 3 hr (Table 2). The reduction of enzyme levels at 3 hr showed a tendency to be more marked in livers from animals which had received the largest amount of ethanol (E-35). The relative increase in enzyme activity seemed smaller in these animals too, compared with the E-22 group (Table 2) but not statistically significant. In both experiments the reduction of enzyme induction caused by ethanol (34 and 28 per cent) was smaller than the earlier reported 50 per cent reduction of dexamethasone induction.[3] The relative protein content showed only minor insignificant variations

TABLE 1. EFFECT OF DIFFERENT DIETS ON RAT BODY AND LIVER WEIGHT

Treatment	Per cent of total calories consumed*				Mean body wt (g)†				Mean liver wt (g)†
	Ethanol	Carbo-hydrate	Protein	Lipid	Initial	2 Weeks	4 Weeks	7 Weeks	7 Weeks
Ethanol (E-22)	22	20	15	43	266·0 ± 4·3	316·0 ± 6·5	339·2 ± 8·6	359·2 ± 11·8	8·64 ± 0·30
Control (C-22)	0	42	15	43	258·0 ± 3·4	293·0 ± 2·1	318·0 ± 1·9	339·8 ± 3·6	8·35 ± 0·18
Ethanol (E-35)	35	25	11	29	283·8 ± 4·0	318·8 ± 5·5	344·7 ± 7·6	374·2 ± 9·2	9·30 ± 0·48
Control (C-35)	0	61	10	29	283·2 ± 3·0	332·2 ± 3·1	368·6 ± 4·5	393·4 ± 5·6	10·02 ± 0·32

* Calculated for the last 4 weeks before assay. The caloric value for ethanol used is 7 cal/g.

† The results are expressed as mean ± S.E.M. No significant differences between ethanol treated rats and their respective controls were found at any time considering levels above 0·05 in Wilcoxon's test as insignificant.

TABLE 2. EFFECT OF ETHANOL TREATMENT ON GLUCAGON INDUCTION OF TAT IN ISOLATED PERFUSED LIVERS*

Treatment	No. of rats	TAT activity (units/g protein) 0 hr	TAT activity (units/g protein) 3 hr	Increase	Increase in % of control
Experiment 1:					
E-22	5	557 ± 92	1046 ± 8	489	72
C-22	5	491 ± 37	1175 ± 31	684	100
Significance	–	NS	0·008	0·043	—
Experiment 2:					
E-35	6	410 ± 39	907 ± 33	497	66
C-35	6	349 ± 35	1102 ± 78	753	100
Significance	–	NS	0·033	0·019	—

* The results are expressed as mean ± S.E.M. The significance levels according to Wilcoxon's test are given, considering levels above 0·05 as insignificant, NS.

among the different groups initially (E-22, 233·6; C-22, 227·8; E-35, 241·9; C-35, 236·8 mg protein/g liver) and after perfusion for 3 hr (E-22, 242·6; C-22, 228·6; E-35, 235·0; C-35 228·2 mg protein/g liver) thus excluding major changes of the effect when referring enzyme activities to wet weight liver.

Glucagon is known to increase TAT-synthesis,[6] and the most likely explanation for the reduced glucagon induction in ethanol-treated livers is therefore that enzyme synthesis is reduced. The enzyme activity measured is, however, also influenced by the rate of enzyme degradation, the rate of enzyme leakage into the perfusate and the degree of activation or inhibition of the existing enzyme molecules. Changes in any of the these parameters due to ethanol treatment could thus be a cause of the effects shown in Table 2. In order to test whether ethanol treatment had any such effect, TAT-activity of mixed assay-homogenates (1:1) from control (925 units/g protein) and from ethanol-treated livers (1091 units/g protein) perfused for 3 hr in the presence of glucagon was measured and found to be 1010 units/g protein, i.e. equal to the calculated mean, 1008 units/g protein. The enzyme activities given represent the mean of five experiments. Since in addition the initial enzyme activities were increased rather than reduced in ethanol-treated animals, the presence of enzyme inhibitors or lack of activators in these livers appears improbable. When protein synthesis was blocked by cycloheximide (20 μg/ml), TAT activity was reduced to the same extent in both control and ethanol-treated livers (Table 3). This observation

TABLE 3. LACK OF EFFECT OF ETHANOL-TREATMENT ON TAT DEGRADATION IN PERFUSED LIVERS AFTER CYCLOHEXIMIDE*

Treatment	No. of rats	TAT activity (units/g protein) 3 hr‡	TAT activity (units/g protein) 4·5 hr†	Reduction	Reduction in % of 3 hr-value
E-35	6	907 ± 33	554 ± 35	353	38·9
C-35	6	1102 ± 78	756 ± 141	346	31·4
Significance	—	0·033	NS (0·057)	NS	NS

* The results are expressed as mean ± S.E.M. The significance levels according to Wilcoxon's test are given, considering levels above 0·05 as insignificant, NS.

† One hr 30 min after the addition of cycloheximide, 20 μg/ml perfusate, final concentration.

‡ These results are also presented in Table 2 (Experiment 2).

makes differences in TAT-leakage and degradation between ethanol-treated and control rats unlikely, if it is assumed that cycloheximide does not interfere with these processes. Since degradation was proceeding at a rate of approximately $t_{1/2} = 2$ hr, which is close to the most rapid rate of degradation reported for TAT,[14,15] it is concluded that cycloheximide did not reduce the rate of degradation in the present experiments. It had been shown earlier that ethanol treatment also did not influence the slower rate of TAT-degradation in livers given dexamethasone or no additions.[3] Thus no effect of ethanol treatment on enzyme degradation, leakage or activation could be detected in the present or earlier experiments.

Could dietary differences apart from ethanol intake mediate the effect on TAT-synthesis? It is known that differences in dietary protein and carbohydrate levels influence TAT-activity and the hormonal induction of the enzyme.[10,16–20] Only carbohydrate levels differed between ethanol and control groups in the present experiments (Table 1). Carbohydrates are known to decrease TAT-activity.[17–20] As soon as this carbohydrate repression is relieved, TAT-activity will increase towards and almost reach a normal value within 6 hr[17] or a longer period of time.[19] When an inducing hormone is given during this period the highest increase of enzyme activity is measured in the animals which previously had the lowest TAT-activity, i.e. the animals which received the largest amount of carbohydrate.[17] Since our ethanol-treated rats received less carbohydrates than controls it might have been possible that the effect seen was due to the carbohydrates given to the control groups rather than the amount of ethanol consumed by the experimental groups. This is, however, unlikely since (a) the diets in both ethanol and control groups were the same for the 24–28 hr (water only) before perfusion, (b) the absolute enzyme levels of controls after perfusion for 3 hr were significantly higher than the respective levels of ethanol-treated rats and (c) other experiments with no hormonal additions[3] showed no increase of neither control nor ethanol-treated liver TAT during perfusion.

The lack of effect of ethanol treatment on hepatic cyclic AMP levels. The reduced glucagon induction of TAT could have been due to reduced cyclic AMP levels in ethanol-treated livers. Hepatic cyclic AMP levels were measured three times during the first hour of perfusion—each time 5 min after the last addition of glucagon (see

TABLE 4. LACK OF EFFECT OF ETHANOL-TREATMENT ON CYCLIC AMP LEVELS IN PERFUSED LIVERS*

Treatment	No. of rats	Cyclic AMP (pmoles/mg liver wet wt) 0 hr	30–60 min (mean)†	30–60 min (max)†	3 hr
Experiment 1:					
E-22	5	1·01 ± 0·23	8·0 ± 1·7	9·9 ± 2·0	—
C-22	5	0·95 ± 0·14	5·2 ± 0·8	7·1 ± 1·0	—
Significance		NS	NS	NS	
Experiment 2:					
E-35	6	1·46 ± 0·27	8·4 ± 1·5	11·7 ± 2·1	2·45 ± 0·33
C-35	6	1·58 ± 0·36	7·6 ± 2·6	9·5 ± 2·6	2·27 ± 0·25
Significance		NS	NS	NS	NS

* The results are expressed as mean ± S.E.M. Significance levels above 0·05 in Wilcoxon's test are considered insignificant, NS.

† c-AMP levels were measured at 30, 45 and 60 min, each time 5 min after the last addition of glucagon; (mean): the mean value at these three times, (max): the maximal of the three values.

Methods)—initially before glucagon was given, and after perfusion for 3 hr. No significant changes were found between ethanol-treated and control rats at any time (Table 4) although values showed a tendency to be higher in the former group. These results indicate that ethanol treatment affects a step beyond the production of cyclic AMP. When adenyl cyclase activity was measured in rat liver homogenates, there was no difference in basal, adrenaline, glucagon or fluoride stimulated activity between ethanol-treated (7 weeks, ethanol providing 31 per cent of total calories) and control rats (T. Christoffersen and J. Mørland, unpublished results). The concentration of cyclic AMP in livers perfused without hormone for 1 hr was 0·95 pmoles/mg (E-35) and 0·88 pmoles/mg (C-35).

Mechanism of ethanol action on TAT-synthesis. Glucagon regulates TAT-synthesis via cyclic AMP as a mediator.[7] Most probably this occurs at a post-transcriptional level[7,21,22] although the induction is inhibited by actinomycin D[5,6,23] and other inhibitors of RNA-synthesis.[23] The induction of TAT due to dexamethasone has been claimed to occur at the transcriptional level,[24,25] as well as at the post-transcriptional level.[26,27] Both possibilities, however, involve an increased amount of functional mRNA. Steroid- and glucagon-mediated induction of TAT utilize possibly at least one common component, probably the pool of mRNA, as suggested by McNamara and Webb.[28] If the effect of ethanol treatment on TAT induction by dexamethasone and glucagon were due to only one mechanism, a step common to glucagon- and steroid-mediated induction must be involved and not the different hormone receptors. The amount or transport of functional mRNA are thus probable targets for the action of ethanol treatment. The definite conclusion with regard to which processes ethanol affects at the molecular level is, however, unsettled at present.

Effect of ethanol treatment on general protein synthesis after glucagon. A reduction of over-all protein synthesis has previously been found in ethanol-treated livers, perfused with dexamethasone.[3] No such effect was seen in livers given glucagon (Fig. 1); only at one point (3 hr, Fig. 1A) was the incorporation significantly lower than the corresponding control value. The general tendency presented by Fig. 1 was an insignificant reduction in incorporation of label. TCA-soluble counts in ethanol-treated rats (Fig. 1) were also reduced, significantly at certain times (45 min, 1 hr and 3 hr, Fig. 1A). However, this reduction probably does not indicate a reduced specific activity of the total amino acid pool in livers subjected to ethanol treatment, since this treatment also reduces the pool-size of unlabelled amino acids by approximately 5–10 per cent, as measured from the decrease in TAC-soluble ninhydrine positive material. A difference in protein degradation during 1 hr and 30 min after the addition of cycloheximide was not found (Fig. 1B). The radioactivity incorporated into perfusate proteins did not differ significantly among the four experimental groups (E-22, 1·10 ± 0·03; C-22, 1·06 ± 0·04; E-35, 1·16 ± 0·05; C-35, 1·33 ± 0·08 × 10^4 counts/min per g initial liver weight).

Glucagon reduced the incorporation of amino acids into hepatic and perfusate proteins compared with experiments in which dexamethasone or no additions were given (unpublished data). Glucagon could therefore possibly reduce liver protein synthesis, an observation consonant with that of Holten and Kenney[6] obtained in the intact animal. TAT is probably an exception in this regard showing increased synthesis, an induction which is inhibited by previous chronical ethanol treatment, as was the induction caused by dexamethasone.[3]

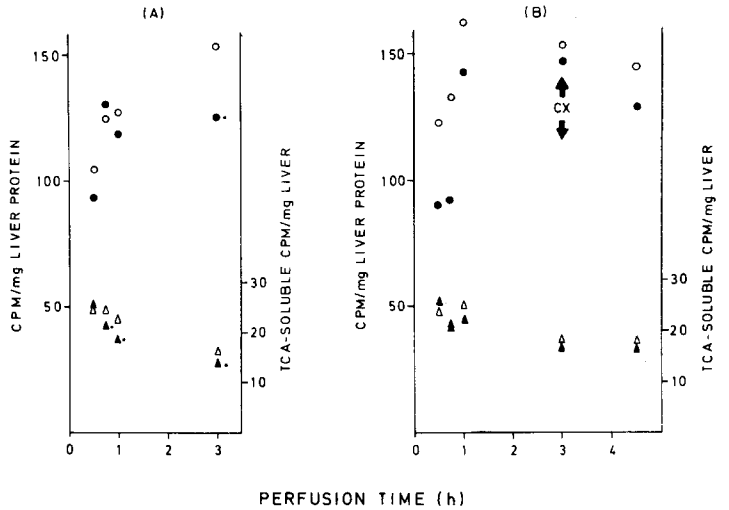

FIG. 1. Effect of chronic ethanol treatment on the incorporation of labelled amino acids into protein. Protein hydrolysate (1·5 μCi, U-^{14}C) was added after perfusion for 15 min. Each point represents the mean incorporation of label into liver proteins (control: ○; ethanol-treated: ●) and TCA-soluble counts (control: △; ethanol-treated: ▲) in five (A) or six (B) experiments. Ethanol treatment lasted for 7 weeks, ethanol substituting 22 per cent (A) or 35 per cent (B) of the total calories. Cx indicates the addition of cycloheximide. Points marked with * were significantly (Wilcoxon, $\alpha < 0{\cdot}05$) lower than the corresponding control values.

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