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Ethanol and Protein Metabolism in the Liver

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MØRLAND, J., A. BESSESEN, A. SMITH-KIELLAND AND B. WALLIN. *Ethanol and protein metabolism in the liver.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 251–256, 1983.—The influence of acute and chronic ethanol administration on liver protein synthesis, secretion and degradation has been studied by various research groups. Acute ethanol administration appeared to have few if any effects on protein synthesis *in vivo*, but reduced the synthetic rates of both stationary and exported proteins in suspensions of isolated rat liver cells. Chronic ethanol intake for more than 4 weeks inhibited protein synthesis *in vivo*, and in cell preparations from treated rats. This inhibitory effect was independent of animal sex, hepatic protein content and diet. The effects of acute and chronic ethanol intake on hepatic protein export are unclear with both inhibition or no effect being reported. The effect of ethanol on liver protein degradation has only been studied to a limited extent, and the results do not indicate clear and marked effects due to ethanol. The inhibitory effect of chronic ethanol intake on hepatic protein synthesis could be of importance in the development of liver injury.

Ethanol metabolism Liver Protein synthesis

THE present paper deals with some effects of ethanol on protein metabolism. It is based mainly on work performed in our research group. Work carried out by other researchers will also be presented, but our report does not intend to give a comprehensive review of the field.

Three main parts of protein metabolism in the liver will be discussed in relation to ethanol. These are protein synthesis, protein export and protein degradation. The processes are connected to different organelles, i.e., free and membrane bound polyribosomes (synthesis), Golgi and secretory vesicles (export) and lysosomes (degradation). The rates of synthesis, export and degradation appear usually to be coupled in some way, although the mechanisms are not too well understood [39]. Thus, knowledge about the rates of two of these processes is needed to estimate the third rate. In other words it is impossible to predict the rate of synthesis from determination of hepatic protein levels only and vice versa.

Another question often raised is whether it is justified to consider all different kinds of proteins as one class of molecules, which is implied in considerations of general synthetic, export and degradation rates as mentioned above. There is apparently no unspecific storage pool of proteins. On the other hand, proteins constitute highly specialized molecules, each with a particular function as enzyme, receptor, membrane channel, structural part of an organelle, etc. It is believed, however, that most proteins share the same synthesizing apparatus as well as the same system for degradation. This implies that proteins to some extent may be considered as a unity [39]. However, there appears to be at least one exception to this generalization. Collagen is subject to specific post-translational synthetic reactions as well as a specific degradation system (collagenase).

METHOD

Single Dose of Ethanol in Vivo

Male Wistar rats (220–265 g body weight) were fasted for 18–21 hr and divided into three weight-matched groups. A triplet consisting of one animal from each group was treated simultaneously, one rat receiving ethanol, the two others receiving either lipid or sucrose in isocaloric amounts by gastric intubation. After 2 hr 20 min (40 min before sacrifice) or 2 hr 40 min (20 min before sacrifice) the rats were given an IP injection containing 187.5 μmol L-valine to which radioactive valine (L-2,3-^{3}H-valine, Amersham U.K.) was added to give a specific radioactivity of 667 mCi·mol^{-1}. Blood, plasma and samples of liver from which labelled valine adhering to the surface was removed, were collected at sacrifice. Radioactivity incorporated into plasma and liver protein was determined by a technique [15] detailed elsewhere [38]. The concentration of free valine was determined in supernatants from plasma and liver samples precipitated with 10% trichloroacetic acid by means of an amino acid analyzer (Kontron Liquimat 3) [24]. Free valine radioactivity was determined in the same supernatants [38]. The concentrations of radioactive and total valine in the hepatic intracellular and extracellular water phase were calculated as described [24]. The specific radioactivity of valyl-tRNA was calculated from these concentrations [37]. The rate of protein synthesis was determined as the increase in protein specific radioactivity in a time interval divided by the mean specific radioactivity of valyl-tRNA in that interval. Protein was measured as described [14], and blood ethanol concentrations were determined by a Perkin-Elmer F-42 head space gas chromatograph.

Long-Term Ethanol Intake

Wistar rats males (190–230 g body weight) and females (170–190 g body weight) were kept on a 12 hr light/12 hr dark cycle, one rat per cage. The rats were divided into weight matched pairs. One rat of each pair received a diet containing ethanol, while the other was pair-fed a control diet. We usually employed a mixed solid/liquid diet as detailed previ-

TABLE 1

EFFECT OF A SINGLE DOSE OF ETHANOL ON RAT LIVER PROTEIN SYNTHESIS IN VIVO*

	Ethanol group	Lipid group	Sucrose group
Rate of protein synthesis stationary liver proteins (pmol·mg protein^{-1}·min^{-1})	145 ± 33	143 ± 32	172 ± 63
Incorporation into plasma proteins (dpm·mg proteins^{-1})	2568 ± 453†	2760 ± 475	3220 ± 583

*Ethanol (2.8 g/kg) or isoenergetic amounts of lipid or sucrose solution was given by gastric tube 2 hr 20 min or 2 hr 40 min before valine (187 μmol, 667 mCi·mol^{-1}) was injected IP as detailed in the Method section. The results are expressed as mean values ± SD, calculated for the period from 20 to 40 min after injection of the precuror (24 rats in each group, 12 killed at 20 min, 12 at 40 min). The mean blood ethanol concentration at sacrifice was 42.1 ± 9.2 mM in the ethanol group.

†Significantly different from sucrose group, $p<0.01$.

ously [18,33]. The solid part of the diet provided 50–60% of the daily energy intake in both the ethanol and the control group, the rest of the energy was supplied by liquid, which was pair-fed. Ethanol rats received a fluid containing 12% (v/v) ethanol and 15% (w/v) sucrose, while the controls obtained isoenergetic amounts of emulsified lipid in some experiments, sucrose in other. Some rat pairs were fed on all liquid diet [8] where carbohydrates replaced ethanol in the control diet. The diets were given for various periods lasting up to approximately 8 weeks. For measurements of the *in vivo* rate of protein synthesis a continuous, intravenous infusion lasting for 32 min was given all rats before sacrifice. The rats were given diethyl ether anaesthesia and a side branch of vena cava inferior was cannulated. The solution to be infused contained 50 μCi ^{3}H-valine and 75 μmol valine per ml (spesific radioactivity 670 mCi·mol^{-1}), otherwise composed and infused as detailed elsewhere [33]. Blood, plasma and liver samples were taken after 12, 22 and 32 min of infusion as described [33]. The samples were analyzed and rates of protein synthesis calculated as described above.

Isolated Hepatocytes

Isolated liver cells were prepared by a two step Ca^{++}-free, Ca^{++}-collagenase perfusion of rat livers as detailed [25,32], and parenchymal as well as nonparenchymal cells were isolated [24]. Cells were incubated [21] for various time periods. Rates of protein synthesis were calculated after (a) addition of tracer amino acids followed by determination of precursor specific radioactivity at various time points [21,24] or (b) addition of labelled valine plus a high concentration (4.2 mM) of unlabelled valine [38]. Protein secretion was measured as the rate of appearance in media of proteins labelled during a 2.5 or 7.5 min period (followed by a chaser dose) [27]. Protein degradation was measured as the release of labelled valine to the media after *in vivo* labeling [21].

RESULTS AND DISCUSSION

Protein Synthesis

Our results are from experiments in which radioactively labelled amino acids are incorporated into proteins synthesized by the liver. To convert incorporation data into protein synthetic rates it is necessary to know the specific radioactivity of the precursor (aminoacyl-tRNA) throughout the incorporation period. This can be achieved by measurement of the concentration and radioactivity of the precursor amino acid inside and outside the liver cell, and then calculating the specific radioactivity of the real precursor [37]. A further prerequisite is that the specific radioactivity of the precursor be rather constant during the incorporation period. Otherwise the calculation of protein synthetic rates will become difficult. Constant precursor specific activity can be obtained in cell suspensions by administering a tracer amino acid, but stable specific activity is obtained more safely if a large amount of unlabelled precursor amino acid is also given simultaneously. In the intact animal it seems impossible to obtain a fairly constant precursor specific activity (s.a.) by injecting a tracer amino acid (maximum s.a. measured 2 min after IP injection, reduced by 60% during subsequent 20 min) while it might be obtained by either administration of a large single dose of amino acid (labelled and unlabelled) or by infusing a mixture of labelled and unlabelled amino acid (maximal s.a. being virtually unchanged during following 20 min).

Single dose of ethanol in vivo. To our knowledge no previous study taking the points discussed above into consideration has been performed on the effects of acute ethanol intake on hepatic protein synthesis *in vivo*. Our main conclusion was that acute administration of ethanol intragastrically, accompanied by blood ethanol levels of 40–60 mM for the following hours, did not influence the synthesis of stationary liver proteins in fasted rats (Table 1, upper line). Synthetic rates of plasma proteins secreted by the liver could not be calculated in the present study, because they were synthesized to a large extent during a period in which the precursor specific radioactivity changed from zero to a value close to 1000 dpm·nmol^{-1}. Assuming that there were no major differences in precursor specific radioactivity among the three treatment groups, since also a large amount of unlabelled precursor amino acid was always given, the incorporation data of Table 1, lower line, can be taken to represent plasma protein synthesis. They demonstrate that ethanol reduced this process compared to controls

TABLE 2

EFFECTS OF ETHANOL AND 4-METHYLPYRAZOLE (4-MP) ON INCORPORATION OF ^{14}C-VALINE AND DISTRIBUTION OF POLYSOMES IN SUSPENSIONS OF ISOLATED HEPATOCYTES*

Group	^{14}C-val incorporation into cell protein (pmoles/mg/min)	(% of control)	Distribution of polysomes (% larger than trisomes)	(% of control)
1 Control	60.5 ± 3	100	50.9 ± 1.6	100
2 Ethanol (80 mM)	40.7 ± 1†	67	45.3 ± 1.6†	89
3 Ethanol (80 mM) + 4-MP (0.5 mM)	49.6 ± 2†	82	51.9 ± 1.9	102

*Parenchymal cells from fasted rats ($6 \cdot 10^6$ cells·ml^{-1}) were incubated for 70 min after the addition of valine (final concentration 2.2 mM, specific radioactivity 46 mCi·mol^{-1}). The values are means ± SE of 6 determinations in each group. For some cell suspensions two parallel analyses of polysomes were performed, and the polysomal values are means of 11 determinations in groups 1 and 2 and 8 determinations in group 3.

†Significantly different from the two other groups, $p<0.05$.

(From Harbitz, Wallin, Hauge and Mørland; unpublished work).

given isocaloric amounts of sucrose, but not compared to animals pair-fed lipids. Therefore, no ethanol-specific effect seemed to exist. Our observations do not exclude that other doses of ethanol might exert effects on protein synthesis. The lack of effect of a single dose of ethanol given *in vivo* is in agreement with earlier results of incorporation experiments [1, 19, 31] and a more recent study [5]. Reduced rates of albumin synthesis [10] and total protein synthesis [28] *in vivo* have been reported in rats receiving ethanol compared to control given no pair-feeding.

Acute administration of ethanol in vitro. Reduced rates of albumin synthesis at ethanol concentrations of 45–50 mM have been found in the perfused liver [30]. In isolated hepatocytes derived from fasted rats, ethanol inhibited the synthetic rates of both stationary and of proteins exported to the cell medium [21]. The effect was present in parenchymal, but not in nonparenchymal liver cells [24]. Ethanol decreased protein synthesis in parenchymal cells at both physiological [21] and high amino acid levels (8 times normal plasma levels, unpublished data). The effect occurred at ethanol concentrations of 10 mM [25], and increased gradually with ethanol concentrations at least up to 100 mM [25]. This observation together with experiments performed with 4-methylpyrazole, propanol and tertiary butanol [25] indicated the presence of two inhibitory mechanisms. One is linked to ethanol metabolism and might be blocked by 4-methylpyrazole. The other appears to be dependent on ethanol *per se*, and to constitute a general alcohol effect. Recent studies on polysome profiles from isolated hepatocytes (Table 2) led us to the preliminary conclusion that the metabolic part of the effect occurs at the level of initiation and might be due to a reduced level of hexose phosphates, while the additional effect caused by ethanol *per se* affects initiation and elongation in a balanced manner, yielding unchanged polysomal distribution (Table 2).

Other studies have shown that hepatocytes derived from fed donors are much more resistant against the inhibitory effect of ethanol than cells from fasted donors [9, 12, 23]. Recently we found that the effect of ethanol could be almost abolished if the cell incubation was performed in the presence of insulin and high glucose levels (20 mM) (unpublished observations). Finally, strict control of medium pH to 7.4 reduced the effect [38]. Taken together, these latter observations could explain why an effect of acute ethanol administration is not seen *in vivo* but is seen under certain circumstances in isolated hepatocytes. Still, however, the *in vitro* observations could be of importance to *in vivo* situations, as extreme fasting, diabetes and acidosis due to hypoxia. With regard to hypoxia the effect of ethanol on protein synthesis has recently been considered of special relevance to the periveneous hepatocytes *in vivo* [3].

Long-term ethanol intake. Earlier studies by our group have indicated that inclusion of ethanol in the diet (25–30 per cent of total calories consumed) was accompanied by reduced protein synthesis after treatment for 30 days or longer periods [22,33], while consumption for 2 weeks or shorter periods had no such consequence [22,34]. The effect of ethanol was seen in comparison with animal groups pair-fed either lipids or carbohydrates [22, 26, 33]. A high intake of dietary protein could not prevent the effect [22]. Still it has been claimed that the effect could be linked to some degree of malnutrition [13]. Recent experiments, however, showed that the effect was present also when the all-liquid diet of Lieber and De Carli was used (Table 3), further supporting a direct role of ethanol in the inhibition of protein synthesis. These results also demonstrated that protein synthesis was inhibited by ethanol independent of its action on hepatic protein levels. The latter were increased after ethanol (685±25 mg × 100 body wt^{-1}) compared to controls (618±10 mg × 100 g body wt^{-1}) in rats fed the all-liquid diet (group 1 in Table 3), while no difference in hepatic protein content was found between ethanol treated and control rats on the mixed solid and liquid diet (groups 2 and 3 in Table 3). In another study we found that the enhanced hepatic protein level due to ethanol measured after 2 weeks disappeared if the treatment period was extended to 8 weeks. Protein synthesis was, however, inhibited at the end of the experiment [34]. The results presented in Table 3 also demonstrate that there was no major sex difference with respect to the inhibition of protein synthesis by ethanol. Other studies have revealed that the inhibitory effect is pres-

TABLE 3

EFFECT OF LONG-TERM ETHANOL TREATMENT ON RATES OF HEPATIC PROTEIN SYNTHESIS (P.S.) IN FASTED MALE AND FEMALE RATS FED VARIOUS DIETS* [35]

	Treatment	Liver protein synthesis nmoles·100 g body wt^{-1}·min^{-1} 12–32 min	(n)
Group 1	Ethanol	91.1 ± 6.2†	(9)
(males)	Control	115 ± 6	(8)
Group 2	Ethanol	78.9 ± 7.7†	(8)
(males)	Control	108 ± 7	(8)
Group 3	Ethanol	102 ± 10†	(8)
(females)	Control	125 ± 4	(8)

*Rats were pair-fed ethanol or control diet for 43–50 days. Group 1 received an all liquid diet, while group 2 and 3 were given a mixture of solid and liquid diet. All rats were fasted for 12–18 hr before giving a 32 min continuous infusion of ^{3}H-valine (specific activity 670 mCi/mol). Blood and liver samples were taken at 10, 20, 32 min and 12, 22, 32 min, respectively. The rates of hepatic p.s. were calculated for each of the two intervals 12–22 min and 22–32 min, and the means are given. For a few animals, rate of p.s. for only one interval was valid, which was then included in the mean. (n) indicates the number of rats. Mean values ± SE are given.

†α<0.05, compared to pair-fed controls, according to Wilcoxon's test (two-sample distribution).

ent when livers from ethanol treated rats are isolated and perfused [18], supporting *in vivo* studies indicating that the actual presence of ethanol is not needed for the effect to occur [22, 26, 33]. In recent experiments on isolated liver cells from animals subjected to long-term ethanol treatment, the inhibition of protein synthesis was present only in parenchymal cells while nonparenchymal cell protein synthesis was resistant to long-term ethanol feeding (unpublished results). Table 4 shows that no particular subcellular organelle was specially influenced with respect to ethanol mediated inhibition of protein synthesis.

The mechanisms underlying the effect of long-term ethanol intake are not known. Obviously the effect is not a consequence of cellular and subcellular destruction since it is not accompanied by morphological alterations [34] and since the effect is rapidly reversible by subjecting the animals to stress [33]. The latter observation might explain why some authors have had difficulties in reproducing the inhibitory effect of long-term ethanol intake on amino acid incorporation into protein, while other groups [2,29] have obtained results similar to ours.

Protein Export

To study the rate of hepatic protein export separated from the rate of protein synthesis, a model is needed which allows labelling of just some molecules during synthesis, followed by subsequent measurements of the passage of this label through the various parts of the liver cell to the exterior. No such model appears to exist, but several experimental designs approach the optimal one. Such designs are, however, often only feasible *in vitro* (see Method Section). Another method, also useful *in vivo*, is to follow the appearance of labelled proteins outside the liver and to express this as a fraction of the total label incorporated. There are some uncertainties with this latter method with regard to the existence of a common precursor pool for stationary and excreted proteins, as to which time period would be the correct base of reference and to the possibility that the treatment studied could also affect synthesis of stationary and export proteins differently. Levels of single export proteins could also be determined inside and outside the liver cell both *in vivo* and *in vitro*. The difficulty here is that changes in rates of synthesis and degradation have to be known to allow meaningful interpretation.

Acute administration of ethanol. After a single dose of ethanol given *in vivo* (see Method Section) and calculating the fraction incorporated into export proteins we found no effect of ethanol: 0.13±0.02 (ethanol), 0.14±0.02 (lipid) and 0.14±0.02 (sucrose), giving mean values±SD for 12 rats in each group measured 40 min after the administration of label. In a similar study an inhibitory effect of ethanol was found when compared to a carbohydrate group [5]. There are some differences with regard to design between these two studies.

TABLE 4

INTRACELLULAR DISTRIBUTION OF ^{3}H-VALINE INCORPORATION INTO PROTEIN AND PROTEIN CONTENT IN LIVERS OF RATS AFTER LONG TERM ETHANOL FEEDING* [34]

	Treatment groups	(n)	Absolute values (N + E)	Percentage values N + E	N	M	P	S	Recovery
Protein incorporation (dpm·g liver wet weight^{-1}·10^{-4})	E	12	54.3 ± 4.4†	100	28.8 ± 0.7	25.0 ± 1.1	27.1 ± 1.2	16.0 ± 1.6	96.8 ± 1.6
	C	10	72.6 ± 6.0	100	28.8 ± 1.4	25.1 ± 1.8	28.3 ± 1.2	15.2 ± 0.3	97.3 ± 2.3
Protein content (mg·g liver wet weight^{-1})	E	12	244 ± 6	100	37.9 ± 0.6	26.0 ± 1.1	10.8 ± 0.4	24.2 ± 0.8	98.9 ± 1.7
	C	10	247 ± 6	100	35.9 ± 1.8	25.7 ± 1.8	12.5 ± 0.6	24.6 ± 1.2	98.3 ± 2.1

*Rats were pair-fed ethanol, E, or control, C, diets for approximately 8 weeks and fasted for the last 12–18 hr. Before sacrifice they were given a 32 min intravenous infusion of ^{3}H-valine (specific activity 670 mCi/mol). Means ± SE are given. (n) gives the number of animals. Subcellular fractions: N=nuclear, E=cytoplasmic extract, M=mitochondrial, P=microsomal, S=cytosol.

†α<0.025, compared to the pair-fed control group according to Wilcoxon's test (two-sample distribution).

In the latter the ethanol concentration was lower (20 mM versus 50–60 mM in our study), albumin was measured (versus total export proteins in our study), fed animals were used (we used fasted) and ether was given as an anaesthetic (we used no anaesthesia). Ether severely impairs the synthesis/export of liver proteins (unpublished observations) and this might be of importance. In another *in vivo* study using a somewhat different technique no effect of ethanol on protein export was found [7].

By a pulse chase method we could not demonstrate any effect of acute ethanol administration on hepatic protein secretion from isolated hepatocytes from fasted and fed donors [27]. Using a similar design and liver slices, other workers were able to show inhibition of glycoprotein secretion by ethanol [36]. In those studies longer pulse periods were used.

Long-term ethanol intake. The fraction of labelled proteins exported was not reduced by chronic ethanol intake [22]. This was also found when proteins secreted by isolated hepatocytes from rats given ethanol for 5–6 weeks were studied (unpublished results). On the other hand it has been reported that long-term ethanol feeding reduced hepatic protein excretion [4]. That study differed from ours with regard to proteins measured, nutritional state (fed versus fasted in our study) and anaesthesia, since ether appeared to be used in that study in contrast to ours.

Protein Degradation

Results from three types of investigation seem to be available. These concern (a) activities of rapidly turning-over enzymes measured after blocked synthesis, (b) levels of branched amino acids (derived from degrading protein), and (c) lysosomal enzyme activities.

Acute administration of ethanol. To our knowledge no studies have so far addressed this question with regard to the intact animal. In isolated perfused liver ethanol reduced the free (non-latent) activity of a lysosomal enzyme [6], and the degradation of the enzyme tyrosine aminotransferase [20]. In suspensions of isolated hepatocytes no effect of ethanol on valine release from protein was recorded [21].

Long-term ethanol intake. After chronic ethanol intake we have measured increased levels of branched amino acids in livers from fasted male rats (unpublished results), but not in livers from fed animals [26]. Chronic ethanol treatment has also been reported to increase lysosomal free (non-latent) enzyme activity [6,16] as well as total activity [16]. We have recently found higher levels of branched amino acids in media of liver cells isolated from animals fed ethanol than in corresponding media of control cells (unpublished observations).

Main Effects of Ethanol on Protein Metabolism

Much work remains to be done, specially to elucidate if there is any consistent acute or chronic effect of ethanol intake on hepatic protein export and degradation. With regard to protein synthesis a general reduction appears to be the result of long-term consumption. The mechanisms underlying this effect are not known. Little is also known about the clinical and biological consequences of the effect.

One possibility is that reduced protein synthesis could reduce the level of some liver enzyme or structural protein below a level critical to normal liver cell function. This could then eventually lead to liver cell necrosis and thus constitute an important event in alcoholic liver damage. In support of this hypothesis is the observation that hepatotoxins very often inhibit protein synthesis or destroy the normal morphology of the endoplasmic reticulum [40]. Of interest is also the reduced number of parenchymal cells compared to the number of nonparenchymal cells (seen after long-term ethanol consumption) [17], when the inhibition of protein synthesis in former cell type and the lack of effect in the latter are born in mind. However, the fact that protein synthesis inhibitors can inhibit the development of hepatic necrosis caused by other hepatotoxins [11], points against a general role of protein synthesis inhibition in the development of hepatic necrosis. On the other hand, decreased synthesis of apolipoproteins could facilitate lipid accumulation in the liver.

Another possibility is that reduced protein synthesis decreases the potential of the liver for rapid adaptation and regulation of metabolic processes. The ability to carry out such rapid adaptive responses is of major importance to the main role the liver plays in regulation of metabolism. After amino acid loads (e.g. meals), amino acid metabolizing enzymes are rapidly induced in the liver to levels several fold the resting level requiring intact protein synthesis. In response to hormone signals, enzyme induction often occurs, again being dependent on normal protein synthetic capacity. It is of interest that the induction of amino acid metabolizing enzymes like tryptophan oxygenase and tyrosine aminotransferase by corticosteroids is reduced in livers of animals chronically fed ethanol [18]. Other examples of metabolic loads that might require rapid protein synthesis is the exposure of the liver to certain metals (metallothionein synthesis) and drugs (synthesis of cytochrome P-450). Lack of protein synthetic capacity in such instances could have important toxicological consequences. Only future studies can evaluate the significance of reduced capacity for adaptation by reduced liver protein synthesis due to ethanol. Conditions like disturbed liver regeneration and possibly central nervous dysfunction caused by excess amino acids being offered to the brain, might be of interest in this respect.

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