Biochemical Pharmacology, Vol. 19, pp. 2097–2103. Pergamon Press. 1970. Printed in Great Britain

LIVER ALCOHOL AND SORBITOL DEHYDROGENASE ACTIVITIES IN HYPO- AND HYPERTHYROID RATS

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(*Received* 4 *November* 1969; *accepted* 9 *January* 1970)

Abstract—A comparative study was made of the effects of prolonged propyl thiouracil or triiodothyronine treatment on rat liver alcohol and sorbitol dehydrogenase activities. The influence of voluntary alcohol consumption on these activities were also investigated. The liver-to-body weight ratio was increased by triiodothyronine treatment and also by voluntary alcohol consumption. Liver alcohol dehydrogenase activity was markedly increased by propyl thiouracil treatment and markedly decreased by triiodothyronine treatment. These changes in enzyme activity were statistically significant whether expressed in relation to liver wet weight, liver soluble protein content or body weight. Simultaneous voluntary alcohol consumption significantly inhibited the increase in alcohol dehydrogenase activity caused by propyl thiouracil treatment. Daily ethanol intake did not increase liver alcohol dehydrogenase activity in the animals. No marked changes were found in liver sorbitol dehydrogenase activity after propyl thiouracil or triiodothyronine treatment. The significance of the two liver enzymes in regulating the *in vivo* elimination rates of ethanol and sorbitol is discussed.

ALCOHOL dehydrogenase and sorbitol dehydrogenase[1] are both liver enzymes dependent on cytoplasmic nicotinamide adenine dinucleotide. Ethanol is oxidized to acetaldehyde in the mammalian organism, mainly by liver alcohol dehydrogenase. Liver sorbitol dehydrogenase oxidizes sorbitol to D-fructose, which affects the redox state of the liver cytosol and the oxidation rate of ethanol.[2] The redox state of the liver is influenced by thyroid hormones.[3]

The effect of ethanol consumption on liver alcohol dehydrogenase is still a matter of controversy.[4] An increase in alcohol dehydrogenase activity has been reported to take place in rats[5] and mice[6] after prolonged alcohol consumption. On the other hand, it has been reported that liver alcohol dehydrogenase activity is directly related to the status of the hepatic parenchymal cells[7] and that ethanol administration does not effect the development of alcohol dehydrogenase activity in foetal or postnatal rat liver.[8]

Propyl thiouracil treatment with simultaneous voluntary alcohol consumption appeared to increase liver alcohol dehydrogenase activity,[9] but the problem of whether this effect was a direct one caused by thiouracil or due to alcohol consumption could not at that time be answered. *In vivo* effects of thyroid hormones on liver alcohol dehydrogenase activity have been found in rats[10] but not in mice.[11] In *in vitro* experiments, alcohol dehydrogenase is inhibited by thyroxine and the mechanism of this inhibition has recently been investigated.[12] However, it has been noted that many enzymes which are inhibited by thyroxine *in vitro* are increased *in vivo* by thyroxine administration.[13]

This study was carried out in order to examine how different thyroid hormone levels in combination with ethanol influence the liver alcohol dehydrogenase and whether there is any interaction between thyroid hormones and ethanol. The *in vivo* elimination rates of ethanol and sorbitol were determined in order to compare these parameters with the measured alcohol dehydrogenase and sorbitol dehydrogenase activities, respectively.

MATERIALS AND METHODS

Male Wistar rats weighing 310–440 g were used in the experiments. Half of the animals were placed in individual cages and given a choice of 10% (v/v) ethanol and tap water. The daily alcohol consumption was recorded. The other half served as controls and received no ethanol. All the rats were given ordinary laboratory food, containing 38 per cent of protein and 0·1 per cent of choline chloride, *ad lib*. Some of the animals received a daily dose, by stomach tube, of 5 mg per 100 g body weight of a 0·5% solution of propyl thiouracil (obtained from Eli Lilly and Co., Indianapolis, USA). Another group was treated daily with 20 μg of 3,3',5-triiodo-L-thyronine (Sigma Chemical Co., Missouri, USA) given intraperitoneally in saline, and a third group of rats received no special treatment.

After a 6-week period of treatment the oxygen consumption of the experimental animals was recorded in basal conditions with a Beckman Oxygen analyser, Model E 2, according to the method presented by Depocas and Hart[14] in order to examine the effect of the treatment. The animals were then weighed and decapitated. The livers were removed carefully and weighed and the liver-to-body weight ratio calculated.

For estimation of the alcohol dehydrogenase and sorbitol dehydrogenase activities, 10% liver homogenate was prepared in ice-cold 0·25 M sucrose containing 1% Triton X-100 (obtained from Rohm & Haas Co., Philadelphia, USA). It has previously been deomonstrated that this procedure gives maximal alcohol dehydrogenase activity in liver supernatants.[15] After centrifugation of the homogenate for 10 min at 5000 *g*, samples were taken from the supernatant for protein determination,[16] and for enzyme assays. Alcohol dehydrogenase activity was estimated by the modified method of Bonnichsen and Brink,[17] with a Beckman DK 1 A Recording Spectrophotometer. Liver supernatant was incubated at 25° with 1·47 mM NAD and 50 mM ethanol in buffer contained 75 mM sodium pyrophosphate, 75 mM semicarbazide and 22 mM glycine adjusted to pH 8·7 with 2N NaOH. The alcohol dehydrogenase activity was expressed in μmoles/min as recommended by the Report of the Commission on Enzymes of I.U.B.[18]

The method adopted for measurement of the sorbitol dehydrogenase was a modification of the alcohol dehydrogenase method. The assay conditions were the same, except that the measurement was carried out at pH 8·0, which has been given as the optimum pH for the enzyme.[1] The enzymic reaction was initiated with sorbitol, the final concentration being 20 mM, instead of ethanol. The activity was expressed as μmoles/min. In the present study both the enzyme activities are expressed as units per g liver wet weight, per 100 g body weight and also per 100 mg liver soluble protein. When the enzyme activities were measured at pH 7·4 and at 38°, the normal temperature of the rat liver, all other assay conditions were as described.

For *in vivo* elimination studies of ethanol and sorbitol the animals were treated similarly with thiouracil and triiodothyronine but were not given ethanol. For

determination of the oxidation rates ethanol was injected in saline intraperitoneally 150 mg/100 g body weight as a 10% (w/v) solution and sorbitol intravenously under light pentobarbital anaesthesia 50 mg/100 g body weight as a 5% (w/v) solution. Blood samples were taken from the tip of the tail. For determination of the elimination rate of sorbitol urine was also collected in special cages for the following twenty hours and its content of sorbitol measured. Ethanol was measured enzymatically by the method described by Bücher and Redetzki[19] and sorbitol was determined colorimetrically by the method of West and Rapoport.[20]

RESULTS

All groups consumed about 8·7 m-moles of ethanol per 100 g of body weight per day. The average basal metabolic rate of rats given thiouracil and ethanol was 31 per cent lower than that of the control group (Table 1). Thiouracil treated rats receiving no ethanol had a metabolic rate 39 per cent lower than controls receiving no ethanol. Both groups of triiodothyronine treated rats had a metabolic rate about 36 per cent higher than the corresponding control animals. The oxygen consumption of the control rats was of the same order in both drinking and non-drinking groups.

TABLE 1. INFLUENCE OF VOLUNTARY ETHANOL CONSUMPTION AND PRETREATMENT WITH PROPYL THIOURACIL OR TRIIODOTHYRONINE ON RAT LIVER CONSTITUENTS AND BASAL OXYGEN CONSUMPTION

Experimental groups		Relative liver weights (g/100 g body wt.)	Liver soluble protein contents (g/g liver wet wt.)	Basal oxygen consumption (ml/hr/100 g)
Control	(9)	2·77 ± 0·13	0·213 ± 0·012	0·64 ± 0·08
Control + Ethanol	(9)	3·09 ± 0·22	0·204 ± 0·012	0·67 ± 0·03
Thiouracil	(9)	2·82 ± 0·16	0·205 ± 0·014	0·39 ± 0·04
Thiouracil + Ethanol	(9)	3·06 ± 0·21	0·212 ± 0·012	0·46 ± 0·04
Triiodothyronine	(9)	4·13 ± 0·78	0·201 ± 0·013	0·87 ± 0·04
Triiodothyronine + Ethanol	(9)	4·41 ± 0·18	0·198 ± 0·011	0·91 ± 0·11

The figures represent the mean ± standard deviation. The figures in parentheses represent the number of animals in each group.

Thiouracil treatment did not influence the liver-to-body weight ratio or liver soluble protein content, as can be seen in Table 1. The significant ($P < 0.001$) increase in the liver-to-body weight ratio after triiodothyronine treatment was caused by the severe weight loss in these animals. The liver soluble protein content was not influenced by the triiodothyronine treatment. Voluntary ethanol intake of 6 weeks' duration increased the liver-to-body weight ratio in all groups (significantly, $P < 0.05$, only in thiouracil treated and control rats) but had no marked effect on the liver soluble protein content.

Table 2 summarizes the liver alcohol dehydrogenase activities of the experimental animals. Liver alcohol dehydrogenase activity is significantly ($P < 0.05$) increased in rats pretreated with thiouracil and significantly ($P < 0.001$) decreased in rats pretreated with triiodothyronine whether expressed in relation to body weight, liver wet

TABLE 2. INFLUENCE OF VOLUNTARY ETHANOL CONSUMPTION AND PRETREATMENT WITH PROPYL THIOURACIL OR TRIIODOTHYRONINE ON RAT LIVER ALCOHOL DEHYDROGENASE ACTIVITY

Experimental groups		Liver alcohol dehydrogenase activity (nmoles × min^{-1} /100 g body wt.)	(nmoles × min^{-1} /g liver wet wt.)	(nmoles × min^{-1} /100 mg liver soluble protein)
Thiouracil	(9)	6145 ± 645	2179 ± 195	1069 ± 130
Thiouracil + Ethanol	(9)	5568 ± 721	1879 ± 270	891 ± 138
Control	(9)	4507 ± 612	1628 ± 226	753 ± 114
Control + Ethanol	(9)	4354 ± 1056	1396 ± 256	680 ± 121
Triiodothyronine	(9)	2623 ± 958	658 ± 287	326 ± 117
Triiodothyronine + Ethanol	(9)	2732 ± 312	621 ± 194	314 ± 46

The figures represent the mean ± standard deviation. The figures in parentheses represent the number of animals in each group.

TABLE 3. INFLUENCE OF VOLUNTARY ETHANOL CONSUMPTION AND PRETREATMENT WITH PROPYL THIOURACIL OR TRIIODOTHYRONINE ON RAT LIVER SORBITOL DEHYDROGENASE ACTIVITY

Experimental groups		Liver sorbitol dehydrogenase activity (nmoles × min^{-1} /100 g body wt.)	(nmoles × min^{-1} /g liver wet wt.)	(nmoles × min^{-1} /100 mg liver soluble protein)
Thiouracil	(9)	2710 ± 301	959 ± 72	471 ± 45
Thiouracil + Ethanol	(9)	2702 ± 280	883 ± 64	417 ± 44
Control	(9)	2435 ± 255	878 ± 61	412 ± 30
Control + Ethanol	(9)	2463 ± 239	797 ± 64	397 ± 33
Triiodothyronine	(9)	3515 ± 837	851 ± 103	420 ± 91
Triiodothyronine + Ethanol	(9)	3197 ± 791	725 ± 142	367 ± 94

The figures represent the mean ± standard deviation. The figures in parentheses represent the number of animals in each group.

weight or liver soluble protein content and irrespective of whether the animals had consumed ethanol or not.

Somewhat lower alcohol dehydrogenase activities were recorded in thiouracil treated and control rats given ethanol as compared to the corresponding controls. This effect of ethanol consumption was statistically significant ($P < 0.05$) only in thiouracil treated rats and when alcohol dehydrogenase activity was not expressed as units per 100 g of body weight. Total liver alcohol dehydrogenase activity was 28% higher in ethanol-treated and respectively 36 per cent higher in control thiouracil-treated rats than in the corresponding controls. Voluntary ethanol consumption had no effect on liver alcohol dehydrogenase activity in triiodothyronine treated animals.

The liver sorbitol dehydrogenase activities are given in Table 3. The total sorbitol dehydrogenase activity of the liver, calculated per 100 g of body weight, seemed to be slightly higher in both triiodothyronine ($P < 0.05$) and thiouracil treated rats than in the control animals; ethanol had no effect on this parameter. When sorbitol

dehydrogenase activity was calculated as units per g of liver wet weight, the order of activity of the groups was thiouracil > control > triiodothyronine, the thiouracil treated group differing significantly ($P < 0.05$) from the other two groups. Ethanol consumption lowered the sorbitol dehydrogenase activity in all groups ($P < 0.05$).

The alcohol dehydrogenase and sorbitol dehydrogenase activities, measured at pH 7·4 and at 38°, and the oxidation rates of ethanol and sorbitol, all expressed in μmoles of substrate oxidized per minute per g of liver wet weight, are given in Table 4.

TABLE 4. THE *IN VIVO* OXIDATION RATES OF ETHANOL AND SORBITOL COMPARED WITH THE LIVER ALCOHOL DEHYDROGENASE AND SORBITOL DEHYDROGENASE ACTIVITIES IN THE EXPERIMENTAL GROUPS

Group		*In vivo* oxidation rate of ethanol	Alcohol dehydrogenase activity	*In vivo* oxidation rate of sorbitol	Sorbitol dehydrogenase activity
Thiouracil treated	(9)	3·8 ± 1·3	2·4 ± 0·2	0·99 ± 0·13	0·43 ± 0·03
Control	(9)	4·5 ± 0·9	1·8 ± 0·3	0·88 ± 0·13	0·38 ± 0·03
Triiodothyronine treated	(9)	3·4 ± 0·7	0·7 ± 0·3	0·75 ± 0·12	0·33 ± 0·04

The enzyme activities are measured at pH 7·4 and at 38°. Both the *in vivo* oxidation rates and the enzyme activities are expressed as μmoles of substrate oxidized/min/g liver wet weight. The figures represent the mean ± standard deviation. The figures in parentheses represent the number of animals in each group.

The *in vivo* oxidation rates of both ethanol and sorbitol were greater than the corresponding enzyme activities of the liver. The *in vivo* oxidation rate of ethanol followed the order: control > thiouracil > triiodothyronine, the triiodothyronine treated group differing significantly ($P < 0.05$) from the control group. When the oxidizing capacity of ethanol of the liver was measured *in vitro* the following order was revealed: thiouracil > control > triiodothyronine. The *in vivo* oxidation rate of sorbitol as well as the sorbitol dehydrogenase activity of the liver followed the order: thiouracil > control > triiodothyronine. Sorbitol was oxidated at a significantly ($P < 0.05$) lower rate in triiodothyronine treated rats when compared with control animals.

DISCUSSION

The liver-to-body weight ratio increased in all experimental groups during a 6-week period of voluntary ethanol consumption. This may be due to accumulation of fat in the liver, since consumption of relatively small amounts of ethanol is known to increase the liver triglyceride content in non-alcoholic volunteers on a standard diet.[21]

No increase in liver alcohol dehydrogenase activity due to ethanol consumption was observable in this study. However, it must be pointed out that the amount of ethanol consumed daily by our animals was not very high.

The results presented indicate that thyroid hormones influence the liver alcohol dehydrogenase activity. The thiouracil treatment increased the alcohol dehydrogenase activity. Suzuki *et al.*[10] have shown that the effect of thyroidectomy is the same, which suggests that the effect of thiouracil on alcohol dehydrogenase activity is not a direct one but mediated through the thyroid gland. The decrease in liver alcohol dehydrogenase activity in thyrotoxicosis induced by triiodothyronine as shown in Table 2 is

confirmed by the similar effect of thyroxine treatment, published while this manuscript was in preparation.[22]

Simultaneous voluntary ethanol consumption and thiouracil treatment appeared to have a slight inhibitory effect on the increase of liver alcohol dehydrogenase activity and on the decrease in basal metabolic rate induced by thiouracil. These effects are interesting, since it has recently been reported that a greater output of hormones by the thyroid gland are induced by prolonged alcohol consumption in rats.[23] The small effect produced by treatment with thouracil or triiodothyronine on liver sorbitol dehydrogenase activity is not incompatible with the data found in our laboratory concerning the sorbitol elimination rate *in vivo*. The liver sorbitol dehydrogenase activity calculated per 100 g body weight has been shown to be uninfluenced by the effect of various diets and hormones.[24]

Attempts have been made to correlate the alcohol dehydrogenase activity determined *in vitro* with the *in vivo* oxidation rate of ethanol.[25, 26] It is generally accepted that at least 90 per cent of the ingested ethanol is oxidized by liver alcohol dehydrogenase. Ethanol oxidation via alcohol dehydrogenase of other tissues and elimination via lung and kidney is quantitatively small. The measured alcohol dehydrogenase activity was only 63, 40 and 21 per cent of the corresponding *in vivo* oxidation rate of ethanol in the thiouracil treated, control and triiodothyronine-treated groups, respectively. These low values agree with the findings of Mourad and Woronick,[26] who compared human liver alcohol dehydrogenase activity with the elimination rate of ethanol from the blood stream. However, it is possible that our method of alcohol dehydrogenase assay did not give all of the alcohol dehydrogenase activity of the liver cell. The greater alcohol dehydrogenase activity of the thiouracil treated group was not accompanied by a greater ethanol oxidation rate. This discrepancy suggests that the regulation of the rate of oxidation of ethanol in rat liver is more complicated and cannot be explained on the basis of mere alcohol dehydrogenase activity. Our findings in rats do not agree with the positive correlation between liver alcohol dehydrogenase activity and the elimination rate of ethanol in mice reported by Schlesinger.[27]

As can be recognized from Table 4, the *in vivo* oxidation rate of sorbitol is just about twice as high in every experimental group as the measured sorbitol dehydrogenase activity. The numerical values of the enzyme activity are of little significance, because ionic strength, substrate concentrations, etc., of the intracellular compartment are different from those of the test tube. However, the parallelism between the *in vivo* oxidation rate and the sorbitol dehydrogenase activity suggests a controlling role of the amount of sorbitol dehydrogenase in sorbitol metabolism.

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