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INHIBITION OF SORBITOL OXIDATION BY ETHANOL IN INTACT RATS PRETREATED WITH TRIIODOTHYRONINE OR PROPYL THIOURACIL

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Abstract—The influence of pretreatment of intact rats with propyl thiouracil or triiodothyronine on the inhibition of sorbitol elimination by ethanol was studied. Ethanol inhibited sorbitol elimination by 60 per cent in hypothyroid animals, by 39 per cent in controls and by 16 per cent in hyperthyroid rats. The capacity of liver tissue to eliminate sorbitol without ethanol was not significantly different in control, hypo- and hyperthyroid rats. Changes in the redox state of the liver cytosol were found to be reflected in ethanol inhibition of sorbitol elimination.

VERRON,[1] in his studies on human subjects, found that ethanol inhibits sorbitol oxidation. According to a recent report, this phenomenon also takes place in intact rats and in rat liver slices.[2] Sorbitol is converted into D-fructose in a nicotinamide adenine dinucleotide (NAD)-dependent reaction catalysed by sorbitol dehydrogenase (L-Idit:NAD oxidoreductase, EC 1.1.1.14). In the rat this enzyme is present only in the liver, kidney and male accessory organs.[3] Moreover, the conversion of ethanol to acetaldehyde is catalysed by an NAD-dependent enzyme, liver alcohol dehydrogenase (Alcohol: NAD oxidoreductase, EC 1.1.1.1). Accordingly, it seems quite logical to expect an increase in the level of reduced nicotinamide adenine dinucleotide ($NADH_2$) in the liver during the oxidation of both sorbitol and ethanol.[4] Since thyroid hormone treatment has been reported to prevent ethanol-induced accumulation of hepatic $NADH_2$,[5] an attempt has been made to elucidate whether this effect is reflected in ethanol inhibition of sorbitol elimination.

MATERIALS AND METHODS

Twenty-seven male Wistar rats, aged 4 months, were divided into three groups of nine animals. The first group was given a daily administration, by stomach tube, of 5 mg per 100 g body weight of a 0·5 per cent solution of propyl thiouracil (PTU, from Eli Lilly and Co., Indianapolis, USA). The second group served as a control group. The third group had a daily administration, by a single i.p. injection, of 20 μg per 100 g body wt. of 3,3',5-triiodo-L-thyronine (T_3, from the Sigma Chemical Company, Missouri, USA) dissolved in slightly alkalinized saline. The PTU and T_3 treatments were continued for a 3-week period. Thereafter the oxygen consumption of the animals in basal conditions was measured with a Beckman Oxygen analyser,[6] Model E2, as a check on the hyper- and hypothyroidal effects of the drugs. For determination of the elimination rate of sorbitol under a heavy ethanol load, all the rats were first

given ethanol in an amount of 250 mg per 100 g body weight, as a 10 per cent (w/v) solution i.p. This concentration of ethanol has been reported not to influence liver oxygen uptake.[7] Fifteen min later sorbitol was injected into the femoral vein, in an amount of 50 mg per 100 g body wt. as a 5 per cent (w/v) solution. The i.v. injection was given under light pentobarbital anaesthesia. The amount of sorbitol mentioned was given alone on determination of the elimination rate of sorbitol without ethanol. Blood samples were drawn from the tip of the tail every 15 min, for determination of the elimination rate of sorbitol. Urine was collected in special cages during the following 20 hr, and its sorbitol content measured. The determination of sorbitol was performed colorimetrically, by the method of West and Rapoport.[8] At the end of the experiment, the animals were decapitated, and the liver-to-body weight ratio determined.

RESULTS

Basal oxygen consumption and the liver-to-body weight ratio of the experimental animals can be seen in Table 1. In the control and PTU-treated rats the liver-to-body weight ratio was not significantly different, but was about 30 per cent higher ($P < 0{\cdot}001$) in the animals treated with T_3. A similar increase in the liver-to-body weight

TABLE 1. BASAL OXYGEN CONSUMPTION AND LIVER TO BODY WEIGHT RATIO OF THE EXPERIMENTAL ANIMALS

Groups	Number of animals	Basal oxygen consumption (ml/hr/100g)	Liver/body weight (g/100g)
PTU-treated	9	0·44 ± 0·04	2·94 ± 0·18
Controls	9	0·64 ± 0·03	2·81 ± 0·14
T_3-treated	9	0·87 ± 0·11	3·63 ± 0·47

The figures represent the mean ± standard deviation.

TABLE 2. ELIMINATION RATE OF SORBITOL ALONE AND WITH ETHANOL IN T_3-TREATED, PTU-TREATED AND CONTROL RATS

Groups	Number of animals	Sorbitol (mg/g liver/hr)	Sorbitol (mg/100 g body weight/hr)	Sorbitol + Ethanol (mg/100 g body weight/hr)	Inhibition (%)
PTU-treated	9	11·2 ± 1·5	33·0 ± 4·4	13·3 ± 2·0	60
Controls	9	10·5 ± 1·4	29·9 ± 4·3	18·3 ± 1·9	39
T_3-treated	9	9·9 ± 1·5	36·0 ± 5·8	30·4 ± 6·9	16

The figures represent the mean ± standard deviation.

ratio induced by thyroxine treatment has been reported by Freedland and Krebs.[8] On the average, the basal oxygen consumption of the rats given PTU was 31 per cent lower than that of the control group; this indicates that the drug has a good thyrostatic effect. The group treated with T_3 exhibited a basal oxygen consumption which was, as a mean, 36 per cent more than that of the control group.

Under normal conditions, the elimination rate of sorbitol in intact rats was not

significantly ($P > 0.05$) influenced by administration of PTU, as is observable from Table 2. T_3 treatment increased the elimination rate of sorbitol significantly ($P < 0.025$). This effect was entirely attributable to the increased liver-to-body weight ratio in this group. The elimination rate of sorbitol, expressed in milligram per gram of liver fresh weight per hour, was not significantly influenced by pretreatment with PTU or T_3.

Ethanol significantly ($P < 0.001$) inhibited sorbitol elimination in control and PTU-treated rats, but the inhibition discovered in T_3-treated rats was not significant ($P > 0.05$). The inhibitory effect of ethanol was higher in the PTU-treated rats than in the controls.

DISCUSSION AND CONCLUSION

According to Hohorst *et al.*,[10] the free $NADH_2/NAD$ ratio is reflected in the lactate/pyruvate ratio in the liver cytosol. It has been reported by Rawat and Lunquist[11] that in comparison with slices from euthyroid rats, the lactate/pyruvate ratio is higher in the incubation medium when liver slices of methyl thiouracil-treated or thyroidectomised rats are incubated in the presence of ethanol. The authors did not observe any change in the lactate/pyruvate ratio after treatment with L-thyroxine. However, Ylikahri *et al.*[12] have subsequently reported that the increase in the lactate/pyruvate ratio induced by ethanol is significantly smaller in liver slices of animals treated with L-thyroxine than it is in slices from euthyroid animals. A similar effect of ethanol upon the lactate/pyruvate ratio in the intact liver of T_3-treated rats has recently been observed in this laboratory.* These observations indicate that T_3 treatment prevents the ethanol-induced increase in the redox state of liver cytosol while PTU treatment has the reverse effect.

The reduced redox state of the liver cytosol during ethanol oxidation explains the inhibitory effect of ethanol on sorbitol elimination. During normal conditions of the liver the balance in the redox pair sorbitol–fructose favours the oxidized substrate. During ethanol metabolism the balance is shifted towards the reduced substrate, sorbitol, and much smaller amounts of fructose are available for the reaction fructose→fructose-1-P. On the basis of the above-described observations concerning thyroid function, ethanol and the redox state of liver cytosol, it was expected that T_3 treatment would diminish and PTU treatment intensify the inhibitory effect of ethanol on sorbitol elimination. This was found to be true and it can be concluded that differences in the redox state of the liver cytosol during ethanol oxidation in hypo- and hyperthyroid rats are reflected in ethanol inhibition of sorbitol elimination. Whether the inhibition is affected by accumulation of free hepatic $NADH_2$ or by lack of free hepatic NAD cannot yet be answered.

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