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EFFECT OF THYROID HORMONES ON THE ACTIVITIES OF HEPATIC ALCOHOL METABOLIZING ENZYMES

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Summary

Treatment with thyroxine or triiodothyronine for 7 days in order to simulate a hyperthyroid state results in an enhanced activity of the microsomal ethanol oxidizing system. Conversely, a decrease of hepatic alcohol dehydrogenase activity was observed under these experimental conditions, whereas hepatic catalase activity remained unchanged. These findings suggest that if chronic ethanol consumption simulates a "hyperthyroid hepatic state", increased rates of ethanol metabolism observed following prolonged alcohol intake might therefore be attributed at least in part to an induction of microsomal ethanol oxidizing system activity in the liver.

The hepatocyte contains three different enzymes capable of oxidizing ethanol to acetaldehyde in vitro: alcohol dehydrogenase (ADH) of the cytosol or the soluble fraction of the cell (1, 2), the microsomal ethanol oxidizing system (MEOS) in the endoplasmic reticulum (2 - 5) and catalase in the peroxisomes (6). A variety of studies have suggested that ADH and MEOS fully account for rates of ethanol metabolism (2, 7), whereas the role of catalase is negligible (2, 8). Following chronic alcohol consumption an adaptive increase of MEOS activity occurs which was considered to contribute to increased rates of alcohol metabolism commonly observed after chronic alcohol consumption (7, 9, 10).

It has been reported, however, that some similarities may exist in the livers of alcohol treated animals compared to those given thyroid hormones especially since hepatic uptake of thyroxine was found to be increased following chronic alcohol consumption (11). Based upon these findings it has been

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suggested that chronic alcohol consumption results in a "hyperthyroid hepatic state", which was considered to be responsible for increased rates of ethanol metabolism (12).

In the present study the effect of thyroid hormones on the activities of the three hepatic enzymes capable of oxidizing ethanol in vitro was therefore investigated to elucidate the underlying mechanism for increased ethanol metabolism under these conditions.

Material and Methods

Female Sprague-Dawley rats with a body weight of 150 - 250 g were obtained from Zentralinstitut für Versuchstierzucht, Hannover, West Germany. They were fed Altromin-R chow ad libitum and had free access to tap water. L-Thyroxine (sodium salt from SERVA Feinbiochemica, Heidelberg, West Germany; 150 µg/100 g BW) and L-3,3',5-triiodothyronine (sodium salt from SERVA Feinbiochemica, Heidelberg, West Germany; 10 µg/100 g BW) dissolved in 0.5 ml alkaline solution (10 mM NaOH in 0.9 % NaCl) or the same amount of vehicle alone (controls) was injected s. c. daily for seven consecutive days. The animals were killed 24 h after the last injection.

The rats were decapitated, their livers in situ perfused with ice-cold 0.15 M KCl through the portal vein, excised, chilled, and a 25 % homogenate was prepared with the same solution. The homogenate was centrifuged at 10,000 g for 30 min, and the resulting supernatant was used for the subsequent preparation of washed microsomes and of liver cytosol (100,000 g supernatant) with an ultracentrifuge (13). Protein concentration was determined by the method of Lowry et al. (14).

Alcohol dehydrogenase activity was measured in the cytosol according to the method of Bonnichsen and Brink (15) with 0.1 M phosphate buffer (pH 7.4) and expressed in nmoles NADH formed per min per mg protein.

Microsomal ethanol oxidizing system (MEOS) activity was determined in the main chamber of 50 ml Erlenmeyer flasks with center wells containing 0.6 ml of semicarbazide hydrochloride (15 mM in 0.1 M phosphate buffer, pH 7.4). Microsomes (3 mg protein/flask) were incubated with ethanol (50 mM), sodium azide (0.1 mM) and a NADPH generating system (13, 16). The activity was expressed as nmoles acetaldehyde formed per min per mg microsomal protein.

Catalatic activity of catalase was determined in the 25 % liver homogenate by measuring the decrease in absorption at 240 nm following addition of H_2O_2 and expressed as units per g liver wet weight according to Lück (17).

Results

To study the effect of a hyperthyroid state on the activities of various alcohol metabolizing enzymes in the liver, female rats were treated with thyroid hormones, and subcellular fractionation of the hepatocyte was subsequently performed.

Table 1

ADH, MEOS AND CATALASE ACTIVITIES AFTER TREATMENT WITH L-THYROXINE (T_4) OR TRIIODOTHYRONINE (T_3)

	ENZYME ACTIVITY		
Treatment	ADH	MEOS	Catalase
Controls	5.0 ± 0.3	4.0 ± 0.2	1.55 ± 0.1
T_4	2.4 ± 0.3 ($p < 0.001$)	5.8 ± 0.6 ($p < 0.005$)	1.63 ± 0.1 NS
T_3	2.5 ± 0.4 ($p < 0.001$)	5.0 ± 0.1 ($p < 0.025$)	1.77 ± 0.2 NS

Female rats were treated for 7 days by s. c. injection of T_4 (150 µg/100 g BW) or T_3 (10 µg/100 g BW) dissolved in 0.5 ml alkaline solution (10 mM NaOH in 0.9 % NaCl). The controls received the same volume of the vehicle. ADH activity was determined in the cytosol and expressed as nmoles NADH formed per min per mg protein. MEOS activity was determined in washed microsomes and expressed as nmoles acetaldehyde formed per min per mg protein. Catalatic activity of catalase was determined in the 25 % homogenate and expressed as Lück units x 10^3 per g liver. The values are derived from 18 experimental animals and represent means ± SEM.

Compared to controls, treatment with L-thyroxine (150 µg/100 g BW) for 7 days resulted in a significant decrease of alcohol dehydrogenase activity by 52 % ($p < 0.001$), as measured in the hepatic cytosol (Table 1). Similarly, a decrease by 50 % ($p < 0.001$) was observed in animals treated with triiodothyronine for 7 days (Table 1). Conversely, under these experimental conditions a pronounced increase of MEOS activity was observed after both T_4 and T_3 treatment by 45 % ($p < 0.025$) and 25 % ($p < 0.005$), respectively, when determined in washed microsomes (Table 1). Finally, when the catalatic activity of catalase was measured in the 25 % homogenate after the same treatment with L-thyroxine or triiodothyronine, catalatic activity remained virtually unchanged when compared to controls receiving the vehicle only (Table 1).

Table 2

EFFECT OF IN VITRO ADDITION OF T_4 OR T_3 ON RAT LIVER ADH, MEOS AND CATALASE ACTIVITIES

Assay Conditions	ENZYME ACTIVITY		
	ADH	MEOS	Catalase
No addition	4.77	4.47	1.52
T_4 (ng)			
100	4.75	4.74	1.14
1.000	4.38	4.63	1.29
10.000	4.28	4.75	1.05
T_3 (ng)			
100	4.19	4.81	1.21
1.000	4.38	4.91	1.19
10.000	4.44	4.90	1.28

Cytosol, washed microsomes and 25 % liver homogenate were obtained from untreated rats. When indicated, L-thyroxine (T_4; sodium salt) or triiodothyronine (T_3; sodium salt) was preincubated with the enzyme for 5 min before the reaction was started. The final incubation volume was 3.0 ml. ADH activity was expressed as nmoles NADH formed per min per mg protein, MEOS activity as nmoles acetaldehyde formed per min per mg microsomal protein and catalatic activity of catalase as Lück units x 10^3 per g liver. The results are means of two experiments.

Since prolonged in vivo treatment of rats with T_4 or T_3 resulted in a striking decrease of ADH activity, a simultaneous induction of MEOS activity and an unaltered catalatic activity of catalase (Table 1), the question arose whether the in vitro addition of these hormones may influence the activities of these three enzymes. T_4 or T_3 were therefore added at various amounts ranging from 100 ng to 10.000 ng and preincubated with the enzyme for 5 min before the reaction was started. The control incubations were carried out without addition of the hormones. Compared to control incubations, T_4 at low doses failed to inhibit ADH activity, whereas higher amounts resulted in a slight decrease of activity (Table 2). T_3 at concentrations

ranging from 100 ng to 10.000 ng exhibited a slight inhibitory effect on ADH activity (Table 2). Moreover, in vitro addition of T_4 or T_3 was without any effect on MEOS activity, irrespective whether low or high amounts of thyroid hormones were employed (Table 2). Finally, catalatic activity of catalase was slightly inhibited in the presence of T_4 or T_3 (Table 2). These experiments therefore show that the in vitro addition of thyroid hormones failed to affect ADH, MEOS and catalase activities to a major extent.

Discussion

There is considerable debate whether increased rates of ethanol metabolism following chronic alcohol consumption are due to alteration of hepatic alcohol metabolizing enzymes activities such as MEOS (7, 9, 10) or to a "hyperthyroid hepatic state" (11). The present study shows that a hyperthyroid state produced by administration of T_4 or T_3 significantly decreases ADH activity and induces MEOS activity, without altering catalase activity (Table 1). These findings therefore suggest that if chronic ethanol consumption simulates a "hyperthyroid hepatic state", increased rates of ethanol metabolism observed following prolonged alcohol intake might therefore be attributed at least in part to an induction of MEOS activity in the liver.

The observation that chronic alcohol consumption resulted in an increased hepatic thyroxine uptake (11) associated with an enhanced activity of hepatic ATP-ase (18) and increased hepatic oxygen consumption (19) led to the hypothesis that a "hyperthyroid hepatic state" may be created under these conditions. This was considered to be responsible for increased rates of ethanol metabolism through increased mitochondrial NADH reoxidation (12) the rate limiting step in the ADH mediated ethanol metabolism (20, 21). Other investigators, however, observed increased alcohol clearance despite normal ATP-ase activity (22). Similarly, O_2 uptake was found by others not to be increased following chronic alcohol consumption (22, 23). Moreover, it is generally accepted that chronic alcohol intake results in mitochondrial injury both by electron microscopical (24) as well as biochemical assessment (25), casting doubts on

a mitochondrial mechanism of increased ethanol clearance. Finally, it has been reported that the acceleration of ethanol oxidation at high ethanol concentrations and a major part of the adaptative increase in ethanol metabolism after chronic ethanol intake can be attributed to the increase of MEOS activity (10).

The present study therefore suggests that irrespective whether chronic alcohol consumption may create a "hyperthyroid hepatic state" or not, increased rates of ethanol metabolism can be ascribed at least in part to an induction of MEOS activity.

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