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Effect of Thyroid Hormone on the Alcohol Dehydrogenase Activities in Rat Tissues

The effects of thyroid hormone on hepatic and gastric alcohol dehydrogenase (ADH) activities (nM of NADH/min/mg of cytosolic protein) have been investigated in male Sprague Dawley rats treated with thyroxine (1 mg/kg, po) for 14 days. Whereas hepatic ADH activity in thyroxine-treated rats decreased by 61.3% of control rats (26.4 vs 43.2, $p<0.001$), gastric ADH activity increased by 262.9% of control rats (4.9 vs 1.9, $p<0.001$). As for the activities of the lung and kidney, thyroxine treatment did not produce any statistically significant changes. These data suggest that thyrotoxicosis causes a decrease of hepatic alcohol metabolism, and that the increase of gastric ADH activity in thyrotoxic rats can partly restore the first-pass metabolism of ethanol.

Key Words : *Alcohol Dehydrogenase; Thyroid Hormones; Thyrotoxicosis*

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INTRODUCTION

Alcohol dehydrogenase (ADH) is the principal enzyme which catalyzes the oxidation of ethanol to acetaldehyde, and present in most of tissues including liver, stomach, intestine, lung, kidney, skin, and urogenital systems (1). Several studies have shown that the hepatic activity of ADH varies with aging (2) and gender (3), and is regulated by various hormones (4) or drugs (5-7). Castration (8) and hypophysectomy (9) were demonstrated to result in an increase of hepatic ADH and rates of ethanol elimination in male rats. The increases of ADH resulted from hypophysectomy and castration were suppressed by the administration of growth hormone and testosterone, respectively. Thyroid hormone also affect the activity of ADH. Thyroxine (10) and triiodothyronine (11) are known to depress hepatic ADH, and thyroidectomy results in a marked increase of hepatic ADH in rat (12, 13).

Although the liver is the chief site of ethanol metabolism, such metabolism also occurs in other tissues such as gastrointestinal tract. The enzyme responsible for ethanol oxidation in stomach is ADH. As it was reported that the activity of gastric ADH correlates with the first-pass metabolism of ethanol (14), and as there have been no reports about the effect of thyroid hormone on gastric ADH activity, we investigated the effect of thyroid hormone on the ADH in gastric mucosa and other tissues by comparing thyroxine treated and control rats.

MATERIALS AND METHODS

Chemicals

NAD^+ (nicotinamide adenine dinucleotide, grade I), dithiothreitol, levothyroxine (T_4) and Bradford reagent were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.).

Animals and sample preparation

Sprague-Dawley male rats initially weighing 210-230 g were obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.). The rats were kept in conventional plastic cages with free access to water and Purina Chow (Purina Mills, Richmond, IN, U.S.A.), and housed in a protected environment at a constant temperature of 23-25℃ with light-dark cycles alternating every 12 hr beginning at 7 a.m. Experimental animals were 20 rats and were divided into two groups such as control and thyroxine-treated groups. The rats of control group were treated with inert vehicle (normal saline, po), and

thyroxine-treated rats were treated by thyroxine (1 mg/kg of body weight, po) for two weeks to induce thyrotoxicosis. Rats were killed by ether anesthesia and serum T_4 levels were measured in trunk blood collected at the time of killing by chemiluminescence method (Chiron Diagnostic ACS: 180, U.S.A.). Liver, lung, and kidney tissues were immediately removed. Stomach was cut and rinsed with cold normal saline, and placed on a cold glass plate, and the mucosa was gently scraped off with a glass slide. All tissues were frozen and stored at -70℃.

Alcohol dehydrogenase activity

Cytoplasmic ADH activity determination as described by Theorell (15) and Bonnichsen (16) is based on spectrophotometric measurement of the amount of 2.4 mM of NAD^+ being reduced NAD^+ (NADH) in 10 min. The tissues obtained from animals were weighed; rinsed by cold normal saline; placed in 0.05 M of Tris-HCl buffer, pH 7.2, containing 0.5 mM of dithiothreitol; homogenized; and centrifuged at 40,000×*g* for 1 hr. The supernatant was used immediately for the determination of ADH activity. The reaction mixture was 1.0 mL in volume and consisted of 0.5 M of Tris-HCl, pH 7.2; 1.5 M of ethanol; 2.8 mM of NAD^+; and 0.03 mL of the tissue supernatant. A blank reaction without ethanol was run in each case. The formation of NADH was monitored at 340 nm for 10 min. The alcohol dehydrogenase activity was expressed as nanomoles of NADH produced per minute, based on extinction coefficient of 6.22 $cm^2/\mu M$ (nM/min/mg of cytosolic protein). The soluble protein concentration in the supernatant fraction was determined with the Bradford assay (17) using bovine serum albumin as a standard. Data were expressed as mean±standard deviation with 10 rats. The statistical significance of the differences in ADH activity was assessed using Student's t-test and nonparametric test (Mann-Whitney test). *P* values under 0.05 were considered to show statistical significance.

RESULTS

Induction of thyrotoxicosis

As expected, the thyroxine-treated rats had a decrease of body weight as compared with the control rats (Table 1); the mean body weight in the thyroxine-treated rats decreased by 23.3 g during the 2-weeks treatment period, but the mean body weight in control rats increased by 27 g. As the T4 assay kit which was basically for human sample was used in this experiment, the exact serum thyroxine concentrations of the rats could not be obtained. However serum thyroxine concentrations with this assay kit showed significant increases in thyroxine-treated rats compared to control rats, which contributed to the distinction of the thyrotoxic group from the control group. Whereas mean serum thyroxine level in thyroxine-treated rats was 8.8±3.3 μg/dL, that in control rats was 5.3±0.4 μg/dL.

Table 1. Effects of thyroid hormone administration (1 mg/kg) for 2 weeks on weight change in Sprague Dawley rats

Group	Body weight (g)		
	Pre treatment	Post treatment	Weight change
Control	215	242	+27
T_4-treated	220	196.7	-23*

*$p<0.01$ versus control by Mann-Whitney test

Change of hepatic ADH activity

The hepatic activity of ADH was 43.19±10.42 nM/min/mg in control rats. The administration of thyroxine resulted in a decrease of hepatic ADH activity (26.39±5.23 nM/min/mg; 61.3% of control, $p<0.001$) (Fig. 1).

Change of gastric mucosal ADH activity

Whereas gastric mucosal ADH activity was 1.86±0.66 nM/min/mg in control rats, the activity in thyroxine-treated rats was 4.89±1.43 nM/min/mg (262.9% of control, $p<0.001$) (Fig. 2).

Change of ADH activity in the lung and kidney

The ADH activity of the lung was 1.68±0.45 nM/min/mg in control rats. The administration of thyroxine

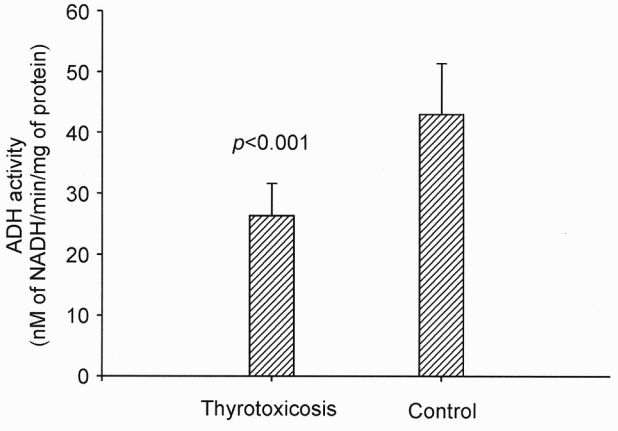

Fig. 1. Effect of thyroid hormone administration on hepatic alcohol dehydrogenase activity (nM of NADH/min/mg of cytosolic protein) in Sprague Dawley rats. Data are means±SD of groups (n=10).

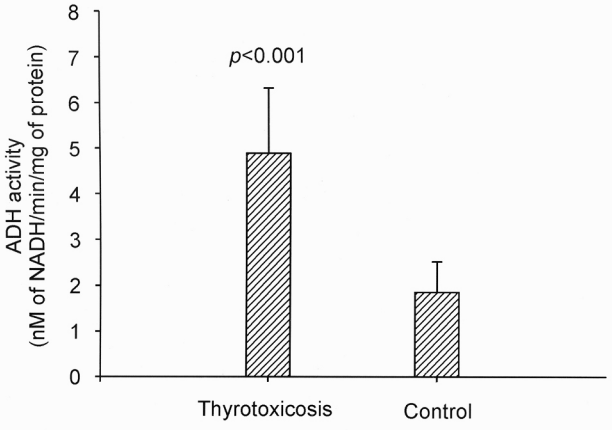

Fig. 2. Effect of thyroid hormone administration on gastric alcohol dehydrogenase activity (nM of NADH/min/mg of cytosolic protein) in Sprague Dawley rats. Data are means±SD of groups (n=10).

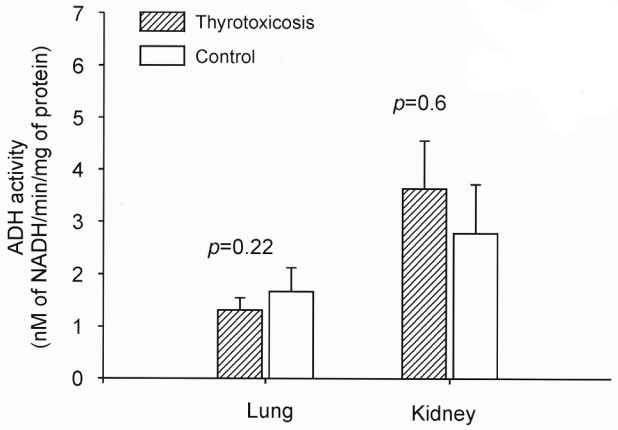

Fig. 3. Effect of thyroid hormone administration on lung and kidney alcohol dehydrogenase activities (nM of NADH/min/mg of cytosolic protein) in Sprague Dawley rats. Data are means± SD of groups (n=10).

decreased the ADH activity of the lung (1.32±0.23 nM/min/mg; 78.6% of control), but showed no statistical significance (p=0.22). The ADH activity of the kidney was 2.79±0.93 nM/min/mg in control rats. The administration of thyroxine resulted in an increase of the ADH activity of kidney with no statistical significance (3.64± 0.92 nM/min/mg; 130.4% of control, p=0.06) (Fig. 3).

DISCUSSION

In this study, thyroid hormone administration decreased hepatic ADH activity and increased gastric ADH activity. The changes in the hepatic ADH activity in the thyrotoxic rats were in agreement with previous reports (13, 18-20). Hyperthyroidism has been known to cause a decrease of liver ADH activity. This is not because thyroid hormone simply acts as an enzyme inhibitor. Thyroid hormone administration decreases the number of ADH molecules and ADH mRNA level (10). Despite a decrease of hepatic ADH activity in hyperthyroidism, rates of ethanol elimination have been found to be increased in hyperthyroid patients (21), and after the administration of thyroxine in some studies (19, 22). The increased rates of ethanol metabolism are attributed at least in part to an enhanced activity of the microsomal ethanol oxidizing system in the liver (22).

Though ethanol exogenously administered is metabolized mostly in the liver, gastric ADH contributes to ethanol metabolism as well, as evidenced by the lower areas under the blood ethanol concentration curve after oral than after intravenous administration of ethanol in rats (23, 24) and in humans (14, 25). First-pass metabolism of alcohol has received considerable attention because of its influence on the bioavailability of ethanol in the systemic circulation after oral ingestion (14, 23). Oral consumption of alcohol results in much lower blood alcohol concentrations than does the same dose administered intravenously, suggesting significant first-pass metabolism. At 33 mM of blood ethanol concentration, gastric ADH activity represented 10% of the liver activity in rat (1). Therefore, oral administration of ethanol results in a higher contribution of the extrahepatic activity than does intravenous or intraperitoneal administration. To date the effects of thyroid hormone on gastric ADH have not been studied. This study shows that gastric ADH activity in thyrotoxic rats markedly increases in the opposite way that hepatic ADH activity does. This raises the possibility that the increase of gastric ADH activity may contribute to the enhanced first-pass metabolism of ethanol in thyrotoxicosis.

Extrahepatic alcohol dehydrogenases are present in most of the tissues and are regulated by various hormones. Orchiectomy, which increases liver ADH activity (8), has been reported to have no effect on the gastric enzyme. Ovariectomy, which does not change liver ADH activity (26), neither has any effect on the gastric enzyme (3). However kidney ADH has been known to be regulated by estradiol (27) and androgen (28). Whereas Dipple et al. (10) reported that kidney ADH responded in the same fashion of hepatic ADH in hyperthyroid rats, our study revealed that thyroid hormone administration did not significantly change the kidney ADH activity, nor the lung ADH activity. Although it has not been clearly studied whether the ADH activities in those tissues play important roles on ethanol metabolism in thyrotoxic rats, the activities are believed to be negligible.

In conclusion, the thyroid hormone administration decreases the hepatic ADH activity and increases the gastric ADH activity in the rat. As the increase of gastric ADH

activity correlates with the enhancement of first-pass metabolism of ethanol, it may at least in part contribute to the maintenance of ethanol metabolism in spite of the decrease of hepatic ADH activity in thyrotoxicosis. However, the exact physiological significance for the effects of the increase of gastric ADH activity in thyrotoxic rats remains to be determined.

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