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Effect of thyroidectomy on liver alcohol dehydrogenase in the female rat

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Liver alcohol dehydrogenase activity is higher in female than in male rats [1, 2]. Hypophysectomy [3], castration [2, 4, 5] and thyroidectomy [6] all result in increases in liver alcohol dehydrogenase in male rats. These increases are suppressed, respectively, by the administration of growth hormone [3], testosterone [2, 4, 5] and triiodothyronine [6], suggesting that the activity of the enzyme is controlled by a variety of hormones. However, it is also possible that the effects observed were are mediated by decreases in circulating testosterone, which occur not only after castration but also after hypophysectomy [7] and thyroidectomy [8]. To evaluate this possibility the effect of thyroidectomy on liver alcohol dehydrogenase activity was determined in female rats where testosterone would not be a factor.

Sixteen thyroidectomized and eight sham-operated female Sprague–Dawley rats weighing between 90 and 110 g were obtained from the Charles River Breeding Laboratories (Wilmington, MA). The animals were placed in separate wire mesh cages in a room at a controlled temperature of 20° with light/dark cycles alternating every 12 hr, beginning at 7:00 a.m., and provided Purina chow *ad lib*. The water given to the thyroidectomized animals contained 1% calcium chloride. Starting 10 days after surgery, eight thyroidectomized animals were given subcutaneous injections of 3,3′,5-triiodo-L-thyronine, sodium salt (Sigma Chemical Co., St. Louis, MO), 15 μg/100 g of body weight twice a day, while the remainder of the thyroidectomized and sham-operated animals received isovolumetric amounts of saline. The injections were given for 10.5 days, and the animals were killed 2 hr after the last injection. Rates of ethanol elimination were determined on injection day 7 following the intraperitoneal administration of ethanol (2.0 g/kg body weight) as described previously [6]. At the time of sacrifice the animals were anesthetized with ether, and blood for the determination of thyroxine, triiodothyronine and testosterone was drawn from the abdominal aorta until the onset of apnea. The livers were removed, rinsed in 1.15% KCl, weighted, and homogenized in 4 vol. of 0.25 M sucrose in 0.1 M Tris–HCl buffer, pH 7.4. The homogenate was centrifuged at 480 *g* for 10 min, and the resulting supernatant fraction was centrifuged at 9,000 *g* for 10 min. The mitochondrial pellet recovered was resuspended in the same buffer and used for the determination of glutamate dehydrogenase activity as described by King and Frieden [9]. The 9,000 *g* supernatant fraction was centrifuged at 106,000 *g* for 60 min. Alcohol dehydrogenase activity was determined in the 106,000 *g* supernatant fraction at 37° in 0.5 M Tris–HCl buffer, pH 7.2, by the method of Crow *et al.* [10]. Lactate dehydrogenase activity was determined in the same supernatant fraction as described by Plagemann *et al.* [11]. The microsomal pellet was resuspended in 0.1 M NaH_2PO_4–K_2HPO_4, pH 7.4, and used for the determination of the activity of the microsomal ethanol oxidizing system by the method of Lieber and DeCarli [12]. Protein concentration was determined by the method of Lowry *et al.* [13] with bovine serum albumin used as a standard. Measurements of plasma thyroxine and triiodothyronine were carried out at the Hazelton Laboratories (Vienna, VA) by radioimmunoassay using the diagnostic kits from the Nuclear Medical Laboratories (Dallas, TX). Radioimmunoassay of plasma testosterone was carried out as described by Harman *et al.* [14]. The results are expressed as means ± S.E. The data were analyzed by Student's two-tailed *t*-test for unpaired results.

The thyroidectomized animals had decreased plasma concentrations of thyroxine and triiodothyronine (Table 1). Plasma testosterone concentrations were not changed by thyroidectomy or the administration of triiodothyronine to the thyroidectomized animals. The values of plasma testosterone in these female animals approximate the testosterone concentration of 26.2 ± 2.22 ng/dl previously reported by us in castrated male Sprague–Dawley rats [4].

Body and liver weights were decreased in the thyroidectomized animals as compared with the sham-operated control animals (Table 2). Administration of triiodothyronine to the thyroidectomized animals resulted in no significant changes in body weight, but increased liver weight to values comparable to those of control animals. Cytosolic protein concentration was increased after thyroidectomy whether or not the animals had received triiodothyronine. Liver alcohol dehydrogenase activity was increased markedly by thyroidectomy whether expressed per mg of protein, per g of wet weight, per kg of body weight (not shown), or per total animal. The 2-fold increase in the enzyme activity expressed per mg of protein caused by thyroidectomy in the female animals was of a magnitude similar to the increase in enzyme activity obtained previously after thyroidectomy in male animals [6]. This increase in enzyme activity in the female occurred despite the higher normal activities of liver alcohol dehydrogenase in the female than in the male rats [15].

Triiodothyronine administration resulted in a suppression of the enhanced enzyme activity to values lower than those found in the sham-operated control animals. In previous studies in male rats, triiodothyronine was found to inhibit liver alcohol dehydrogenase activity after its admin-

Table 1. Plasma concentrations of thyroid hormones and testosterone in the experimental animals*

	Thyroxine (μg/dl)	Triiodothyronine (ng/dl)	Testosterone (ng/dl)
Control	4.1 ± 0.25	84.0 ± 6.82	35.6 ± 3.43
Thyroidectomy	0.9 ± 0.05†	42.6 ± 2.29†	29.4 ± 2.17
Thyroidectomy + T_3	1.7 ± 0.12†‡	2696.6 ± 342.62†‡	31.2 ± 4.80

* All values are expressed as means ± S.E. of eight animals. T_3 denotes triiodothyronine.
† Significantly different from control at $P < 0.001$.
‡ Significantly different from thyroidectomy alone at $P < 0.001$.

istration *in vivo* [6] or *in vitro* after its addition to liver cytosol [6]. The inhibition of ethanol oxidation demonstrated *in vitro* was found to be competitive with respect to NAD^+ and uncompetitive with respect to ethanol [6]. The most likely mechanism for the inhibition is that triiodothyronine interferes with coenzyme binding by blocking the binding site of the ADP-ribose portion of the coenzyme [16].

Thyroidectomy and the administration of triiodothyronine did not affect the K_m of alcohol dehydrogenase for ethanol and NAD^+. The K_m values in the three groups of animals studied ranged from 0.98 to 1.43 mM for ethanol and from 46 to 57 μM for NAD^+.

The activity of the microsomal ethanol oxidizing system was not changed by thyroidectomy (Table 3); however, the administration of triiodothyronine to the thyroidectomized animals increased the activity of this enzyme system. This latter effect agrees with the observations of Moreno *et al.* [17] of an increase in this enzyme system in female rats after the administration of either thyroxine or triiodothyronine. It differs from the lack of a similar effect of triiodothyronine when administered to male rats [6].

A unique effect of thyroidectomy in increasing liver alcohol dehydrogenase activity is supported by the opposite effect of thyroidectomy in decreasing the activities in both cytosolic lactate dehydrogenase and mitochondrial glutamate dehydrogenase (Table 3). Previous studies showed that thyroidectomy decreased lactate dehydrogenase in rabbits [18] and glutamate dehydrogenase in rats [19]. The administration of triiodothyronine in this study restored the activity of lactate dehydrogenase to a value greater than the normal value, while it depressed further the activity of glutamate dehydrogenase.

The rates of ethanol elimination were not changed significantly by thyroidectomy or by the administration of triiodothyronine to the thyroidectomized animals. The rates of ethanol elimination were 8.53 ± 0.830 mmoles per kg body weight per hr in the thyroidectomized animals and 10.96 ± 1.405 mmoles per kg body weight per hr in the thyroidectomized animals which received triiodothyronine. This compares with a rate of 9.16 ± 0.789 mmoles per kg body weight per hr in the sham-operated control animals. The lack of a change in the rate of ethanol elimination in the thyroidectomized animals with the enhanced liver alcohol dehydrogenase activity is most likely caused by a limitation in *in vivo* reoxidation of NADH. A decreased transport of NADH into mitochondria [6] and decreases in mitochondrial function and oxygen consumption [20, 21] have been demonstrated after thyroidectomy.

To our knowledge this is the first demonstration of an experimentally induced increase in liver alcohol dehydrogenase activity in female rats. The increase in liver alcohol dehydrogenase in female rats after thyroidectomy supports a direct effect of thyroid hormone on this enzyme. It rules out the possibility that the effect of thyroidectomy previously demonstrated in male rats was mediated by a decrease in androgens. Also, a mediation of the effect of thyroidectomy by a decrease of estrogens is not a possibility since ovariectomy did not result in any changes in the enzyme activity [15].

In conclusion, the similar increases in the liver alcohol dehydrogenase activities in female and in male rats indicate

Table 2. Effect of thyroidectomy on liver alcohol dehydrogenase activity in the female rat*

Determinations	Control	Thyroidectomy	Thyroidecomy + T_3
Body weight (g)	155.5 ± 2.65	133.0 ± 3.00†	138.6 ± 5.45‡
Liver weight (g)	7.5 ± 0.20	4.8 ± 0.15§	6.8 ± 0.35‖
(g/100 g body wt)	4.8 ± 0.17	3.5 ± 0.14§	4.9 ± 0.19‖
Cytosolic protein (mg/g)	74.3 ± 1.35	81.1 ± 1.82‡	82.6 ± 1.30†
Alcohol dehydrogenase			
(μmoles/mg protein/hr)	2.31 ± 0.201	4.84 ± 0.398§	1.66 ± 0.151‡‖
(μmoles/g liver/hr)	170.4 ± 13.80	391.3 ± 31.47§	136.3 ± 11.59‡‖
(mmoles/rat/hr)	1.27 ± 0.109	1.94 ± 0.160†	0.92 ± 0.090‡¶

* All values are expressed as means ± S.E. of eight animals. T_3 denotes triiodothyronine.
† Significantly different from control at $P < 0.01$
‡ Significantly different from control at $P < 0.05$.
§ Significantly different from control at $P < 0.001$.
‖ Significantly different from thyroidectomy alone at $P < 0.001$.
¶ Significantly different from thyroidectomy alone at $P < 0.01$.

Table 3. Effect of thyroidectomy on the activities of the microsomal ethanol oxidizing system, lactate dehydrogenase and glutamate dehydrogenase in the female rat*

	MEOS (nmoles/mg protein/min)	LDH (μmoles/mg protein/min)	GDH (μmoles/mg protein/min)
Control	4.92 ± 0.279	4.36 ± 0.205	0.48 ± 0.015
Thyroidectomy	5.08 ± 0.289	2.87 ± 0.141†	0.39 ± 0.035‡
Thyroidectomy + T_3	7.44 ± 0.615†§	6.52 ± 0.244‖¶	0.29 ± 0.024§‖

* All values are expressed as means ± S.E. of eight animals. T_3 denotes triiodothyronine; MEOS, microsomal ethanol oxidizing system; LDH, lactate dehydrogenase; and GDH, glutamate dehydrogenase.
† Significantly different from control at $P < 0.01$.
‡ Significantly different from control at $P < 0.05$.
§ Significantly different from thyroidectomy alone at $P < 0.05$.
‖ Significantly different from control at $P < 0.001$.
¶ Significantly different from thyroidectomy alone at $P < 0.001$.

that the thyroid gland has regulatory effect on the enzyme which is independent of the previously demonstrated effects of a reduction in androgens on the enzyme.

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Xanthine accumulation by normal and SV40-transformed WI-38 fibroblasts*

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The human diploid fibroblast WI-38 and an SV40-transformant of WI-38 (VA13) have been used extensively as model systems for studying the regulation of cyclic nucleotide metabolism [1–9]. Both cell types were observed to respond to catecholamines and prostaglandins with dramatic increases in intracellular cyclic AMP levels. Concentrations of methylxanthines which potentiated the effects of agonists on WI-38 cyclic AMP content were, nevertheless, found to have no significant effects on the pattern of cyclic AMP accumulation by hormone-stimulated VA13 cultures [2, 4, 9]. In addition, recent work with MIX† and three MIX analogs, i.e. 7-benzyl MIX, 8-*t*-butyl MIX, and 1-isoamyl-3-isobutylxanthine, has shown that the ability of the xanthines to alter the cyclic AMP responses of WI-38 and VA13 cells cannot be predicted on the basis of their potencies as inhibitors of fibroblast phosphodiesterase activities [9]. In view of the observation of Barber and Butcher that the turnover constant for cyclic AMP is greater in WI-38 than in VA13 cultures [6], it seems likely that the different sensitivities of the fibroblasts to the xanthines are a function of higher phosphodiesterase activity in the normal cells. However, discrepancies between the potencies of the xanthines as inhibitors of fibroblast phosphodiesterase activity and their effects on cyclic AMP accumulation by intact WI-38 or VA13 cells cannot be readily explained. It is conceivable that phosphodiesterase activities in fibroblast supernatant fractions differ significantly from the enzyme activities of the intact cells (see Ref. 10). Alternatively, the effects of some of the xanthines on WI-38 and/or VA13 cyclic AMP content may be limited by slow penetration of fibroblast membranes. The experiments described in this report were conducted to evaluate the latter hypothesis by determining the capacities of intact WI-38 and VA13 fibroblasts for the uptake of radiolabeled theophylline, 7-benzyl MIX [11], 8-*t*-butyl MIX [12], and 1-isoamyl-3-isobutylxanthine [12] from incubation media.

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† Abbreviations: MIX, 1-methyl-3-isobutylxanthine; PGE_1, prostaglandin E_1; DMSO, dimethyl sulfoxide; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; and MEM, minimum essential medium.