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The effect of bile salts on thyroxine 5'-monodeiodination in rat liver homogenate[1]

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Summary. Unconjugated and conjugated bile salts inhibited the conversion of thyroxine to 3,3',5 triiodothyronine in rat liver homogenate.

The conversion of thyroxine(T_4) to 3,3',5 triiodothyronine (T_3) has been demonstrated to occur in the liver[2-4], kidney[5,6] and pituitary[7] of animals. Factors affecting this conversion are known to include fasting[4], thyroid state[8], sulfhydryl groups[7,9] and drugs[2]. However, little information is available about the significance of bile acids in this conversion. Our previous study in vivo[10] demonstrated that deoxycholate(DCA) feeding lowered the plasma T_3 levels in rats, and this was restored by additional cholestyramine feeding, suggesting that bile acid might affect the conversion of T_4 to T_3. In order to ascertain this assumption, the effect of bile salts such as sodium deoxycholate(SDCA), sodium cholate(SCA) and their tauro-conjugates on the in vitro conversion of T_4 to T_3 by rat liver homogenate was examined in this study.

Materials and methods. Sprague-Dawley male 70-day-old rats were fed a diet AO, which was prepared by removal of KI from the composition of diet A[11] in order to decrease iodine content to about 0.3 µg of iodine per g diet[12], for 7 days. These rats were decapitated to remove the liver. The liver was homogenized with 0.15 M phosphate buffer (pH 7.5) at 4 °C and the homogenate was incubated with a substrate, 50 µg T_4/ml, for 1 h at 37 °C, according to the method of Chopra[2].

SDCA and SCA were obtained from Nakarai Chemicals, Ltd. Sodium taurodeoxycholate(STDCA) and sodium taurocholate(STCA) were purchased from Sigma Chemical Co. Propylthiouracil (PTU) was donated by Tokyo Tanabe Co. Addition of these chemicals to the incubation medium resulted in no change of pH.

The amount of T_3 generated during incubation was measured by radioimmunoassay[13]. This assay system was not affected by any test substances in the concentrations used in this study.

Results. Both SDCA and SCA in concentrations between 10^{-1} M and 10^{-4} M significantly inhibited the conversion of T_4 to T_3 in rat liver homogenate. These effects were dose-related, but insignificant at a concentration of 10^{-6} M (table 1). In addition, SDCA was consistently less potent than SCA. The effect of PTU (2×10^{-5} M) in inhibiting T_4 to T_3 conversion was less than that of SCA at a concentration of 10^{-1} M ($p < 0.001$), but not significantly different from that of SDCA (10^{-1} M). The effects of conjugated bile salts on T_4 to T_3 conversion were also inhibitory (table 2), being significant at a concentration of 10^{-6} M. There was no significant difference between the effects of STDCA and STCA in all concentrations used.

Discussion. The present study demonstrates that both unconjugated and conjugated bile salts exert inhibitory effects on the hepatic conversion of T_4 to T_3 in the rat and that conjugated forms may be more potent than those of

Table 1. Effect of unconjugated bile salts and PTU on T_4 to T_3 conversion in rat liver homogenate

Bile salts	T_3 generated during incubation (ng/µg T_4/h/g-eq. tissue) SDCA	SCA	p-value (SDCA vs SCA)
10^{-1} M	9.82±0.27*	7.65±0.25*	<0.001
10^{-3} M	12.31±0.59*	10.50±0.45*	<0.05
10^{-4} M	15.50±0.25*	12.13±0.30*	<0.001
10^{-6} M	21.24±0.24**	19.23±0.70**	>0.05
PTU 2×10^{-5} M	9.41±0.25		
Control	20.33±0.30		

Values are expressed as the mean±SE in each group of 4 rats. Differences from control are shown by * $p<0.001$ and ** $p>0.05$.

Table 2. Effect of conjugated bile salts on T_4 to T_3 conversion in rat liver homogenate

Bile salts	T_3 generated during incubation (ng/µg T_4/h/g-eq. tissue) STDCA	STCA	p-value (STDCA vs STCA)
10^{-1} M	6.50±0.96*	5.50±0.65*	NS
10^{-4} M	12.00±0.71*	10.75±0.48*	NS
10^{-6} M	18.50±0.28**	18.30±0.24**	NS
Control	20.25±0.63		

Values are expressed as the mean±SE in each group of 4 rats. Differences from control are shown by * $p<0.001$ and ** $p<0.05$. NS indicates no significant difference ($p>0.05$).

unconjugated forms. The latter finding may be consistent with the study[14], which showed better uptake of conjugated bile acids by hepatocytes compared to the uptake of unconjugated bile acids. These effects may be interpreted as indicating the physiological significance of bile acids in hepatic regulation of T_4 5′-monodeiodinating activity, if the concentration of bile acids in the portal venous blood (56 μM)[15] and bile (32.5 mM)[16] are taken into consideration. The in vitro results presented here may be consistent with our previous study[10], which showed lowering of plasma T_3 levels by DCA feeding in rats. The present data on SDCA and STDCA could help to explain the high plasma T_3 levels found in germfree rats[17], which are characterized by large pools of bile acids[18] and lack of DCA[19], but the data on SCA and STCA could not. Therefore, the thyroid hormone economy in germfree rats must be carefully explained by other factors including the enterohepatic circulation of thyroid hormones[20] and lipids[11,18,19].

It has been reported that the enzyme system[2-6] which catalyzes T_4 to T_3 by 5′-monodeiodination, may be located in particulate subcellular fractions such as microsomes and mitochondria[3], where large amounts of bile acids are also distributed[21]. The interaction between the converting enzyme, T_4 5′-monodeiodinase, and the bile acids might occur in the hepatic subcellular organelles. The enzyme system involved in T_4 to T_3 conversion is thought to be affected by unconjugated and conjugated bile salts in some ways which need to be elucidated.

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Effects of duration of gonadectomy, sex and age on adrenal steroid 5α-reductase activity in the rat

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Summary. Within 6-8 weeks, gonadectomy in male and female rats leads to an increase in adrenal 5α-steroid reductase activity. However, long-term post-orchiectomy enzyme activity decreases to the control level and this effect is not related to the age of animals.

One of the intraadrenal factors controlling corticosterone output by the rat adrenal gland is steroid 5α-reductase (EC 1.3.1.4), an enzyme responsible for the conversion of corticosterone to dihydro- and tetrahydrocorticosterone[2,3]. The activity of this enzyme in the adrenals of intact male and female rats is very low; gonadectomy results in an increase, while estradiol or testosterone replacement results in a decrease in enzyme activity[2,4-7]. After prepubertal or neonatal gonadectomy adrenal 5α-reductase increased only after the normal age of puberty and this effect is not related to the duration of gonadal hormone deficiency nor to chronological age. On the contrary, hypophysectomy in prepubertal rats resulted in a rapid increase in enzyme activity[8]. Stimulation of adrenal 5α-steroid reductase activity due to postpubertal gonadectomy is visible within 2 weeks after the removal of the gonads, and activity increases progressively up to 6-8 weeks after surgery[2,4-8]; however, longer times were not investigated. The aim of the present study was to investigate the long-term effects of gonadectomy on adrenal 5α-reductase activity in the rat.

Materials and methods. Gonadectomy was performed on rats of the Wistar strain at the age of 2.5-3 months or in appropriate control age groups 41-44 days before autopsy. Some of the gonadectomized rats received a single dose of testosterone cypionate (5 mg/100 g b.wt) or estradiol cypionate (100 μg/100 g b.wt) 2 weeks before autopsy. Investigations were carried out in the 8-month experiment on 231 (females) or 233 (males) days after surgery, while in the 16-month experiment measurements were made after 528 or 526 days, respectively.

Adrenals were homogenized in 0.154 M KCl. 5α-Steroid reductase activity and the corticosterone output were assayed in whole adrenal homogenates as described by Kitay et al.[2,9]. Corticosterone was determined by sulfuric acid fluorescence[10]. Results were evaluated statistically by the multiple-range test of Duncan[11].

Results and discussion. In the 8-month experiment (table 1) both long- and short-term orchiectomy lowered the corticosterone output by adrenal homogenates and testosterone had an opposite effect. In the 16-month experiment short-term but not long-term orchiectomy increased, and testosterone replacement lowered, corticosterone output by adrenal homogenates. In both experimental groups after long-term orchiectomy or testosterone replacement adrenal 5α-reductase activity did not differ from that in control groups. On the contrary, in appropriate groups of rats, within 6