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

Table 1. The effects of long-term orchiectomy on body and adrenal weights, corticosterone production by whole adrenal homogenates and adrenal 5α-steroid reductase activity in the rat. Results are expressed as means ± SE

Group (No.)	n	Body weight (g)	Adrenal weight Absolute (mg)	Relative (mg/100 g b.wt)	Corticosterone production (nmoles/10 mg of tissue/60 min)	5α-Reductase activity (nmoles/10 mg of tissue/60 min)
8-month experiment						
Control (1)	8	385 ± 13	42.5 ± 1.2	11.1 ± 0.4	3.06 ± 0.17	0.32 ± 0.17
Orchiectomy 8 months (2)	8	380 ± 5	41.6 ± 1.0	11.0 ± 0.3	2.51 ± 0.11^{I}	1.30 ± 0.75
Orchiectomy 6 weeks (3)	7	375 ± 15	$48.5 \pm 1.6^{I,II}$	$13.6 \pm 0.3^{I,II}$	2.31 ± 0.11^{I}	$4.62 \pm 1.12^{I,II}$
Orchiectomy 8 months + testosterone (4)	8	376 ± 13	44.9 ± 1.4	11.9 ± 0.2^{III}	$2.97 \pm 0.14^{II,III}$	0.63 ± 0.46^{III}
16-month experiment						
Control (1)	6	392 ± 4	50.2 ± 1.1	12.8 ± 0.2	3.78 ± 0.26	0.11 ± 0.11
Orchiectomy 16 months (2)	6	380 ± 4	57.7 ± 1.3^{I}	15.2 ± 0.5^{I}	4.18 ± 0.11	0
Orchiectomy 6 weeks (3)	8	393 ± 8	58.2 ± 1.8^{I}	14.8 ± 0.4^{1}	4.65 ± 0.32^{1}	$6.58 \pm 1.33^{I,II}$
Orchiectomy 16 months + testosterone (4)	6	381 ± 10	53.4 ± 1.4^{3}	14.1 ± 0.5^{1}	$3.12 \pm 0.26^{2,III}$	0.23 ± 0.23^{III}

n, Number of animals. Statistical evaluation of the results by the Duncan test: difference from the value of group (within the same experiment) marked by numerals: arabic numerals $p < 0.05$; roman numerals $p < 0.01$.

Table 2. The effects of long-term ovariectomy on body and adrenal weights, corticosterone production by whole adrenal homogenates and adrenal 5α-steroid reductase activity in the rat. Results are expressed as means ± SE

Group (No.)	n	Body weight (g)	Adrenal weight Absolute (mg)	Relative (mg/100 g b.wt)	Corticosterone production (nmoles/10 mg of tissue/60 min)	5α-Reductase activity (nmoles/10 mg of tissue/60 min)
8-month experiment						
Control (1)	8	219 ± 8	54.5 ± 2.4	24.9 ± 0.6	4.13 ± 0.52	0
Ovariectomy 8 months (2)	8	282 ± 8^{I}	46.2 ± 1.1^{I}	16.4 ± 0.3^{I}	3.72 ± 0.20	4.56 ± 1.44^{I}
Ovariectomy 6 weeks (3)	8	238 ± 4^{II}	49.0 ± 1.3	$20.6 \pm 0.4^{I,II}$	3.00 ± 0.14^{1}	2.28 ± 0.69
Ovariectomy 8 months + estradiol (4)	7	$263 \pm 8^{I,3}$	$58.5 \pm 2.7^{II,III}$	$22.3 \pm 1.0^{I,II}$	3.98 ± 0.20^{3}	0.52 ± 0.29^{II}
16-month experiment						
Control (1)	7	246 ± 8	56.9 ± 2.4	23.1 ± 0.3	7.71 ± 0.37	8.80 ± 1.67
Ovariectomy 16 months (2)	6	309 ± 17^{I}	50.7 ± 1.6	16.5 ± 0.6^{I}	5.92 ± 0.66^{1}	7.68 ± 0.69
Ovariectomy 6 weeks (3)	8	255 ± 7^{II}	55.5 ± 2.4	21.8 ± 0.7^{II}	5.14 ± 0.29^{1}	5.48 ± 1.10
Ovariectomy 16 months + estradiol (4)	5	273 ± 15	59.1 ± 2.5^{2}	21.7 ± 0.8^{II}	6.35 ± 0.81	$3.38 \pm 1.33^{1,2}$

n, Number of animals. Statistical evaluation of the results by Duncan test: difference from the value of group (within the same experiment) marked by numerals: arabic numerals $p < 0.05$; roman numerals $p < 0.01$.

weeks after removal of the testes an increase in adrenal 5α-steroid reductase activity was found.

These results show that long-term post-orchiectomy activity of adrenal 5α-steroid reductase, which increases progressively during the initial 6–8 weeks[4,7,8], did not differ from that in corresponding intact male rats. This decrease in enzyme activity is not an age-related phenomenon, because in rats of the same age 6 weeks after testes removal a high 5α-reductase activity is observed. These data show that in the rat, intraadrenal 5α-steroid reductase plays a transitory role in the adaptation of the adrenals to orchiectomy.

With female rats (table 2) in both age groups corticosterone output by adrenal homogenates obtainded 6 weeks after ovariectomy was lower than in control groups. This output was also lower 16 months after ovariectomy if compared with that in control females. In the 8-month experiment in long-term gonadectomized rats a high 5α-reductase activity was found and this effect was partially prevented by estradiol. In this experiment, 6 weeks after ovariectomy only a statistically insignificant increase in the enzyme activity was found. On the contrary, in the 16-month experiment in both groups of gonadectomized females as well as in intact control rats a high reductase activity was observed and again estradiol inhibited this activity.

These observations show a different responses of adrenal 5α-reductase to long-term gonadectomy in male and female rats and it seems that the effect of ovariectomy is more persistent than that of orchiectomy. In the 16-month experiment high reductase activity was found even in intact control females. This effect may be related to aging changes in these animals and suggests hormonal insufficiency or dysfunction of the ovaries. This supposition is proved by the inhibitory effect of estradiol on adrenal 5α-reductase activity in these old female rats.

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