Effect of age, gonadectomy and hypophysectomy on mitochondrial hydroxylation of vitamin D₃ (cholecalciferol) and of 5 β -cholestane-3 α ,7 α ,12 α -triol in female and male rat liver

Kristin SAAREM and Jan I. PEDERSEN

Institute for Nutrition Research, School of Medicine, University of Oslo, 0316 Oslo 3, Norway

In a previous study we found that liver mitochondrial side-chain hydroxylation of vitamin D₃ (cholecalciferol) and of 5 β -cholestane-3 α ,7 α ,12 α -triol was higher in female than in male rats [Saarem & Pedersen (1987) *Biochem. J.* **247**, 73–78]. The present paper describes the effects of age, gonadectomy and hypophysectomy on these activities. The sex difference became manifest above the age of 7 weeks. Ovariectomy and/or injection of oestradiol valerate had no effect on the hydroxylase activities in adult females. Castration increased, and subsequent testosterone treatment decreased, the hydroxylase activities in adult males. Hypophysectomy had no effect in females, but increased the hydroxylase activities in males. Testosterone treatment had no effect in hypophysectomized females or males. Injection of oestradiol valerate had no effect on the hydroxylase activities in hypophysectomized females. In hypophysectomized males this treatment had no effect on the vitamin D₃ 25-hydroxylase activity, but decreased the C₂₇-steroid 27-hydroxylase activity in males. Microsomal 1 α -hydroxyvitamin D₃ 25-hydroxylase activity was lower in females than in males in all age groups. Castration or hypophysectomy decreased the activity in male rats. It is concluded that, in adult female rats, the mitochondrial side-chain hydroxylation of vitamin D₃ and of 5 β -cholestane-3 α ,7 α ,12 α -triol is independent of sex hormones. In males these activities are regulated by influence of sex hormones on the hypophysis, probably by the presence of androgens in the neonatal period. Different effects on the two hydroxylases indicate the presence of at least two different cytochromes *P*-450 in rat liver mitochondria.

INTRODUCTION

We have recently shown that mitochondrial 25-hydroxylation of vitamin D₃ and 27-hydroxylation of 5 β -cholestane-3 α ,7 α ,12 α -triol were significantly higher in female- than in male-rat liver [1] (note: the enzyme responsible for this activity was previously termed a 'C₂₇-steroid 26-hydroxylase'; according to an investigation on the stereospecificity of the enzyme [1a] the correct name should be a 'C₂₇-steroid 27-hydroxylase'). Sex differences in microsomal metabolism of steroids and drugs in rat liver have long been known (for reviews, see [2,3]). Many of the microsomal sex-differentiated reactions are catalysed by cytochromes *P*-450 [4]. Mitochondrial vitamin D₃ (cholecalciferol) 25-hydroxylation and C₂₇-steroid 27-hydroxylation are catalysed by cytochrome(s) *P*-450 [5–7]. So far sex-differentiated forms of purified liver mitochondrial cytochromes *P*-450 have not been reported.

The sexual differences in microsomal steroid metabolism do not occur until after puberty [8] and are abolished by hypophysectomy of adult rats [9]. One of the factors determining the sexual differences has been shown to be the pattern of growth-hormone release, which is different in males and in females [10,11]. A prerequisite for a male pattern of steroid metabolism in the adult rat is exposure of the neonatal male to testicular androgens (for a review, see [12]).

Injection of oestradiol valerate feminized the mitochondrial vitamin D₃ 25-hydroxylase and C₂₇-steroid

27-hydroxylase activities in male rats [1]. A similar feminizing effect of oestrogen in males has been reported for microsomal steroid metabolism [13].

The present work was undertaken to study the mechanism by which the sex hormones regulate mitochondrial side-chain hydroxylase activities. To test if these activities were influenced by sex hormones directly, or were under some form of hypothalamic-hypophyseal-gonadal control, the effects of age, of castration and of hypophysectomy on the mitochondrial side-chain hydroxylation of vitamin D₃ and of 5 β -cholestane-3 α ,7 α ,12 α -triol were monitored in female and male rats.

The microsomal fractions were analysed for 1 α -hydroxyvitamin D₃ 25-hydroxylase activity to monitor the effects of age, castration and hypophysectomy on a known sex-differentiated cytochrome *P*-450-dependent enzyme system [14–16].

EXPERIMENTAL

Chemicals

ATP, potassium malate, Mops, Hepes and EGTA were purchased from Sigma Chemical Co., St. Louis, MO. *NN'*-Diphenylphenylenediamine (DPPD) (used to prevent peroxidation of vitamin D₃ and 1 α -hydroxyvitamin D₃ [17]) was from Eastman-Kodak Co., Rochester, NY, U.S.A. Sep-Pak C₁₈ cartridges were purchased from Waters Associates, Milford, MA,

U.S.A. Primogyn Depot (oestradiol valerate) was from Schering A.G., Berlin, Germany, and Triolandrene (slow-release testosterone preparation) from CIBA-Geigy A.G., Basel, Switzerland. All chemicals were standard commercial high-purity materials.

Unlabelled steroids

1 α -Hydroxyvitamin D₃ was a gift from Løvens Kemiske Fabrik, Copenhagen, Denmark. Vitamin D₃ was obtained from Sigma Chemical Co. 1 α -Hydroxyvitamin D₃ and vitamin D₃ were dissolved in ethanol to a concentration of 1 mg/ml or 15 mg/ml (respectively) just before use. 1,25-Dihydroxyvitamin D₃ was a gift from Roche Norge A/S, and 25-hydroxyvitamin D₃ was purchased from Philips-Duphar B. V., Veenendaal, The Netherlands. 5 β -Cholestane-3 α ,7 α ,12 α -triol was generously given by Dr. I. Björkhem (The Research Centre at the Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden).

Labelled steroids

1 α , 25-Dihydroxy[23,24(n)-³H]vitamin D₃ (80–120 Ci/mmol) and 25-hydroxy[23,24(n)-³H]vitamin D₃ (91 Ci/mmol) were obtained from Amersham International, Amersham, Bucks, U.K., and purified by h.p.l.c. before use. [7 β -³H]5 β -Cholestane-3 α ,7 α ,12 α -triol (7 Ci/mol), synthesized as described [18], was generously given by Dr. I. Björkhem (The Research Centre at the Karolinska Institute).

Rat liver material

Normal, gonadectomized and hypophysectomized female and male rats of the Wistar strain were purchased from Møllegaard Breeding Laboratory, Ejby, Denmark. The animals were fed a commercial pellet diet (Ewos R3, Møllesentralen A/S, Oslo, Norway). They were housed in plastic cages (Danish design with galvanized lids), two to four in each cage and had free access to food and water. The climatic conditions were: 23 °C, 60% relative humidity, and a 12 h light period (07:00–19:00 h). Treatment started 5 days after their arrival at the laboratory. Hormones were administered as one intramuscular injection 14–20 days before they were killed. After killing the animals by decapitation, the livers were homogenized in 5 vol. (w/v) of 0.25 M-sucrose/15 mM-Hepes (pH 7.4)/0.1 mM-EGTA. The numbers of animals and liver weights in each experimental group are shown in Table 1. Mitochondrial and microsomal fractions were prepared from each liver essentially as described [1,19]. The mitochondrial fractions (8700 g pellets) were washed twice in the homogenization buffer. The microsomal fractions (100000 g pellets) were used without washing. The subcellular fractions were resuspended in the incubation buffer to a protein concentration of 10 mg/ml.

Treatment

Male and female rats of various ages (3–12 weeks) were used without any special treatment. Ovariectomy of female rats was performed at the age of 7 weeks. Hormone-treated normal and ovariectomized females were injected with 200 μ g of Primogyn depot in 100 μ l of sesame oil. Normal controls and untreated ovariectomized rats received the vehicle only. Castration of male rats was performed at the age of 7 weeks. Hormone-treated normal and castrated males received 5 mg of

Table 1. Numbers (*n*) of animals and liver weights [means $\pm$ S.D.(*n*)] in each group of rats included in the present study

Group	Sex ...	Liver wt. (g)	
		Female	Male
(a) Age (weeks)			
3		2.6 $\pm$ 0.2 (3)	2.2 $\pm$ 0.3 (3)
4		5.6 $\pm$ 0.6 (5)	6.1 $\pm$ 1.2 (5)
7		8.1 $\pm$ 0.4 (4)	10.0 $\pm$ 1.5 (5)
9		8.5 $\pm$ 0.4 (5)	12.1 $\pm$ 0.9 (4)
12		9.1 $\pm$ 0.7 (3)	16.1 $\pm$ 0.9 (3)
(b) Gonadectomy			
Normal		8.9 $\pm$ 1.1 (5)	13.9 $\pm$ 1.4 (5)
+ Oestrogen		9.6 $\pm$ 1.0 (6)	
+ Testosterone			14.2 $\pm$ 1.8 (5)
Gonadectomized		10.0 $\pm$ 1.4 (5)	12.6 $\pm$ 1.5 (5)
+ Oestrogen		9.6 $\pm$ 1.1 (6)	
+ Testosterone			14.6 $\pm$ 0.7 (5)
(c) Hypophysectomy			
Normal		10.0 $\pm$ 0.6 (5)	15.2 $\pm$ 1.4 (10)
Hypophysectomized		5.2 $\pm$ 0.5 (6)	5.3 $\pm$ 1.0 (12)
+ Oestrogen		4.3 $\pm$ 0.5 (6)	3.4 $\pm$ 1.0 (5)
+ Testosterone		5.3 $\pm$ 0.4 (7)	6.1 $\pm$ 1.1 (13)

Triolandrene in 100 μ l of sesame oil. Normal controls and untreated castrated rats received the vehicle only. Hypophysectomy was performed at the age of 7 weeks. Hormone-treated hypophysectomized rats were injected with 5 mg of Triolandrene or 200 μ g of Primogyn Depot in 100 μ l of sesame oil. Normal controls and untreated hypophysectomized rats received the vehicle only.

Incubation, extraction and chromatographic procedures

Liver mitochondria from one animal from each experimental group (total of four animals) were prepared at the same time and immediately incubated. The microsomal fractions were stored at –20 °C until analysed. Incubations were performed in duplicate. The incubation mixture contained the following (in 1 ml): 250 μ mol of sucrose, 25 μ mol of Mops buffer, pH 7.4, 10 μ mol of MgCl₂, 1 mg of subcellular protein and an NADPH-generating system that was either 3 μ mol of isocitrate (incubations with mitochondrial fractions), or 0.7 μ mol of NADP, 0.6 μ mol of glucose 6-phosphate and 0.5 unit of glucose-6-phosphate dehydrogenase (incubations with microsomal fractions). The reaction was started by adding a saturating amount of substrate that was either 150 μ g of vitamin D₃ (mitochondria), 10 μ g of 1 α -hydroxyvitamin D₃ (microsomes) or 10 μ g of 5 β -cholestane-3 α ,7 α ,12 α -triol containing 100000 c.p.m. of ³H-labelled steroid (mitochondria). With vitamin D₃ or 1 α -hydroxyvitamin D₃ as substrate, 2 μ g of DPPD in ethanol was added. After 60 min (vitamin D₃ or 1 α -hydroxyvitamin D₃) or 40 min (5 β -cholestane-3 α ,7 α ,12 α -triol) at 37 °C with gentle shaking, the incubations were stopped with 1 ml of acetonitrile. When vitamin D₃ or 1 α -hydroxyvitamin D₃ was used as substrate, about 3000 c.p.m. of [³H]25-hydroxyvitamin D₃ or [³H]1 α ,25-dihydroxyvitamin D₃ respectively was added to correct for recovery during extraction and chromatographic procedures. The precipitated protein was removed by centrifugation, and the supernatant was

transferred to a Sep-Pak C₁₈ cartridge prewashed with 3 ml of methanol and 3 ml of water. The cartridge was washed with 3 ml of water/methanol (3:7, v/v) before elution of the sample with 3 ml of acetonitrile. The acetonitrile was evaporated under N₂ and the residue dissolved in 100 µl of methanol. The extracts of incubations with vitamin D₃ and with 1α-hydroxyvitamin D₃ were purified by two h.p.l.c. systems. The products were partially purified and separated from the substrates on a Supelcosil LC 8 column (Supelco, Bellefonte, U.S.A.) (4.6 mm × 250 mm) with 10% (vitamin D₃) or 13% (1α-hydroxyvitamin D₃) water in methanol (v/v) as elution solvent. The retention time for 25-hydroxyvitamin D₃ was 4.1 min and for 1α,25-dihydroxyvitamin D₃ 4.0 min at a flow rate of 1.8 ml/min. The product peaks were collected, evaporated and redissolved in 5% or 15% (v/v) propan-2-ol respectively in n-hexane. The final purification and quantification step was performed on a Merck LiChrospher Si60/II cartridge (Merck, Darmstadt, Germany) (4.6 mm × 250 mm) using 5% (25-hydroxyvitamin D₃) or 15% (1α,25-dihydroxyvitamin D₃) propan-2-ol in n-hexane as eluting solvent. The samples were injected by means of a Waters WISP model 710B autoinjector. Integration of the peaks at 254 nm was performed by a Spectra Physics (San Jose, CA, U.S.A.) SP4270 integrator. Product formation was calculated by comparing the peak areas with areas of known amounts of authentic 25-hydroxyvitamin D₃ or 1α,25-dihydroxyvitamin D₃ respectively, after correction for recovery of added ³H-labelled metabolite. The identity of 25-hydroxyvitamin D₃ or 1α,25-dihydroxyvitamin D₃ had previously been confirmed by g.c.-m.s. [20,21].

Portions of the incubation mixtures containing 5β-cholestane-3α,7α,12α-triol were separated by h.p.l.c. The extracts were injected on a Supelcosil LC 8 column (4.6 mm × 250 mm) with 10% water in methanol as eluting solvent at a flow rate of 1.8 ml/min. Retention time for the 27-hydroxylated tetrol was 4 min and for the substrate 7–8 min. Product formation was calculated by percentage distribution of the radioactivity in the collected fractions (one/min).

Other methods

Protein was determined by the method of Lowry *et al.* [22], with bovine serum albumin as standard.

Statistical analysis

A two-sample *t* test or a Mann-Whitneys non-parametric test (MINITAB statistical program; Statistical Department, Pennsylvania State University, University Park, PA 16802, U.S.A.) were used to evaluate the significance of difference between the groups. *P* values (given in the Figure legends) of greater than 0.05 were taken as not significant.

RESULTS

Effects on mitochondrial hydroxylase activities

The vitamin D₃ 25-hydroxylase activity increased with age up to 7 weeks for females and 4 weeks for males (Fig. 1a). The C₂₇-steroid 27-hydroxylase activity increased with age up to 4 weeks and thereafter decreased for both sexes (Fig. 1b). For both substrates the side-chain hydroxylase activity was higher in females than in males in 7 weeks old (*P* < 0.03) and older animals (*P* < 0.05 when 9- and 12-week-old animals were combined).

Fig. 1. Influence of age on mitochondrial 25-hydroxylation of vitamin D₃ (a) and 27-hydroxylation of 5β-cholestane-3α,7α,12α-triol (b) in female (□) and male (■) rat liver

Incubations were performed as described in the Experimental section. The numbers of animals in each group are shown in Table 1. The data represent the mean activity ± s.d. Significance: *significantly different from the activity in females of same age (*P* < 0.03); **significantly different from the activity in females for the combined groups of 9- and 12-week-old animals (*P* < 0.05).

Ovariectomy of female rats at 7 weeks of age, and/or injection of oestradiol valerate had no effects on the side-chain hydroxylase activities studied (Figs. 2a and 2b). Castration of male rats at 7 weeks of age increased the vitamin D₃ 25-hydroxylase activity (*P* < 0.03) (Fig. 2a). A similar trend was seen for 27-hydroxylation of 5β-cholestane-3α,7α,12α-triol (Fig. 2b), but the difference from normal males was not statistically significant. Injection of testosterone had no effect on the hydroxylase activities in normal males, decreased the vitamin D₃ 25-hydroxylase activity in castrated males to that of untreated males (Fig. 2a), but had no effect on the 27-hydroxylase activity in castrated males.

Hypophysectomy at 7 weeks of age had no effect on the hydroxylase activities in females (Figs. 3a and 3b). Neither testosterone nor oestradiol valerate affected the hydroxylase activities in hypophysectomized females (Figs. 3a and 3b). In males, hypophysectomy at 7 weeks

Fig. 2. Effect of hormone treatment on mitochondrial 25-hydroxylation of vitamin D₃ (a) and 27-hydroxylation of 5β-cholestane-3α,7α,12α-triol (b) in normal and gonadectomized female (□) and male (▨) rat liver

Abbreviations: N, normal controls; GX, gonadectomized animals; NH, hormone-treated controls; GH, hormone-treated gonadectomized animals. Hormone-treated females received 200 μg of Primogyn Depot (oestradiol valerate) and hormone-treated males 5 mg of Triolandrene (testosterone) in 100 μl of sesame oil as one intramuscular injection 14–20 days before they were killed. The untreated animals received the vehicle only. Incubations were performed as described in the Experimental section. The numbers of animals in each group are shown in Table 1. The data represent the mean activity ± S.D. Significance: *significantly different from normal males (N) ($P < 0.03$) and hormone-treated castrated males (GH) ($P < 0.05$).

Fig. 3. Effect of hypophysectomy and hormone treatment on mitochondrial 25-hydroxylation of vitamin D₃ (a) and 27-hydroxylation of 5β-cholestane-3α,7α,12α-triol (b) in female (□) and male (▨) rat liver

Abbreviations: N, normal controls; HX, hypophysectomized animals; HT, hypophysectomized animals treated with 200 μg of Primogyn Depot (oestradiol valerate). Hormones were administered in 100 μl of sesame oil as one intramuscular injection 14–20 days before the rats were killed. The untreated animals received the vehicle only. Incubations were performed as described in the Experimental section. The numbers of animals in each group are shown in Table 1. The data represent mean activity ± S.D. Significance: *significantly different from normal males ($P < 0.03$); **significantly different from hypophysectomized males ($P < 0.001$).

of age increased the side-chain hydroxylation activities ($P < 0.03$) (Figs. 3a and 3b). Testosterone treatment of hypophysectomized males had no effect (Figs. 3a and 3b). Injection of oestradiol valerate had no effect on the vitamin D₃ 25-hydroxylase activity (Fig. 3a), but decreased the C₂₇-steroid 27-hydroxylase activity in hypophysectomized males ($P < 0.001$) (Fig. 3b).

Effects on microsomal hydroxylase activities

Microsomal 25-hydroxylation of 1α-hydroxyvitamin D₃ was higher in males than in females in 7-week-old and older animals ($P < 0.005$) (Fig. 4). The 25-hydroxylase

activity increased with age in males, but not in females (Fig. 4).

Ovariectomy of adult females and/or injection of oestradiol valerate decreased the 25-hydroxylation activity in females ($P < 0.05$) (Fig. 5). Castration of adult males decreased the 25-hydroxylase activity ($P < 0.03$) (Fig. 5). Testosterone treatment had no effect in normal males, but restored the activity in castrated males to that found in normal males (Fig. 5).

Hypophysectomy decreased the 25-hydroxylase activity in both females ($P < 0.05$) and males ($P < 0.001$). Injection of testosterone or oestradiol valerate

Fig. 4. Influence of age on microsomal 25-hydroxylation of 1 α -hydroxyvitamin D₃ in female (□) and male (▨) rat liver

Incubations were performed as described in the Experimental section. The numbers of animals in each group are shown in Table 1. The data represent the mean activity $\pm$ s.d. Significance: *significantly different from control females of same age ($P < 0.005$).

Fig. 5. Effect of gonadectomy and hypophysectomy on microsomal 25-hydroxylation of 1 α -hydroxyvitamin D₃ in female (□) and male (▨) rat liver

Abbreviations: N, normal controls; GX, gonadectomized animals; NH, hormone-treated controls; GH, hormone-treated gonadectomized animals; HX, hypophysectomized animals; HT, hypophysectomized animals treated with 5 mg of testosterone; HE, hypophysectomized animals treated with 200 μ g of Primogyn Depot (oestradiol valerate). The animals were treated as described in the legends to Figs. 2 and 3. The data represent the mean activity $\pm$ s.d. A relative activity of 100 corresponds to 0.254 nmol of 1,25-dihydroxyvitamin D₃ formed/min per mg of protein. Significance: *significantly different from normal males ($P < 0.03$); **significantly different from normal females ($P < 0.05$).

normalized the 25-hydroxylase activity in females, but had no effect on the 25-hydroxylase activity in males (Fig. 5).

DISCUSSION

In the present study we have shown that the sex difference in mitochondrial side-chain hydroxylation of vitamin D₃ and of 5 β -cholestane-3 α ,7 α ,12 α -triol, described in our previous work [1], becomes manifest after puberty (Fig. 1). From the pattern observed (Scheme 1) it can be concluded that the mitochondrial side-chain hydroxylase activities are regulated by sex hormones in males, but not in females. A direct effect of sex hormones on the mitochondria cannot explain the sex difference, because of the different effects of these hormones, especially of oestrogen (Fig. 2 and [1]), in females and in males.

It can be concluded that the hypophysis must be involved in the regulation of mitochondrial side-chain hydroxylation of vitamin D₃ and of 5 β -cholestane-3 α ,7 α ,12 α -triol, at least in male rats, as the sex difference was diminished in hypophysectomized rats (Fig. 3), and the effect of oestradiol valerate was different in normal [1] and in hypophysectomized males (Fig. 3).

The differences in mitochondrial hydroxylase activities between females and males in the present study (Fig. 1) were much smaller than those reported for reactions catalysed by sex-specific forms of cytochrome P-450 in liver microsomes [14–16]. The existence of sex-specific forms of cytochromes P-450 in rat liver mitochondria is, therefore, unlikely. Other factors must be involved, like differences in substrate availability and the amount, and/or modification(s), of the enzyme(s).

The pituitary factor responsible for the sex differences in steroid metabolism in liver microsomes has been identified as the different pattern of growth-hormone secretion in females and in males [11]. Growth hormone has been reported to have a pronounced effect on vitamin D metabolism and calcium homeostasis in the rat [23,24]. There are no reports, however, on the effect of growth hormone on liver metabolism of vitamin D. Effects of growth hormone have been demonstrated on mitochondrial protein synthesis [25] and on membrane lipid distribution [26] in male rat liver. These effects may be different in females and males. Further studies are needed to elucidate the effects of growth hormone on mitochondrial side-chain hydroxylations in female and male rat liver.

The different effects on vitamin D₃ 25-hydroxylase and C₂₇-steroid 27-hydroxylase activities of sex, age and oestrogen treatment of hypophysectomized males strengthen previous indications of multiple forms of cytochrome P-450 in rat liver mitochondria [6,27]. A mitochondrial cytochrome P-450 catalysing 27-hydroxylation of C₂₇-steroids has been purified from male-rabbit liver mitochondria [28]. It was not reported, however, whether that cytochrome preparation also catalysed 25-hydroxylation of vitamin D₃.

The pattern observed for the microsomal 1 α -hydroxyvitamin D₃ 25-hydroxylase activity (Figs. 4 and 5) is in contrast with what has been reported for microsomal steroid hydroxylation, where a male pattern of hydroxylase activities was observed after ovariectomy and hypophysectomy of female rats [9,12]. Similar findings have been reported for the purified male-specific cyto-

Scheme 1. Changes (*italicized*) in liver mitochondrial vitamin D₃ 25-hydroxylase activity as a function of age (a) and of different treatments (b) of 9-week-old female and male rats

Mitochondrial C₂₇-steroid 27-hydroxylase activity paralleled the vitamin D₃ 25-hydroxylase activity, except in oestrogen-treated hypophysectomized males (*), where the 27-hydroxylase activity decreased compared with that in the hypophysectomized males.

chrome P-450 [14–16]. The male-specific cytochrome P-450 [14] cannot therefore be the only enzyme catalysing 25-hydroxylation of 1 α -hydroxyvitamin D₃. This is supported by the findings by Hansson *et al.* [29], who presented evidence for more than one 25-hydroxylase in liver microsomes from male rats, and by Andersson & Jörnvall [14], who reported that 25-hydroxylation of vitamin D₃ could be detected in female-rat liver microsomes when incubating partially purified cytochrome P-450. The difference between males and females in the latter study [14] was, however, much larger than that found in the present study, indicating that one or more forms of cytochrome P-450 might have been lost during the purification procedure [14].

The reciprocal activities of mitochondrial and microsomal vitamin D₃ 25-hydroxylase activities in female and male-rat liver must be taken into account when discussing the relative physiological importance of these two hydroxylases in the rat. We did not find any difference between the serum concentration of 25-hydroxyvitamin D₃ in 9-week-old females and males [24.6 ± 11.8 ng/ml (*n* = 14) as against 26.9 ± 9.1 ng/ml (*n* = 18) respectively; J. I. Pedersen & K. Saarem, unpublished work]. Recently Dahlbäck & Wikvall [30] also reported that the serum concentration of 25-hydroxyvitamin D₃ was about the same in female and male rats, in spite of a hardly detectable 25-hydroxylase activity in female liver microsomes. They concluded [30] that the mitochondrial vitamin D₃ 25-hydroxylase is most important in 25-hydroxylation of vitamin D₃. The present study supports this conclusion for female rats, but the relative importance of the two 25-hydroxylases in male rat liver is still uncertain.

In conclusion, we have shown that mitochondrial vitamin D₃ 25-hydroxylase and C₂₇-steroid 27-hydroxylase activities (both cytochrome P-450-dependent reactions) in male rats, are regulated by effects of testosterone on the hypophysis, presumably during the neonatal period. The activities in females seem to be independent of sex hormones. Our results indicate existence of more than one species of cytochrome P-450 in rat liver mitochondria.

The excellent technical assistance of Torhild Morsund is greatly appreciated. This work was supported by the Norwegian Research Council for Science and the Humanities.

REFERENCES

1. Saarem, K. & Pedersen, J. I. (1987) *Biochem. J.* **247**, 73–78
- 1a. Atsuta, Y. & Okuda, K. (1981) *J. Biol. Chem.* **256**, 9144–9146
2. Kato, R. (1974) *Drug. Metab. Rev.* **3**, 1–32
3. Colby, H. D. (1980) *Adv. Sex Horm. Res.* **4**, 27–71
4. Kato, R. & Kamataki, T. (1982) *Xenobiotica* **12**, 787–800
5. Pedersen, J. I., Holmberg, I. & Björkhem, I. (1979) *FEBS Lett.* **98**, 394–398
6. Björkhem, I., Holmberg, I., Oftebro, H. & Pedersen, J. I. (1980) *J. Biol. Chem.* **255**, 5244–5249
7. Pedersen, J. I., Björkhem, I. & Gustafsson, J. (1979) *J. Biol. Chem.* **254**, 6464–6469
8. Stenberg, Å. (1976) *J. Endocrinol.* **68**, 265–272
9. Gustafsson, J. & Stenberg, Å. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1462–1464

10. Edén, S. (1979) *Endocrinology* (Baltimore) **105**, 555–560
11. Mode, A., Gustafsson, J.-Å., Jansson, J.-O., Edén, S. & Isaksson, O. (1982) *Endocrinology* (Baltimore) **111**, 1692–1697
12. Gustafsson, J.-Å., Mode, A., Norstedt, G. & Skett, P. (1983) *Annu. Rev. Physiol.* **45**, 51–60
13. Skett, P. (1978) *Biochem. J.* **174**, 753–760
14. Andersson, S. & Jörnvall, H. (1986) *J. Biol. Chem.* **261**, 16932–16936
15. Waxman, D. J. (1984) *J. Biol. Chem.* **259**, 15481–15490
16. Morgan, T., MacGeoch, C. & Gustafsson, J.-Å. (1985) *Mol. Pharmacol.* **27**, 471–479
17. Bhattacharyya, M. H. & DeLuca, H. F. (1974) *Arch. Biochem. Biophys.* **160**, 58–62
18. Cronholm, T. & Johansson, G. (1970) *Eur. J. Biochem.* **16**, 373–381
19. Saarem, K., Bergseth, S., Oftebro, H. & Pedersen, J. I. (1984) *J. Biol. Chem.* **259**, 10936–10940
20. Björkhem, I. & Holmberg, I. (1978) *J. Biol. Chem.* **253**, 842–849
21. Björkhem, I., Holmberg, I., Kristiansen, T. & Pedersen, J. I. (1979) *Clin. Chem.* **25**, 584–588
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
23. Pahuja, D. N. & DeLuca, H. F. (1981) *Mol. Cell. Endocrinol.* **23**, 345–350
24. Wongsurawat, N., Armbrrecht, H. J., Zenser, T. V., Forte, L. R. & Davis, B. B. (1984) *J. Endocrinol.* **101**, 333–338
25. Maddaiah, V. T., Sharma, R., Balachandar, V., Rezvani, I., Collipp, P. J. & Chen, S. Y. (1973) *J. Biol. Chem.* **248**, 4263–4268
26. Clejan, S. & Maddaiah, V. T. (1986) *Lipids* **21**, 677–683
27. Oftebro, H., Björkhem, I., Srede, S., Schreiner, A. & Pedersen, J. I. (1980) *J. Clin. Invest.* **65**, 1418–1430
28. Wikvall, K. (1984) *J. Biol. Chem.* **259**, 3800–3804
29. Hansson, R., Holmberg, H. & Wikvall, K. (1981) *J. Biol. Chem.* **256**, 4345–4349
30. Dahlbäck, H. & Wikvall, K. (1987) *Biochem. Biophys. Res. Commun.* **142**, 999–1004

Received 24 June 1987/26 October 1987; accepted 9 December 1987