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NEONATAL ANDROGENIC PROGRAMMING OF HEPATIC STEROID METABOLISM IN RATS

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SUMMARY

Hepatic metabolism of steroid hormones in the rat is sexually differentiated at birth by irreversible programming ("imprinting") by testicular androgens. The regulation of sex-dependent enzyme activities in the rat has been shown to be under hypothalamico-hypophyseal control. Probably this control is mainly exerted *via* a pituitary "feminizing factor" the secretion of which is suppressed in the male rat by a hypothalamic inhibiting factor. The production of this inhibiting factor is "turned on" by neonatal androgenic imprinting.

INTRODUCTION

During the neonatal period the hypothalamus in the rat becomes programmed by testicular androgens resulting in an acyclic secretion of LH in adult life [1]. Recently, considerable interest has been focused on neonatal programming by androgens of various other physiological and biochemical functions, *e.g.* sexual behaviour [2, 3] and hepatic steroid metabolism [4–7]. The latter phenomenon has the advantage of making it possible to study neonatal sexual differentiation with exact biochemical methods and the present paper will summarize our recent findings on neonatal programming of hepatic steroid metabolism in the rat.

EXPERIMENTAL

Male and female rats of the Sprague–Dawley strain were castrated, hypophysectomized or sham-operated at various ages from birth until adulthood. In certain experiments, castrated newborn male and female rats were given 500 μg of testosterone propionate subcutaneously at birth. In other experiments, castrated, postpubertal adult rats were treated for two weeks by intramuscular injections of 100 μg of testosterone propionate or 100 μg of estradiol benzoate in 0·5 ml of propylene glycol. Control rats received vehicle only.

The rats were killed by cervical dislocation and the liver was excised quickly and cooled in a modified Bucher medium, pH 7·4 [8]. Liver homogenates, 20% (w/v), were prepared with a Potter–Elvehjem homogenizer equipped with a loosely fitting Teflon pestle. The microsomal and cytosol (105,000 *g* supernatant) fractions were prepared using differential centrifugation and the microsomal fraction was resuspended. Microsomal suspension from 0·10 g of liver or cytosol from 0·60 g of liver was incubated in 3·0 ml of Bucher medium with 500 μg of 4-[4-^{14}C]-androstene-3,17-dione at 37°C for 8 min in the presence of an NADPH-regenerating system [6]. Incubations were also carried out with 5α-[4-^{14}C]-androstane-3α,17β-diol and 5α-[1,2-^{3}H] androstane-3α,17β-diol 3,17-disulphate using 200 μg of each substrate and microsomal suspension from 0·20–0·50 g liver in 4·0 ml of Bucher medium containing the same amount of NADPH-regenerating system as in the incubations with 4-[4-^{14}C]-androstene-3,17-dione.

The incubation mixtures were extracted and analyzed by thin-layer chromatography-radioautography (the sulphurylated steroids were solvolyzed in acidified ethyl acetate prior to application on thin-layer plates). The radioactive zones were localized, scraped off separately and measured for radioactivity in a Packard Liquid Scintillation Spectrometer, Model 4322. The steroid metabolites in the various zones were identified by gas chromatography—mass spectrometry (LKB 9000 instrument) and by radio–gas chromatography (Hewlett–Packard Gas Chromatograph, Model 402, equipped with a Barber–Colman Radioactivity Monitoring System Model 5190).

RESULTS AND DISCUSSION

General findings on neonatal programming of hepatic metabolism of 4-androstene-3,17-dione, 5α-androstane-3α,17β-diol and 5α-androstane-3α,17β-diol 3,17-disulphate

Table 1 summarizes the metabolites formed after incubation of liver microsomal and supernatant fractions with 4-[4-^{14}C]-androstene-3,17-dione, 5α-[4-^{14}C]-androstane-3α,17β-diol and 5α-[1,2-^{3}H]-androstane-3α,17β-diol 3,17-disulphate. References are given to earlier publications where the identifications of formed metabolites are described in detail. By measuring the formation of these products it was possible to assay the following enzyme activities: the 5α- and 5β-reductases (sum of total 5α- and 5β-reduced metabolites formed, respectively), 17β- and 17α-hydroxysteroid reductases, and 6β-, 7α- and 16α-hydroxylases active on 4-androstene-3,17-dione, the 2α-, 2β-, 7α-, 7β- and 18-hydroxylases active on 5α-androstane-3α,17β-diol and the 15β-hydroxylase active on 5α-

Table 1. Metabolites formed after incubation of liver microsomal and supernatant fractions with 4-[4-^{14}C]-androstene-3,17-dione, 5α-[4-^{14}C]-androstane-3α,17β-diol and 5α-[1,2-^{3}H]-androstane-3α,17β-diol 3,17-disulphate

Steroid substrate and cellular subfraction	Identified metabolites	References to literature where identification is described
4-Androstene-3,17-dione, microsomal fraction	5α-Androstane-3,17-dione	5
	3α-Hydroxy-5α-androstan-17-one	5
	3β-Hydroxy-5α-androstan-17-one	5
	17β-Hydroxy-4-androsten-3-one	5
	17α-Hydroxy-4-androsten-3-one	6
	6β-Hydroxy-4-androstene-3,17-dione	5
	7α-Hydroxy-4-androstene-3,17-dione	5
	16α-Hydroxy-4-androstene-3,17-dione	5
4-Androstene-3,17-dione, supernatant fraction	5β-Androstane-3,17-dione	6
	3α-Hydroxy-5β-androstan-17-one	6
5α-Androstane-3α,17β-diol, microsomal fraction	5α-Androstane-2α,3α,17β-triol	9
	5α-Androstane-2β,3α,17β-triol	9
	5α-Androstane-3α,7α,17β-triol	9
	5α-Androstane-3α,7β,17β-triol	9
	5α-Androstane-3α,17β,18-triol	9
	5α-Androstane-3β,17β,18-triol	9
5α-Androstane-3α,17β-diol 3,17-disulphate, microsomal fraction	5α-Androstane-3α,15β,17β-triol	10

References are given to publications where identifications are described in detail.

androstane-3α,17β-diol 3,17-disulphate. It was difficult to assay the 3α- and 3β-hydroxysteroid reductase activities under the conditions used since these enzymes use the 4-androstene-3,17-dione metabolite 5α-androstane-3,17-dione as substrate. However, the 3α- and 3β-hydroxysteroid reductase activities were estimated by forming the ratio of 3α-/5α-reduced and 3β-/5α-reduced metabolites formed, respectively.

The findings on regulation of hepatic metabolism of 4-androstene-3,17-dione, 5α-androstane-3α,17β-diol and 5α-androstane-3α,17β-diol 3,17-disulphate are summarized in Table 2. Based on these findings the hepatic steroid-metabolizing enzyme activities may be grouped into three classes with regard to the mechanisms regulating their activity: (I) Enzymes irreversibly programmed or "imprinted" by androgens during the neonatal period and reversibly influenced by sex hormones postpubertally (the 2α-hydroxylase active on 5α-androstane-3α,17β-diol, the 15β-hydroxylase active on 5α-androstane-3α,17β-diol 3,17-disulphate and the 16α-hydroxylase, the 5α- and 5β-reductases and the 3β- and 17α-hydroxysteroid reductases active on 4-androstene-3,17-dione); (II) enzymes reversibly influenced by sex hormones (the 2β-, 7β- and 18-hydroxylases active on 5α-androstane-3α,17β-diol and the 6β-hydroxylase active on 4-androstene-3,17-dione); and (III) enzymes almost or completely sex hormone-independent (the 7α-hydroxylase active on 5α-androstane-3α,17β-diol and the 7α-hydroxylase and the 3α- and 17β-hydroxysteroid reductases active on 4-androstene-3,17-dione) [5].

Table 2 also shows that castrated female rats responded markedly less to treatment with testosterone propionate than castrated male rats with respect to liver enzyme activities. In one series of experiments it was demonstrated that this sexual difference in androgen responsiveness was due to neonatal imprinting by testicular secretion products. Thus, liver microsomal 2β-, 7β- and 18-hydroxylation of 5α-androstane-3α, 17β-diol and 6β-hydroxylation of 4-androstene-3,17-dione were considerably more stimulated in male rats castrated 14 days after birth than in male rats castrated at birth when treated with testosterone propionate as adult. These two experimental groups of rats also responded quite differently to testosterone propionate administration with respect to hepatic 5β-reductase activity; whereas a fourfold increase in enzyme activity was noted in the neonatally castrated animals, the animals castrated at 14 days of age responded by a decrease of about 20% in enzyme activity [7].

Regulation and properties of the sex-specific 15β-hydroxylase system in female rats active on steroid sulphates

When 5α-androstane-3α,17β-diol 3,17-disulphate is incubated with liver microsomes from female rats it is hydroxylated in position 15β, as indicated above. The 15β-hydroxylase system cannot be detected in liver microsomes from male rats and is at least 3000 times more active in female rats. When the binding of the sulphurylated substrate to liver microsomal cytochrome P-450 was measured with an Aminco-Chance spectrophotometer, the spectral dissociation constant (K_s) was found to be 9·52 μM for male liver microsomes and 23·8 μM for female liver microsomes. The apparent K_m value calculated for female liver microsomes was 25·8 μM. Incubations carried out in the presence of carbon monoxide led to more than 98% inhibition of the 15β-hydroxylase system, indicating that 15β-hydroxylation is a cytochrome P-450-dependent process [10].

Table 2. Regulation of hepatic enzyme activities

Enzyme activity	Group I: intact adult rats	Group II*: adult ♂ gonad-ectomized at 7–14 days of age or later	Group III*: adult ♂ neonatally gonad-ectomized	Group IV*: adult ♀ gonad-ectomized neonatally or later	Group II†: treated with testosterone propionate when adult	Groups III and IV‡: treated with testosterone propionate when newborn	Group II†: treated with estradiol benzoate when adult	Groups III and IV‡: treated with testosterone propionate when adult	Group IV§: treated with estradiol benzoate when adult
Active on 4-androstene-3,17-dione in microsomal fraction									
5α-Reductase	♂ < ♀	↑	↑↑	⇣	↓	↓	↑↑	⇣	⇡
17β-Hydroxysteroid reductase	♂ = ♀	—	—	—	—	—	—	—	—
17α-Hydroxysteroid reductase	♂ > ♀	↓	↓↓	—	↑	↑	↓↓	⇡	—
3β/5α-Reduced metabolites	♂ > ♀	↓	↓↓	—	↑	↑	↓↓	⇡	—
3α/5α-Reduced metabolites	♂ = ♀	—	—	—	—	—	—	—	—
6β-Hydroxylase	♂ > ♀	↓	↓	—	↑	—	↓	⇡	—
7α-Hydroxylase	♂ ≤ ♀	⇡	↑	—	⇣	⇣	↑	⇣	—
16α-Hydroxylase	♂ > ♀	↓	↓↓	—	↑	↑	↓↓	⇡	—
Active on 4-androstene-3,17-dione in supernatant fraction									
5β-Reductase	♂ > ♀	↑	↓↓	—	↓	↑	↓↓	↑↑	—
Active on 5α-androstane-3α,17β-diol in microsomal fraction									
2α-Hydroxylase	♂ > ♀	↓	↓↓	—	↑	↑	↓↓	⇡	—
2β-Hydroxylase	♂ > ♀	↓	↓	—	↑	—	↓	⇡	—
18-Hydroxylase	♂ > ♀	↓	↓	—	↑	—	↓	⇡	—
7β-Hydroxylase	♂ > ♀	↓	↓	—	↑	—	↓	⇡	—
7α-Hydroxylase	♂ ≤ ♀	↑	↑	—	⇣	⇣	↑	⇣	—
Active on 5α-androstane-3α,17β-diol 3,17-disulphate in microsomal fraction									
15β-Hydroxylase	♂ ≪ ♀	—	↑↑	⇣	—	↓↓	↑	⇣	⇡

* Compared to Group I.
† Compared to Group II.
‡ Compared to Groups III and IV.
§ Compared to Group IV.

Symbols: ↑ and ↓ represent an increase and decrease, respectively, of the enzyme activity in question when compared to the situation in intact rats or the appropriate control group; ⇡ and ⇣ indicate slight changes; ↑↑ and ↓↓ indicate very pronounced changes in activity. No effect is indicated by —.

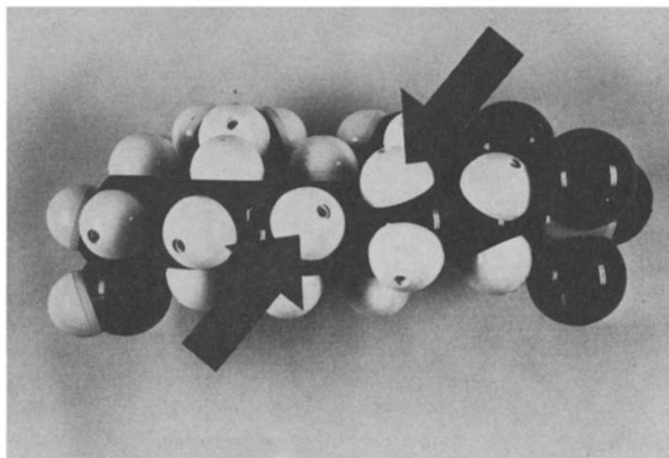

Fig. 1. CPK model of 5α-androstane-3α,17β-diol 17-sulphate with arrows indicating the positions of the 7β- and 15β-hydrogen atoms.

In order to study the specificity of the 15β-hydroxylase system further, eighteen different C_{18}, C_{19}, C_{21} and C_{27} steroid sulphates and the corresponding free steroids were incubated with microsomal preparations from male and female rats. The sulphate-specific enzyme system was only present in preparations from female rats and primarily catalyzed hydroxylation in position 15β but also in position 7β. As seen in the molecular model shown in Fig. 1 the 7β- and 15β-hydrogen atoms have a very close spatial relationship and the 7β- and 15β-hydroxylation reactions may well be catalyzed by the same active site on the enzyme. In contrast, male liver microsomes were more efficient than female liver microsomes in hydroxylating free steroids; these were hydroxylated in positions 2α, 2β, 6α, 6β, 7α, 7β, 16α and 18. The sulphate-specific hydroxylase system in female liver microsomes was found to have rigid requirements concerning the structure of ring D in the substrate molecule; only 17β-sulphates (C_{19} steroids) and 21-sulphates (C_{21}-steroids) were hydroxylated (Fig. 2). Less rigid criteria, however, exist concerning the structure of ring A; the enzyme system was active on both 5α- and 5β-steroids, estrogens, 3-sulphurylated compounds and steroids with axial and equatorial hydroxyl groups at C-3. These results indicate the existence in the female rat liver of a hydrophilic species of cytochrome P-450, specifically catalyzing the hydroxylation of steroid sulphates and with quite different properties from the "bulk" of cytochrome P-450 participating in hydroxylations of hydrophobic substrates.

The unique character of the 15β-hydroxylase system in female liver microsomes when compared to other microsomal hydroxylase systems is also evident from studies on its regulation. Treatment of female rats with the well-known hydroxylase inducers phenobarbital and 3-methylcholanthrene led to a significant decrease in the activity of the 15β-hydroxylase system, and treatment with the "catatoxic" steroid 16α-cyanopregnenolone [10] did not significantly affect the enzyme activity. Biliary drainage did not affect the 15β-hydroxylase activity whereas the 2α- and 7α-hydroxylase activities (using free 5α-androstane-3α,17β-

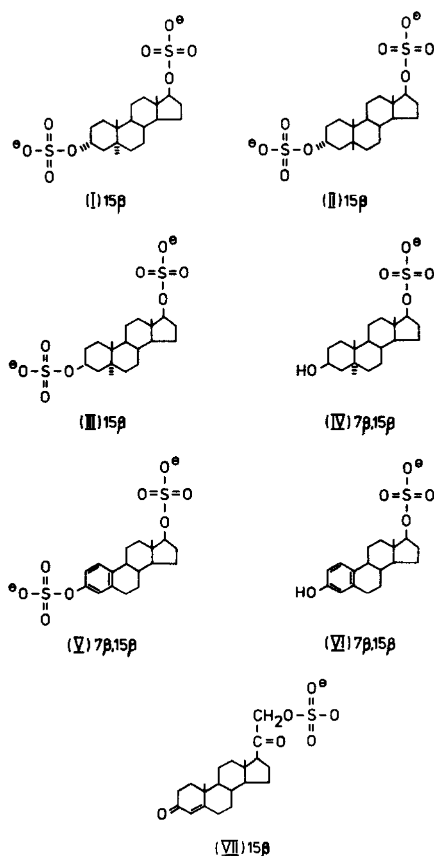

Fig. 2. Steroid sulphates that are substrates for the sulphate-specific hydroxylase system (7β- and 15β-hydroxylases) present in female rat liver microsomes. I = 5α-androstane-3α,17β-diol 3,17-disulphate; II = 5β-androstane-3α,17β-diol 3,17-disulphate; III = 5α-androstane-3β,17β-diol 3,17-disulphate; IV = 5α-androstane-3β,17β-diol 17-sulphate; V = estradiol 3,17-disulphate; VI = estradiol 17-sulphate; VII = deoxycorticosterone 21-sulphate.

diol as substrate) decreased. Ligation of the common bile duct led to a depression of the 2α-, 7α- and 18-hydroxylase activities but to an increase in the 15β-hydroxylase activity. Administration of 5α-androstane-3α,17β-diol 3,17-disulphate stimulated the 15β-hydroxylase activity whereas no significant changes were seen in the activities of the 2α-, 7α- and 18-hydroxylase systems. These results indicate that the 15β-hydroxylase system in female liver microsomes active on steroid sulphates fulfilling certain structural criteria is regulated by other control mechanisms than the hydroxylase systems active on free 5α-androstane-3α,17β-diol.

Hypophyseal control of hepatic enzyme activities

When female rats were hypophysectomized, the 15β-hydroxylase activity was completely abolished. As shown in Table 2, induction of the 15β-hydroxylase activity in castrated male rats was achievable by treatment with estradiol benzoate; in contrast to this, hypophysectomized male rats were unresponsive towards this effect of estradiol benzoate. These results indicate that the hypophysis is necessary for the maintenance of the 15β-hydroxylase activity in liver microsomes from female rats and for the inducing effect of estradiol benzoate on the 15β-hydroxylase activity in liver microsomes from male rats. It seems reasonable to assume that the hypophysis secretes some kind of feminizing factor that simulates 15β-hydroxylation of steroid sulphates. The mechanism of this stimulation may be induction of the 15β-hydroxylase system by increased synthesis of the species of cytochrome P-450 involved. However, the K_s value of 9·52 μM for male and 23·8 μM for female liver microsomes with 5α-androstane-3α,17β-diol 3,17-disulphate as substrate might possibly indicate that the postulated hypophyseal feminizing factor stimulates 15β-hydroxylation by increasing the synthesis of an essential factor necessary for the participating cytochrome P-450 species in its catalytic function rather than by increasing the synthesis of the cytochrome P-450 species itself.

Hypophyseal control of hepatic steroid hormone metabolism is not confined to the sulphate-specific 15β-hydroxylase system. Hypophysectomy was found to lead to an over-all masculinization of hepatic steroid metabolism in female rats (increased activities of the 2α-, 2β-, 7β- and 18-hydroxylase systems active on 5α-androstane-3α,17β-diol and of the 6β- and 16α-hydroxylase systems, 3β- and 17α-hydroxysteroid reductase and 5β-reductase enzymes active on 4-androstene-3,17-dione). These findings indicate the existence of a hypophyseal feminizing factor that feminizes a basic masculine level of the hepatic sex-dependent enzyme activities. The masculinizing and feminizing effects on liver enzyme activities observed following treatment with testosterone propionate and estradiol benzoate, respectively (cf. Table 2), were not seen in hypophysectomized rats. The estrogen unresponsiveness characterizing these rats may be explained by the lost capacity to induce secretion of a hypophyseal feminizing factor. It may be speculated that the androgen unresponsiveness in hypophysectomized rats is due to the loss of a hypophyseal factor necessary for the action of androgens in the liver cell [11].

In order to define more precisely the nature of the hypophyseal control of hepatic steroid hormone metabolism, adult male and female rats were castrated, hypophysectomized and transplanted under the kidney capsule with a hypophysis from a male rat. Control rats that were only castrated and hypophysectomized displayed the expected masculinization of hepatic steroid metabolism whereas transplanted rats of both sexes showed a female type of liver metabolism. These experiments indicate that the hypophysis *in situ* in both male and female rats has a basic feminizing factor secreting tonus which is modulated by central influences. In order to give support to this theory, efforts were made to relieve the male hypophysis from the hypothesized inhibiting control of the secretion of the feminizing factor. With the help of an electrothermic lesion in the median eminence the connections between the hypothalamus and the pituitary gland were partially destroyed in male rats. When the hepatic enzyme activities were measured 1 week after the operation, they were found to be partially feminized (*e.g.* induced 15β-hydroxylase system active on 5α-androstane-3α,17β-diol 3,17-disulphate, increased activity of the 5α-reductase active on 4-androstene-3,17-dione and decreased activities of the 6β- and 16α-hydroxylases active on 4-androstene-3,17-dione and of the 2α-, 2β-, 7β- and 18-hydroxylases active on 5α-androstane-3α,17β-diol) [12].

Figure 3 outlines our present concept of the hypothalamico-hypophyseal control of sex-dependent steroid hormone metabolism in rat liver. Neonatal testicular secretion of androgens results in imprinting of the hypothalamus which becomes programmed to secrete feminizing factor inhibiting factor (FFIF) in adult life. FFIF prevents the male hypophysis from secreting liver feminizing factor (FF) which results in a basic, masculine type of hepatic steroid hormone

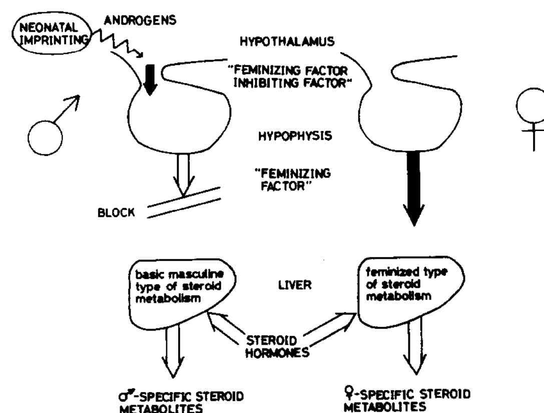

Fig. 3. Current concept of neonatal programming of sex-dependent hepatic enzyme levels. For explanation, see text.

metabolism. The lack of neonatal androgenic imprinting in female rats leads to an uninhibited secretion of FF in adult female animals with a consequently feminized type of hepatic steroid hormone metabolism.

The hypothalamic regulation of the secretion of FF has certain similarities to that of the secretion of prolactin and the question may be asked whether FF is identical to any of the known gonadotrophic hormones. However, experiments where the influence of prolactin, FSH and LH upon liver enzyme activities has been studied give no indications that these hormones are involved in feminization of hepatic enzyme levels.

Neonatal imprinting of the postulated FFIF-secreting centre in rat hypothalamus

A hereditary type of male pseudohermaphroditism in rats was described in 1964 by Stanley and Gumbreck[13]. The affected rats were phenotypic females with an xy-karyotype and bilateral inguinal testes [14]. Bardin *et al.* have suggested that the androgen insensitivity that characterizes the male pseudohermaphroditic rat is due to an inherited abnormality of a regulatory protein which renders the nucleus of the cell incapable of concentrating androgens at their proposed intracellular site of action [15]. With 4-androstene-3,17-dione and 4-pregnene-3,20-dione as substrates, the hepatic microsomal metabolism of these animals was found to be identical to that of female but different from that of male littermate rats [16]. The female character of the liver metabolism of steroid hormones in male pseudohermaphroditic rats is probably due to the lack in these rats of a neonatal androgen "imprinting" of the hypothalamic FFIF-secreting centre.

During recent years, much effort has been devoted to studies on male pseudohermaphroditism in rats experimentally induced by pre- and postpubertal treatment with cyproterone acetate [3]. These animals possess outer genitalia with female characteristics including a vagina and also display a partially feminized sexual behaviour. When hepatic metabolism of 4-androstene-3,17-dione, 5α-androstane-3α,17β-diol and 5α-androstane-3α,17β-diol 3,17-disulphate was studied in treated male rats, they were found to have partially feminized enzyme levels, whereas the liver enzyme activities in treated female rats generally were not affected [17]. These results are probably best explained in the following way: treatment with the anti-androgen during the neonatal period results in less efficient imprinting of the hypothalamic FFIF-secreting centre leading to less pronounced masculine setting of sex-dependent enzyme levels and also to a relative androgen unresponsiveness.

When two groups of neonatally castrated female rats were treated with single subcutaneous injections of 0·145 or 1·45 μmoles of testosterone propionate, respectively, only the group receiving the higher dose of androgen showed a masculine setting of "imprintable" enzyme levels in the adult period [6]. This indicates that the hypothalamic FFIF-secreting centre is different from the centre regulating cyclic gonadotrophin secretion which is sensitive to neonatal programming by testosterone propionate in the dose range of 0·145 μmol [18]. Furthermore, it could be shown that dihydrotestosterone propionate and estradiol benzoate were as efficient in inducing masculine differentiation of the liver in neonatally castrated male rats as testosterone propionate itself. Masculine differentiation of the hypothalamus by neonatal exposure to estrogens is well-known [19] but dihydrotestosterone cannot induce acyclic gonadotrophin secretion, nor can it imprint masculine behavioural patterns [20, 21]. These results further support the theory that the hypothalamic FFIF-secreting centre is different from previously known or hypothesized, neonatally programmed hypothalamic centres.

General considerations

The question may be asked why the hepatic metabolism of steroid hormones in the rat is regulated by such elaborate hypothalamico-hypophyseal mechanisms. What is for example the physiological function of the highly sex- and sulphate-specific 15β-hydroxylase system in female liver which represents a unique species of cytochrome P-450, both with respect to general properties and to type of regulation? It seems reasonable to believe that the specific character and regulation of this enzyme system is associated with a specific function. One obvious possibility is that the 15β-hydroxylase acts as a protective mechanism in female rats ensuring efficient deactivation of potentially androgenic steroids. In analogy, the male-specific products of hepatic steroid metabolism (*e.g.* 3β- and 17α-hydroxy-C_{19} steroids) may have specific functions as androgen effectors in target organs yet to be defined.

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REFERENCES

1. Barraclough C. A.: *Recent Prog. Horm. Res.* **22** (1966) 503–539.
2. Phoenix C. H., Goy R. W., Gerall A. A. and Young W. C.: *Endocrinology* **65** (1959) 369–382.
3. Neumann F.: *Gynec. Invest.* **2** (1971) 150–179.
4. DeMoor P. and Denef C.: *Endocrinology* **82** (1968) 480–492.
5. Einarsson K., Gustafsson J.-Å. and Stenberg Å.: *J. biol. Chem.* **248** (1973) 4987–4997.
6. Gustafsson J.-Å. and Stenberg Å.: *J. biol. Chem.* **249** (1974) 711–718.
7. Gustafsson J.-Å. and Stenberg Å.: *J. biol. Chem.* **249** (1974) 719–723.
8. Bergström S. and Gloor U.: *Acta chem. scand.* **9** (1955) 34–38.
9. Berg A. and Gustafsson J.-Å.: *J. biol. Chem.* **248** (1973) 6559–6567.
10. Gustafsson J.-Å. and Ingelman-Sundberg M.: *J. biol. Chem.* **249** (1974) 1940–1945.

11. Gustafsson J.-Å. and Stenberg Å.: *Endocrinology*, **95** (1974) 891–896.
12. Gustafsson J.-Å., Hökfeldt T., Ingelman-Sundberg M. and Stenberg Å.: to be published.
13. Stanley A. J. and Gumbreck L. G.: In *Progr. 46th Meet. Endocrine Soc.* (1964) p. 40.
14. Allison J. E., Stanley A. J. and Gumbreck L. G.: *Anat. Rec.* **153** (1965) 85–87.
15. Bardin C. W., Bullock L., Blackburn W. R., Sherins R. J. and Vanha-Perttula T.: *Birth Defects*: Original Article Series **7** (1965) 185–192.
16. Einarsson K., Gustafsson J.-Å. and Goldman A. S.: *Eur. J. Biochem.* **31** (1972) 345–353.
17. Gustafsson J.-Å., Ingelman-Sundberg M., Stenberg Å. and Neumann F.: *J. Endocr.* In the press (1974).
18. Gorski R. A.: In *Frontiers in neuroendocrinology* (Edited by L. Martini and W. F. Ganong). Oxford University Press, New York (1971) pp. 237–290.
19. McDonald P. G. and Doughty C.: *J. Endocr.* **61** (1974) 95–103.
20. Luttge W. G. and Whalen R. E.: *Horm. Behav.* **1** (1970) 265–281.
21. Brown-Grant K., Munck A., Naftolin F. and Sherwood M. R.: *Horm. Behav.* **2** (1971) 173–182.