Proc. Natl. Acad. Sci. USA
Vol. 73, No. 5, pp. 1462–1465, May 1976
Biochemistry

On the obligatory role of the hypophysis in sexual differentiation of hepatic metabolism in rats

(pituitary transplant/sex hormonal effects/hepatic steroid metabolism/neonatal androgen imprinting /hypothalamico-pituitary control)

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Communicated by Jan G. Waldenström, February 4, 1976

ABSTRACT **The hepatic metabolism of 4-[4-^{14}C]androstene-3,17-dione and 5α-[4-^{14}C]androstane-3α,17β-diol was studied in castrated and hypophysectomized male rats with a transplanted pituitary under the kidney capsule. The effects of testosterone propionate and estradiol benzoate on liver metabolism were also studied in these experimental animals. It was found that the autonomous pituitary secreted a "feminizing factor" that transformed the male type of steroid metabolism characteristic of hypophysectomized rats into a female type of metabolism. Hypophysectomized rats were unresponsive towards androgen action on the liver and did not respond with feminized hepatic metabolism after treatment with estradiol benzoate. It is concluded that estrogenic action on liver enzymes is mediated via modulation of the secretion of a central (probably hypothalamic) "feminizing factor inhibiting factor" and that sex hormonal effects on hepatic metabolism only occur in the presence of a pituitary *in situ*.**

The metabolism of steroid hormones in rat liver shows large sexual differences (1–5). This differentiation of hepatic metabolism, which begins at about 4 weeks of age and is almost completed at about 8–10 weeks of age, is manifested due to active processes in both sexes (6–9). The female metabolic type is maintained by a pituitary factor, without gonadal influence, whereas the male metabolic type seems to be maintained by testicular androgens acting directly upon the liver in the presence of an intact pituitary (2, 10–13).

The male type of hepatic metabolism is irreversibly determined or "programmed" at birth by testicular androgen (5, 14, 15); if castrated during the neonatal period, male rats develop a female type of metabolism when adult. In animals exposed to androgen at birth (i.e., normal males and androgenized females), the male type of hepatic metabolism is temporarily feminized after treatment with estrogen (5, 6). Castration of adult male rats leads to partial feminization of certain enzyme activities in the liver whereas other enzymes retain their male characteristics (5, 12). The male type of metabolism is completely restored by androgen administration (5, 6, 12).

In order to better understand the nature of the hypophyseal control of hepatic metabolism we have implanted a pituitary under the renal capsule of hypophysectomized rats and studied the effects on liver metabolism of this type of autonomous pituitary. In this investigation we have also studied the effects of sex hormone administration on liver metabolism in such experimental animals.

MATERIALS AND METHODS

Animals. Male rats of the Sprague-Dawley strain were castrated at 7 weeks of age. Some of these rats were given a pituitary transplant from a female rat of the same age 1 week later. Hypophysectomy was performed at 9 weeks of age. Some of the hypophysectomized and hypophysectomized plus transplanted animals were left 4–5 days after the final operation and were then treated for a week with daily intramuscular injections of 100 μg of testosterone propionate or 10 μg of estradiol benzoate in 20 μl of propylene glycol. Each group of rats contained at least four animals.

Incubations. The animals were killed by cervical dislocation and the liver was excised and chilled to 0–+4°. From a 20% (wt/vol) homogenate in a modified Bucher medium (16), microsomal and soluble enzyme fractions were prepared by differential centrifugation (6). The microsomes were resuspended in the buffer. Cytosol from about 0.6 g and microsomal suspension from about 0.1 g of liver tissue were incubated at 37° in the presence of an NADPH-regenerating system (5) with 500 μg of 4-[4-^{14}C]androstene-3,17-dione (specific activity 0.49 μCi/mg). 5α-[4-^{14}C]Androstane-3α,17β-diol (specific activity 3.0 μCi/mg), 200 μg, was incubated with microsomal suspension from about 0.5 g of liver tissue. The conditions of the incubations were designed to give conversions linear with time and enzyme concentration. Small changes in substrate and cofactor amounts did not affect the total conversion of substrate.

The steroids were extracted from the incubation medium with chloroform/methanol, 2:1 (vol/vol). Steroids were separated by thin-layer and radio-gas chromatography (12). Identifications were based on chromatographic mobility and gas chromatographic-mass spectrometric data (5, 12). Enzyme activities were related to the amount of protein incubated. Student's *t*-test was used for statistical evaluation of the results.

RESULTS

Effects of an autonomous pituitary

In control experiments castrated male and spayed female animals were compared to animals that were castrated and hypophysectomized (Table 1). The hepatic metabolism of castrated male rats was similar to that of hypophysectomized rats of both sexes (Table 1). However, large differences were seen in the hepatic metabolism of spayed female rats and that of hypophysectomized animals (Table 1). Similar differences were observed when the hepatic metabolism of male rats with a transplanted pituitary was compared to that of hypophysectomized rats (Table 2). These results indicate that the autonomous pituitary secretes factor(s) capable of feminizing the male type of liver metabolism characteristic of hypophysectomized animals.

Attempts to affect the action of the autonomous pituitary

No effects on hepatic enzyme activities were observed after administration of testosterone propionate to hypophysectomized rats (Table 3). When hypophysectomized animals with a transplanted pituitary were treated in the same way, no significant or consistent effects were noted either (Table 4). Es-

Table 1. Hepatic enzyme activities in castrated male and spayed female rats, in castrated and hypophysectomized male rats, and in spayed and hypophysectomized female rats

Enzyme activity†	Castrated males	Castrated + hypox males	Spayed females	Spayed + hypox females
Enzymes active on 4-androstene-3,17-dione				
5α-Reductase	112 ± 60	29 ± 12*	140 ± 21	33 ± 2.00***
5β-Reductase	6.20 ± 0.50	6.40 ± 1.60	1.30 ± 0.10	4.90 ± 2.20*
17β-Hydroxysteroid reductase	5.69 ± 1.69	3.43 ± 0.99	4.32 ± 0.89	4.25 ± 0.71
3β/5α-Reduced metabolites	0.13 ± 0.04	0.16 ± 0.10*	0.006 ± 0.005	0.09 ± 0.05
6β-Hydroxylase	9.05 ± 4.50	8.14 ± 3.10	6.16 ± 1.39	11.1 ± 1.50**
7α-Hydroxylase	4.94 ± 1.94	4.83 ± 0.47	8.11 ± 2.12	8.70 ± 3.41
16α-Hydroxylase	6.82 ± 2.84	3.05 ± 1.88	0	2.59 ± 2.94***
Enzymes active on 5α-androstane-3α,17β-diol				
2α-Hydroxylase	3.62 ± 1.90	2.41 ± 1.05	0.50 ± 0.08	2.12 ± 0.03***
2β-Hydroxylase	0.60 ± 0.08	1.05 ± 0.03*	0	0.78 ± 0.43**
18-Hydroxylase	1.38 ± 1.01	1.05 ± 0.24	0	1.05 ± 0.20***
7β-Hydroxylase	0.63 ± 0.38	0.38 ± 0.23	0	0.80 ± 0.04***
7α-Hydroxylase	2.30 ± 0.68	1.83 ± 0.10	4.20 ± 0.33	1.81 ± 1.10*

Hypox = hypophysectomized.
* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ when compared to animals that had not been hypophysectomized.
† 5α-Reductase (4,5α-dihydrocortisone: NADP+ Δ^4-oxidoreductase, cortisone α-reductase, EC 1.3.1.4); 5β-reductase (4,5β-dihydrocortisone: NADP+ Δ^4-oxidoreductase, cortisone β-reductase, EC 1.3.1.3); 17β-hydroxysteroid reductase [17β-hydroxysteroid: NADP+ 17-oxidoreductase, testosterone 17β-dehydrogenase (NADP+), EC 1.1.1.64].

tradiol benzoate administration resulted in increases in activities of several hydroxylases active on 4-androstene-3,17-dione and 5α-androstane-3α,17β-diol (Table 4). These effects, however, indicate a masculinizing action of estradiol in the rats with an autonomous pituitary, different from the feminizing action of estradiol seen in the intact rats (5, 12).

DISCUSSION

If castrated male and female rats are hypophysectomized, the sexual differences with respect to hepatic enzyme activities disappear. Whereas the metabolic characteristics of the castrated male rats remain essentially unchanged after hypophysectomy, the female rats develop a male type of hepatic metabolism. The feminizing effect of the female pituitary has led us to postulate the existence of a "feminizing factor" that mediates the feminization effect (11). This factor is not likely to be identical to prolactin, lutropin, or follitropin since these hormones do not "feminize" liver metabolism in male rats (17, 18).

The present investigation has demonstrated a significant feminization of the hepatic metabolic pattern in hypophysectomized rats by a pituitary transplant placed under the kidney capsule. The most likely explanation for this finding is that the release of "feminizing factor" from the pituitary of the intact

Table 2. Effect of pituitary transplant on hepatic enzyme activities in castrated, hypophysectomized male rats

Enzyme activity	Castrated + hypox males	Castrated + hypox males with pituitary transplant
Enzymes active on 4-androstene-3,17-dione		
5α-Reductase	17.7 ± 6.50	46 ± 9.0**
5β-Reductase	6.17 ± 0.41	5.19 ± 0.18*
17β-Hydroxysteroid reductase	10.3 ± 1.40	7.15 ± 1.90
3β/5α-Reduced metabolites	0.34 ± 0.05	0.15 ± 0.06**
6β-Hydroxylase	8.63 ± 0.46	2.00 ± 0.63***
7α-Hydroxylase	3.84 ± 0.13	3.12 ± 0.47
16α-Hydroxylase	4.02 ± 0.90	1.03 ± 0.51**
Enzymes active on 5α-androstane-3α,17β-diol		
2α-Hydroxylase	4.09 ± 2.04	0.55 ± 0.29*
2β-Hydroxylase	2.27 ± 0.48	0.54 ± 0.11***
18-Hydroxylase	3.25 ± 0.62	0.21 ± 0.43***
7β-Hydroxylase	1.65 ± 0.17	0***
7α-Hydroxylase	2.97 ± 1.17	2.35 ± 0.27

Hypox = hypophysectomized.
* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ when compared to nontransplanted animals.

Table 3. Effect of treatment with testosterone propionate on hepatic enzyme activities in castrated, hypophysectomized male rats

Enzyme activity	Castrated + hypox males	Castrated + hypox males treated with testosterone propionate
Enzymes active on 4-androstene-3,17-dione		
5α-Reductase	16 ± 3.0	20 ± 4.0
5β-Reductase	6.27 ± 1.14	6.42 ± 0.73
17β-Hydroxysteroid reductase	6.52 ± 2.08	6.16 ± 2.08
3β/5α-Reduced metabolites	0.22 ± 0.06	0.26 ± 0.04
6β-Hydroxylase	5.65 ± 1.48	5.29 ± 1.43
7α-Hydroxylase	1.78 ± 0.24	1.84 ± 0.33
16α-Hydroxylase	3.49 ± 1.48	2.87 ± 1.14
Enzymes active on 5α-androstane-3α,17β-diol		
2α-Hydroxylase	7.05 ± 2.98	2.61 ± 1.42*
2β-Hydroxylase	2.48 ± 2.29	1.13 ± 0.43*
18-Hydroxylase	2.22 ± 0.35	2.49 ± 1.95
7β-Hydroxylase	1.66 ± 1.50	1.08 ± 0.46
7α-Hydroxylase	2.50 ± 1.45	1.09 ± 0.63

Hypox = hypophysectomized.
* = $P < 0.05$ when compared to untreated hypophysectomized animals.

Table 4. Effect of treatment with estradiol benzoate and testosterone propionate on hepatic enzyme activities in castrated, hypophysectomized, transplanted male rats

Enzyme activity	Castrated + hypox + transplanted males	Castrated + hypox + transplanted males treated with estradiol benzoate	Castrated + hypox + transplanted males treated with testosterone propionate
Enzymes active on 4-androstene-3,17-dione			
5α-Reductase	172 ± 29	127 ± 31	115 ± 9.0*
5β-Reductase	1.48 ± 0.04	1.52 ± 0.32	1.68 ± 0.22
17β-Hydroxysteroid reductase	6.59 ± 1.60	5.76 ± 0.24	4.86 ± 1.40
3β/5α-Reduced metabolites	0.017 ± 0.019	0.012 ± 0.013	0.006 ± 0.002
6β-Hydroxylase	2.21 ± 0.50	1.80 ± 0.07	1.54 ± 0.22
7α-Hydroxylase	2.99 ± 0.24	3.59 ± 1.32	2.14 ± 0.20*
16α-Hydroxylase	0	0	0
Enzymes active on 5α-androstane-3α,17β-diol			
2α-Hydroxylase	0.42 ± 0.09	0.96 ± 0.82	0.36 ± 0.14
2β-Hydroxylase	0.22 ± 0.10	0.56 ± 0.76	0.17 ± 0.08
18-Hydroxylase	0.31 ± 0.07	0.89 ± 1.05	0.20 ± 0.05
7β-Hydroxylase	0	0	0
7α-Hydroxylase	3.62 ± 0.55	3.41 ± 0.88	2.72 ± 0.49

Hypox = hypophysectomized.
* = $P < 0.05$ when compared to untreated operated animals.

male rat is inhibited or decreased by an inhibiting factor secreted from the brain, probably the hypothalamus. Liberated from this influence, the transplanted autonomous pituitary starts to secrete "feminizing factor". This contention is further supported by unpublished experiments where feminization of the hepatic metabolism of male rats was observed after an electrothermic lesion in the median eminence, partially destroying the connections between the hypothalamus and the pituitary gland.

It could be speculated that neonatal androgen exposure of male and female rats permanently induces the release of a "feminizing factor inhibiting factor" from the hypothalamus of the adult animal. The feminization of liver metabolism after estrogen administration to adult male and neonatally androgenized female rats could then be explained by an estrogenic effect on the hypothalamus resulting in decreased release of "feminizing factor inhibiting factor" (Fig. 1).

Recently, Posner *et al.* have described a sex-specific pituitary

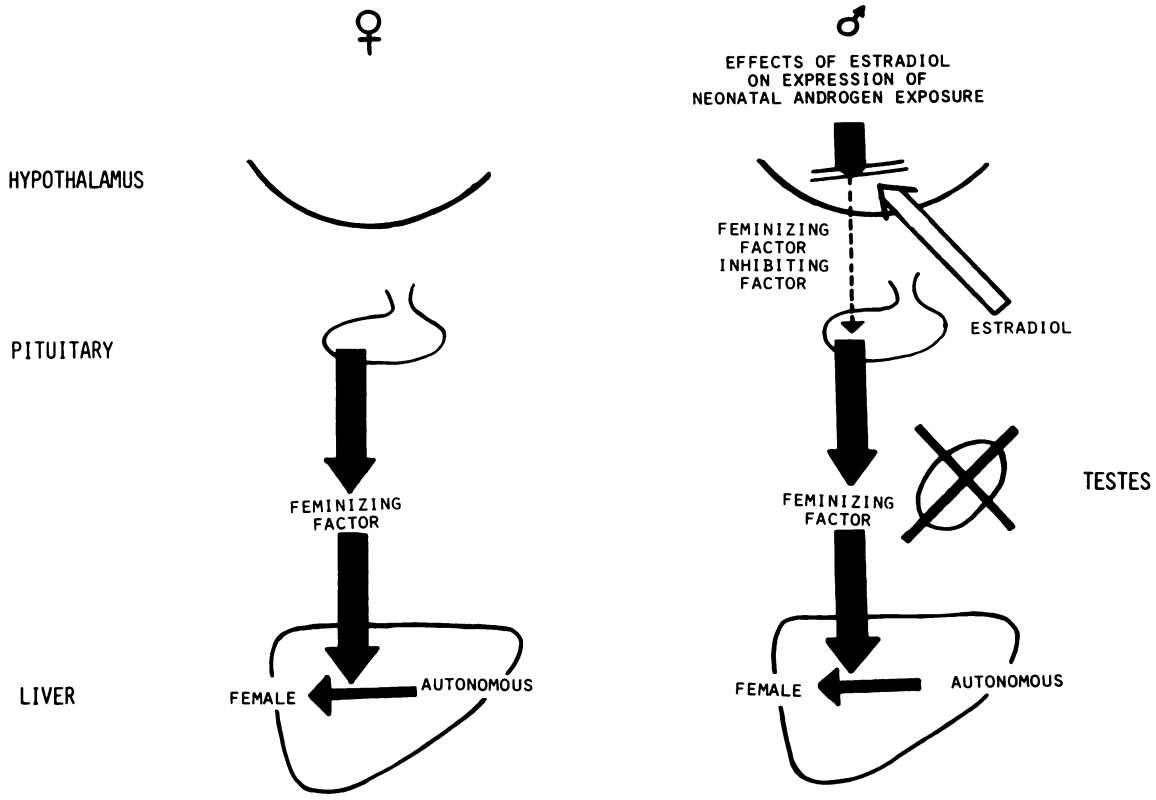

FIG. 1. The figure shows hypothetical schemes for the regulation of male and female types of hepatic steroid metabolism. A hypothetical scheme is also suggested for the effects of estradiol on the neonatally androgen-imprinted hypothalamic center participating in regulation of hepatic steroid metabolism.

factor in female rats responsible for the induction of a lactogenic receptor in liver (19). This pituitary factor appeared in female rats at puberty and could be induced in male animals by estrogen treatment. This type of regulation is identical to that of the "feminizing factor," and the question may be raised if the two pituitary factors are related or in fact identical.

Previous experiments have shown that effects of androgens and estrogens on hepatic metabolism of steroids in rats are observed only in animals with an intact pituitary (11). The lack of effect of estrogens in hypophysectomized animals may be understood as due to absence of production of "feminizing factor" (Fig. 1). Because receptor proteins for estrogens are present in the pituitary (20), the effects of estradiol on liver metabolism could be brought about via direct action of the hormone on the release of "feminizing factor" from the pituitary. The present investigation shows that the secretion of "feminizing factor" from the autonomous pituitary is not significantly or consistently affected by estradiol administration. These results would seem to exclude the possibility of direct estrogenic regulation of hypophyseal release of "feminizing factor" and indicate that estrogenic effects on liver metabolism are mediated via modulation of hypothalamic secretion of "feminizing factor inhibiting factor" (Fig. 1).

Androgenic and estrogenic effects on the liver are not limited to steroid metabolism; metabolism of xenobiotic compounds is also affected by sex hormones (21). The present investigation has demonstrated the obligatory role of a pituitary *in situ* in mediating sex hormonal action on liver metabolism of steroids. Most probably this conclusion can be generalized to encompass also androgenic and estrogenic effects on hepatic metabolism of xenobiotics. It seems reasonable to assume that the existence of pituitary hormones participating in the control of hepatic enzyme activities is not limited to rats. In man, sexual differences with respect to liver metabolism of steroids have recently been described (22), and other sexual differences with respect to hepatic lipid metabolism also occur in man (23). In this connection it is also of interest that Kappas and collaborators recently have indicated the possibility of pituitary regulation of hepatic heme biosynthesis (24, 25). The isolation and characterization of pituitary hormones involved in control of hepatic metabolism may prove to be of great physiological significance.

This work was supported by grants from the Swedish Medical Research Council (03X-2819) and from Harald and Greta Jeanssons Stiftelse.

1. Forchielli, E. & Dorfman, R. I. (1956) *J. Biol. Chem.* **223,** 443–448.
2. Forchielli, E., Brown-Grant, K. & Dorfman, R. I. (1958) *Proc. Soc. Exp. Biol. Med.* **99,** 594–596.
3. Yates, F. E., Herbst, A. L. & Urquhart, J. (1958) *Endocrinology* **63,** 887–902.
4. Leybold, K. & Staudinger, H. (1959) *Biochem. Z.* **331,** 389–395.
5. Einarsson, K., Gustafsson, J.-Å. & Stenberg, Å. (1973) *J. Biol. Chem.* **248,** 4987–4997.
6. Berg, A. & Gustafsson, J.-Å. (1974) *J. Biol. Chem.* **249,** 6559–6567.
7. Carlstedt-Duke, J. & Gustafsson, J.-Å. (1973) *Eur. J. Biochem.* **36,** 172–177.
8. Gustafsson, J.-Å. & Ingelman-Sundberg, M. (1974) *J. Biol. Chem.* **249,** 1940–1945.
9. Gustafsson, J.-Å. (1974) *Biochem. J.* **144,** 225–229.
10. Denef, C. (1974) *Endocrinology* **94,** 1577–1582.
11. Gustafsson, J.-Å. & Stenberg, Å, (1974) *Endocrinology* **95,** 891–896.
12. Gustafsson, J.-Å. & Stenberg, Å. (1974) *J. Biol. Chem.* **249,** 711–718.
13. Gustafsson, J.-Å & Stenberg, Å (1974) *J. Biol. Chem.* **249,** 719–723.
14. DeMoor, P. & Denef, C. (1968) *Endocrinology* **82,** 480–492.
15. Kraulis, I. & Clayton, R. B. (1968) *J. Biol. Chem.* **243,** 3546–3547.
16. Bergström, S. & Gloor, U. (1955) *Acta Chem. Scand.* **9,** 34–38.
17. Gustafsson, J.-Å. & Stenberg, Å. (1975) *Acta Endocrinol.* **78,** 545–553.
18. Gustafsson, J.-Å. & Stenberg, Å. (1975) *Endocrinology* **96,** 501–505.
19. Posner, B. I., Kelly, P. A. & Friesen, H. G. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 2407–2411.
20. Inman, D. R., Banfield, R. E. W. & King, R. J. B. (1965) *J. Endocrinol.* **32,** 17–25.
21. Conney, A. H., Schneidman, K., Jacobsohn, M. & Kuntzman, R. (1965) *Ann. N.Y. Acad. Sci.* **123,** 98–109.
22. Engelhardt, D., Eisenburg, J., Unterburger, P. & Karl, H. J. (1974) *Klin. Wochenschrift* **49,** 439.
23. Bortz, W. M. (1974) *Ann. Int. Med.* **30,** 738–760.
24. Paul, S., Bickers, D. R., Levera, R. D. & Kappas, A. (1974) *FEBS Lett.* **41,** 192–194.
25. Rifkind, A. B., Sassa, S., Merkatz, I. R., Winchester, R., Harber, L. & Kappas, A. (1974) *J. Clin. Invest.* **53,** 1167–1177.