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THE EFFECT OF VARIOUS CENTRALLY-ACTING DRUGS ON HEPATIC STEROID METABOLISM IN MALE AND FEMALE RATS

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Abstract—The effects of various centrally-acting drugs on steroid metabolism in the liver and pituitary hormone concentration in the serum of male and female rats were investigated using well characterized and tested methods. It was seen that picrotoxin, morphine and LSD had a general stimulatory action on hepatic steroid metabolism (morphine only in the female) whereas thiosemicarbazide was without effect. Clonidine gave a move towards a more "female" type of metabolism in the male animals. All drugs tested except thiosemicarbazide had effects on pituitary hormone secretion although not always the effects that have been previously reported. Discrepancies from previous reports are discussed. Correlations between changes in hepatic steroid metabolism and changes in pituitary hormone secretion can be observed but are not consistent. It is concluded that, of the drugs tested, only clonidine may have an effect on "feminizing factor" secretion indicating that this may be controlled in part by the noradrenergic system of the brain.

The control of hepatic steroid metabolism has been well studied with regard to gonadal steroid action [1–8] and effect of pituitary hormones [9–16]. It is still, however, unclear as to which hormone or combination of hormones from the pituitary is responsible for the marked sex differences seen. In a number of publications we have suggested the existence of a "feminizing factor", released from the pituitary of female rats, which maintains the typical "female" pattern of metabolism [12, 15–18]. This factor does not seem to be identical to any of the previously tested pituitary hormones [17].

Recently, certain areas of the brain have been postulated to be involved in the control of "feminizing factor" secretion [19, 20]. The results of the above work indicate the possible involvement of the suprachiasmatic nucleus or surrounding areas.

The brain is made up of a number of independent and overlapping transmitter systems, many of which seem to be involved in the control of pituitary secretion [21, 22] probably via an effect on the neurons producing the release- and release-inhibiting factors. In order to study the various effects of these transmitters, more or less specific agonists and antagonists have been developed. In this study the effects of a number of these drugs on hepatic steroid metabolism have been tested in order to ascertain whether any of the tested transmitter systems are involved in the control of "feminizing factor" secretion. Serum concentrations of pituitary hormones were measured in the same animals to show that the drugs involved actually affected pituitary secretion.

MATERIAL AND METHODS

Sprague–Dawley rats (Anticimex, Stockholm), seven weeks old at the start of the experiment, were used throughout the study. The animals were kept in a light- and temperature-controlled room (lights on 06:00 to 20:00, temperature 23 ± 1°) and given water and food *ad libitum*.

The following drugs were used: picrotoxin (Schuchardt, A. G., West Germany), thiosemicarbazide (Merck, A. G., West Germany), clonidine (Boehringer-Ingelheim A. G., West Germany), morphine (ACO Läkemedel AB, Stockholm, Sweden), phenoxybenzamine (Smith, Kline & French Ltd., Herts., England) and LSD (lysergic acid diethylamide—LSD-25; Sandoz S.A., Basle, Switzerland). All drugs were dissolved in 0.9% (w/v) sodium chloride solution (adjusted to pH 3 with 1 M hydrochloric acid in the case of morphine and phenoxybenzamine) at a concentration to give an injection volume of 100 μl. Picrotoxin was injected i.p. four times a day at a dose of 2.5 mg/kg in the male and 0.6 mg/kg in the female, clonidine i.p. twice daily at 0.2 mg/kg, thiosemicarbazide i.p. at a dose of 2.5 mg/kg, morphine i.m. at 1 mg/kg and LSD i.m. at 100 μg/kg, all once daily. Control animals were injected with vehicle only. Treatment was continued for seven days. On the morning of the eighth day, the animals were killed by decapitation and trunk blood collected. The livers were quickly excised and transferred to ice-cold Bucher medium [23]. After homogenization of the liver in a Potter–Elvehjelm homogenizer, a microsomal fraction was prepared by differential centrifugation as described previously [1, 7, 8].

The microsomal metabolism of 4-[4-^{14}C]-androstene-3,17-dione (New England Nuclear Corp., GmbH, Dreieicheinhain, West Germany; diluted to 0.18 Ci/mole) was investigated as described in previous publications [7, 8].

The collected blood was allowed to clot at +4° and subsequently centrifuged. The resultant serum was used for analysis of pituitary hormones according to the schedules of the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), as previously published [24]. The measurement ranges for the various hormones were as follows: prolactin, 2.5–500 μg/l; FSH, 100–1000 μg/l; LH, 25–1000 μg/l; and soma-

totropin (GH), 2.5–250 μg/l. Interassay and intraassay variation were below 15 per cent and 10 per cent, respectively.

All results were expressed as mean ± S.D. for each group of animals. Statistical analysis was performed using Student's t test and the level of significance set at $P < 0.05$.

RESULTS AND DISCUSSION

Using the results obtained from the incubation of the microsomal fraction with 4-[4-^{14}C]androstene-3,17-dione, the following enzyme activities could be calculated: 5α-reductase, 17-hydroxysteroid oxidoreductase (no attempt was made to separate the 17α- and 17β-isomers of 17-hydroxy-4-androsten-3-one), 16α-, 6β- and 7α-hydroxylases.

In experiment 1 (Table 1) the effect of picrotoxin (a γ-aminobutyric acid antagonist [25]) on the hepatic steroid metabolism and serum hormone levels of male and female rats were tested. Picrotoxin caused an increase in 6β-hydroxylase activity in male animals while causing an increase in 16α- and 7α-hydroxylase activities in female animals (all $P < 0.05$). No other enzyme activities were significantly affected.

In respect to the serum concentration of pituitary hormones picrotoxin caused a marked increase in LH in the male ($P < 0.01$) while decreasing prolactin and LH concentration in the female ($P < 0.05$).

The effect of picrotoxin on steroid metabolism in the male can possibly be explained by the effect on LH secretion. The elevated LH level increases production of testosterone by the testis, thereby increasing the androgen-dependent 6β-hydroxylase activity [7, 8]. In the female, the 16α- and 7α-hydroxylase activities were significantly elevated although the changes were quite small. The decrease in prolactin serum concentrations in female animals was similar to previous reports [26] indicating that the treatment schedule used was effective. The differential effect of picrotoxin on pituitary secretion in male and female rats could indicate a sex-differentiated control of these hormones as suggested by Neill [27]. It would thus appear that picrotoxin was without effect on "feminizing factor" release from the pituitary.

In experiment 2 (Table 2) the effect of thiosemicarbazide (a compound blocking the synthesis of γ-aminobutyric acid [28] and thus acting as a γ-aminobutyric acid antagonist) and clonidine (a selective noradrenergic agonist [29–32]) on hepatic steroid metabolism and serum concentration of pituitary hormones was tested. There were no significant effects of thiosemicarbazide on steroid metabolism or pituitary hormone levels. This should be contrasted to the effect of picrotoxin, a direct γ-aminobutyric acid antagonist. The differences in effect, however, could be explained by the effect of picrotoxin on another brain transmitter, 5-hydroxytryptamine (5-HT) [26], or even by a direct effect of picrotoxin on the liver. Clonidine caused a marked increase of the 5α-reductase ($P < 0.001$) and a decrease of the 6β-hydroxylase activities ($P < 0.05$) in the male while increasing the 7α-hydroxylase activity slightly in the female ($P < 0.05$). All of these changes can be considered as moves towards a more "female"-type of metabolism but it is not a complete "feminization" of hepatic steroid metabolism. In contrast to previous reports [29, 30], clonidine decreased serum prolactin levels in the female. The measurement of clonidine effects acutely and in ovariectomized animals in the previous publications could, however, account for the different results. In the human male, clonidine caused an increase in GH secretion but had no effect on prolactin, LH and FSH [30]. In the male rat, however, clonidine caused a decrease ($P < 0.05$) in the serum concentration of FSH. The results with clonidine would seem to indicate that a noradrenergic nerve system may be involved in part in the control of the factor responsible for "feminization" of hepatic steroid metabolism in the rat. LH, FSH and prolactin serum levels were not increased in these animals, suggesting that none of these hormones are involved. The fact that these hormones are not involved has been postulated previously [12,

Table 1. The effect of a γ-aminobutyric acid antagonist (picrotoxin) on the *in vitro* metabolism of 4-[4-^{14}C] androstene-3,17-dione by liver microsomes from and serum levels of pituitary hormones in male and female rats

Enzymes	Control *♂	Control♀	Treated♂	Treated♀
5α-reductase	3.6 ± 2.8†	21.4 ± 3.8	3.7 ± 0.4	26.7 ± 6.3
17-OH steroid oxidoreductase‡	0.97 ± 0.23	0.47 ± 0.07	1.12 ± 0.15	0.43 ± 0.05
16α-hydroxylase	0.99 ± 0.15	n.d.§	1.31 ± 0.51	0.05 ± 0.03‖
6β-hydroxylase	1.17 ± 0.17	0.32 ± 0.05	2.21 ± 0.41‖	0.35 ± 0.03
7α-hydroxylase	0.54 ± 0.11	0.34 ± 0.04	0.56 ± 0.12	0.43 ± 0.05‖
Hormone				
Prolactin	4 ± 2††	19 ± 10	9 ± 7	<2.5‖
LH	26 ± 7	154 ± 51	67 ± 14¶§	52 ± 17‖
FSH	723 ± 222	451 ± 235	408 ± 241	241 ± 76

* Treated with vehicle only.
† nmol product min^{-1} mg $protein^{-1}$; mean ± 1 S.D. ($n = 4$).
‡ Sum of 17α- and 17β-hydroxysteroid oxidoreductase activity.
§ <0.01 nmol min^{-1} mg $protein^{-1}$.
‖ = $P < 0.05$; ¶ = $P < 0.01$; ** = $P < 0.001$ compared to respective controls.
†† ng ml^{-1}; mean ± 1 S.D. ($n = 4$).

Table 2. The effect of an inhibitor of γ-aminobutyric acid synthesis (thiosemicarbazide) and a noradrenergic agonist (clonidine) on the *in vitro* metabolism of 4-[4-^{14}C] androstene-3,17-dione by liver microsomes from and pituitary hormone serum levels in male and female rats

Enzyme	Control♂	Control♀	Thiosemicarbazide-treated♂	Thiosemicarbazide-treated♀	Clonidine-treated ♂	Clonidine-treated ♀
5α-reductase	0.7 ± 0.1	7.5 ± 1.3	1.0 ± 0.1	9.6 ± 1.4	2.1 ± 0.2**	9.9 ± 1.2
17-OH steroid oxidoreductase	0.52 ± 0.05	0.27 ± 0.04	0.55 ± 0.02	0.38 ± 0.04	0.63 ± 0.03	0.40 ± 0.08
16α-hydroxylase	0.86 ± 0.21	0.09 ± 0.02	0.72 ± 0.08	0.13 ± 0.05	0.64 ± 0.05	0.12 ± 0.02
6β-hydroxylase	0.46 ± 0.09	0.13 ± 0.03	0.36 ± 0.05	0.14 ± 0.03	0.29 ± 0.08‖	0.13 ± 0.02
7α-hydroxylase	0.18 ± 0.03	0.32 ± 0.01	0.22 ± 0.03	0.33 ± 0.04	0.21 ± 0.04	0.40 ± 0.02‖
Hormone						
Prolactin	10 ± 4	4 ± 1	4 ± 2	7 ± 5	6 ± 4	<2.5‖
LH	43 ± 14	62 ± 21	54 ± 18	67 ± 15	53 ± 14	42 ± 9
FSH	347 ± 64	<100	264 ± 105	138 ± 43	151 ± 61‖	<100

For further explanations see Table 1.

17, 18, 33], although a number of reports have indicated effects of these hormones [10, 11, 34].

In experiment 3 (Table 3), morphine and D-lysergic acid diethylamide (LSD-25) were tested for their effect on hepatic steroid metabolism and serum concentration of pituitary hormones. Morphine, a drug which binds to enkephalin receptors in the brain [35–37], had no effect on hepatic steroid metabolism in male animals whereas it had a general stimulatory effect in the female, increasing the activity of all the enzymes studied. The effect of morphine on serum pituitary hormones was similar in male and female animals—a decrease in prolactin (significant [$P < 0.05$] for females). This sex dissociation of the effect on hepatic metabolism and pituitary hormone secretion would indicate that none of the tested pituitary hormones are involved in the changes seen in steroid metabolism. Morphine has been reported to have a direct effect on the liver, increasing the cytochrome P450 and cytochrome *c*-reductase content of mouse liver microsomes [38]. This could explain the effect of morphine seen in this experiment. The effects of morphine on pituitary hormone-secretion are not consistent with previous findings [39–41] but this difference could be due to different treatment schedules. LSD, which can act as a 5-HT agonist or antagonist in various situations [26, 30], had a general stimulatory effect on hepatic steroid metabolism in both male and female animals. The effect was greater than that of morphine in the female, LSD had a similar effect to morphine on prolactin secretion in both sexes. FSH serum concentrations were reduced in both sexes by the action of LSD whereas there was an elevation in the serum level of GH. It is possible that the changes in serum concentration of prolactin, GH and/or FSH are involved in the stimulation of hepatic steroid-metabolizing activity. This is unlikely, however, owing to the results obtained with morphine, where different effect on hepatic metabolism was observed with similar changes in GH, FSH and prolactin serum concentrations. Neither morphine nor LSD gave any move towards a "female"-type metabolism indicating the lack of involvement of enkephalins and 5-HT in the control of "feminizing factor".

As is evident from Tables 1–3, the control animals of the same sex differed from experiment to experiment with regard to certain enzyme activities and hormone

Table 3. The effect of LSD-25 and morphine on the *in vitro* metabolism of 4-[4-^{14}C]androstene-3,17-dione by liver microsomes from and serum concentrations of pituitary hormones in male and female rats

Enzyme	Control ♂	Control ♀	LSD-treated ♂	LSD-treated ♀	Morphine-treated ♂	Morphine-treated ♀
5α-reductase	1.2 ± 0.4	4.7 ± 0.7	3.4 ± 0.8¶	11.2 ± 0.1**	1.7 ± 0.9	7.5 ± 1.6‖
17-OH steroid oxidoreductase	0.75 ± 0.22	0.27 ± 0.04	1.08 ± 0.11‖	0.52 ± 0.06**	0.77 ± 0.19	0.43 ± 0.08‖
16α-hydroxylase	1.40 ± 0.52	0.11 ± 0.02	1.50 ± 0.17	0.22 ± 0.04¶	1.02 ± 0.34	0.20 ± 0.04¶
6β-hydroxylase	0.65 ± 0.21	0.12 ± 0.02	0.86 ± 0.14	0.26 ± 0.08¶	0.71 ± 0.22	0.19 ± 0.02¶
7α-hydroxylase	0.37 ± 0.12	0.31 ± 0.02	0.63 ± 0.03¶	0.53 ± 0.08¶	0.43 ± 0.14	0.48 ± 0.07¶
Hormone						
Prolactin	69 ± 17	58 ± 10	28 ± 15‖	11 ± 7¶	32 ± 14	18 ± 14‖
GH	206 ± 65	35 ± 16	>250	232 ± 35**	224 ± 52	40 ± 40
LH	29 ± 5	<25	27 ± 4	<25	<25	27 ± 4
FSH	688 ± 202	442 ± 64	312 ± 87‖	304 ± 63‖	642 ± 194	561 ± 391

For further explanations see Table 1.

levels. This phenomenon probably reflects variations with respect to the several factors (dietary constituents, estrus cycle, diurnal rhythm, stress, intestinal microflora) known to influence liver enzymes [42] and pituitary hormone levels in blood.

In conclusion, of the drugs tested, only clonidine caused any degree of "feminization" of hepatic steroid metabolism, whereas the other drugs had no effect or only a general stimulatory effect. This would suggest that, of the systems studied, only the α-adrenergic system may be involved in the control of the factor responsible for "feminization" of hepatic steroid metabolism.

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