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CHANGES IN THE ACTIVITIES OF MICROSOMAL ENZYMES INVOLVED IN HEPATIC STEROID METABOLISM IN THE RAT AFTER ADMINISTRATION OF ANDROGENIC, ESTROGENIC, PROGESTATIONAL, ANABOLIC AND CATATOXIC STEROIDS

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Abstract—Several steroids (5β-dihydrotestosterone, 19-nortestosterone, methyltrienolone, norethisterone, medroxyprogesterone acetate, cyproterone acetate, chlormadinone acetate and 16α-cyanopregnenolone) were tested for their ability to influence the activities of three sexually differentiated hepatic microsomal enzymes (3α- and 3β-hydroxysteroid dehydrogenase and 5α-reductase) in male and female gonadectomized and intact female rats. Of the steroids tested only 5β-dihydrotestosterone was completely ineffective. The other tested steroids elicited varying degrees of "masculinization" with a distinct gradation of effect according to the enzyme activity measured and animal model used. 5α-Reductase was the most sensitive enzyme activity and 3α-hydroxysteroid dehydrogenase the least. Male castrates responded better than female castrates, and these in turn better than intact females. The mechanism of action of three of the steriods (methyltrienolone, medroxyprogesterone acetate and norethisterone) was examined. Both flutamide and estradiol were able to block the action of methyltrienolone and medroxyprogesterone acetate, but not that of norethisterone. It is concluded that methyltrienolone and medroxyprogesterone acetate probably masculinize the enzyme activities by the same mechanisms as androgens, whereas the repression of 5α-reductase activity elicited by norethisterone administration involves a different route.

The liver is the organ principally responsible for the metabolism of drugs and steroids in mammals. The enzymes involved in these metabolic pathways show an interesting biochemical feature, namely that many of their activities show well-defined sex differences. Endocrine gland ablation and substitution treatment has shown that most of these sex differences *in animals with intact pituitaries* are due to the presence of androgens, either during the perinatal period and/or after the onset of puberty [1, 2], whereas a few enzyme activities are obviously regulated by estrogens [3]. However, the development and maintenance of androgen-dependent activity levels in male rats can be modified by estrogens in a number of ways, one of which is their ability to antagonize androgen action in postpubertal animals [4].

Androgens and estrogens are not the only types of steroid that can influence these androgen-dependent enzyme activities in hypophysis-intact rats. Other hormonally active steroids such as progestins [5–7], antiandrogens [8, 9], and anabolic steroids [10], as well as hormonally inactive steroids like 16α-cyanopregnenolone [11, 12] and methylcholanthrene [12, 13] affect the activities of these enzymes in manners which may be described as "masculinizing" or "feminizing" depending on whether the action mimics that of androgens or estrogens, respectively.

In the present investigation we have examined a number of steroids (5β-dihydrotestosterone, 19-nortestosterone, methyltrienolone, norethisterone, medroxyprogesterone acetate, cyproterone acetate, chlormadinone acetate and 16α-cyanopregnenolone) for their ability to mimic the action of 5α-dihydrotestosterone or estradiol on three androgen-dependent enzyme activities of hepatic steroid metabolism, namely microsomal 3α- and 3β-hydroxysteroid dehydrogenase (3α- and 3β-HSDH) and 5α-reductase.* In order to gain insight into the mechanism of action of some of these steroids we compared the effects of two hepatic androgen antagonists (flutamide and estradiol) on these activities in 5α-dihydrotestosterone-treated rats [4] with those in methyltrienolone-, medroxyprogesterone acetate- and norethisterone-treated animals. In some of these experiments we also studied the behaviour of a typical drug metabolizing enzyme, aminopyrine-*N*-demethylase.

MATERIALS AND METHODS

Chemicals. [1α, 2α(*n*)-^{3}H]5α-Androstane-3α,17β-diol was obtained from Amersham Buchler (Braunschweig, F.R.G.), [4-^{14}C]5α-dihydrotestosterone from the Commissariat à L'Énergie Atomique

* 3α-Hydroxysteroid dehydrogenase (3α-HSDH) = 3α-hydroxysteroid:NADP$^+$ oxidoreductase (EC 1.1.1.50); 3β-hydroxysteroid dehydrogenase (3β-HSDH) = 3 (or 17) β-hydroxysteroid:NADP$^+$ oxidoreductase (EC 1.1.1.51); 5α-reductase = 4,5α-dihydrocortisone:NADP$^+$ Δ^4-oxidoreductase (EC 1.3.1.4).

(Fa. Zinsser, Frankfurt, F.R.G.) and [dimethyl-^{14}C] aminopyrine from New England Nuclear (Dreieich, F.R.G.). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADH and NADP were purchased from Boehringer Mannheim GmbH (Mannheim, F.R.G.), bovine serum albumin from Behringwerke AG (Marburg, F.R.G.), medroxyprogesterone acetate and 19-nortestosterone from Sigma Chemie GmbH (München, F.R.G.), Unisolve I from Zinsser (Frankfurt, F.R.G.), and aminopyrine from EGA Chemie (Steinheim, F.R.G.). Cyproterone acetate, chlormadinone acetate and norethisterone were generously provided by Prof. Dr. F. Neumann (Schering AG, Berlin, F.R.G.). We would also like to thank Dr. R. Neri (Schering Corporation, Bloomfield, U.S.A.) for kindly supplying us with flutamide, Dr. J. P. Raynaud (Roussel UCLAF, Romainville, France) for the methyltrienolone, and finally Dr. J. C. Babcock (Upjohn Company, Kalamazoo, U.S.A.) for the 16α-cyanopregnenolone. All other chemicals, biochemicals and hormones were obtained from Merck AG (Darmstadt, F.R.G.) and were of the highest analytical grade obtainable.

Animals. Male and female SPRD (syn: Sprague–Dawley) rats were obtained from the Zentralinstitut für Versuchstierkunde (Hannover, F.R.G.). Animals were maintained in a 12 hr light–dark cycle (07.00–19.00 hr) at constant relative humidity (55 ± 5%) and temperature (21 ± 2°C). The rats were fed Altromin 1320 and water *ad libitum*. Gonadectomy (orchiectomy–scrotal route; ovariectomy–abdominal route) was performed under ether narcosis on day 25 of life.

In the first series of experiments 75 day-old rats received 15 daily s.c. injections of either the appropriate volume of vehicle alone or containing 0.5 mg 5β-dihydrotestosterone, 0.5 mg 19-nortestosterone, 0.5 mg methyltrienolone, 5 mg norethisterone, 5 mg medroxyprogesterone acetate, 5 mg chlormadinone acetate, 5 mg cyproterone acetate or 5 mg 16α-cyanopregnenolone. Experiments were performed in parallel on three animal models: male castrates, female castrates and intact females. On the day before starting the administration schedule the animals were divided into groups of six and weighed. For some experiments more than one control group was used. 5β-Dihydrotestosterone, 19-nortestosterone and methyltrienolone were administered at the appropriate dosage in 0.1 ml propandiol, whereas norethisterone, medroxyprogesterone acetate, chlormadinone acetate, cyproterone acetate and 16α-cyanopregnenolone were administered in 0.25 ml sesame oil–benzoyl benzoate (4:1, v/v).

In the second series of experiments rats receiving either 0.5 mg methyltrienolone, 5 mg medroxyprogesterone acetate or 5 mg norethisterone were treated simultaneously s.c. with either 5 mg flutamide (in 0.25 ml sesame oil–benzoyl benzoate, 4:1, v/v), 5 μg estradiol (in 0.1 ml propandiol) or the appropriate vehicle. Animals treated only with vehicle were also included for comparison. All other details were as for the first series.

For some animal groups of the first two series aminopyrine-*N*-demethylase activity was determined. In order to provide reference values for purposes of comparison some 5α-dihydrotestosterone-treated rats (0.5 mg 5α-dihydrotestosterone in 0.1 ml propandiol s.c./day/rat/15 days) were included in this study.

On the morning following the last injection rats were stunned by a blow on the head and decapitated. The liver was removed and placed in ice-cold 0.25 mol/l sucrose until it could be weighed and processed further. Seminal vesicles, levator ani and uteri were also taken and their weights used as a measure of the biological efficacy of the treatments.

Determination of enzyme activities. The preparation of the microsomal fractions and the determination of 3α-HSDH, 3β-HSDH and 5α-reductase activity have been described previously [4]. 3α-HSDH activity was determined as the rate of production of [4-^{14}C]5α-androstane-3α,17β-diol from [4-^{14}C]5α-dihydrotestosterone with NADH as coenzyme. 3β-HSDH activity was measured as the conversion rate of 4-androstene-3β,17β-diol to 3-oxo-4-en-steroid products in the presence of NADP. The disappearance of testosterone in the presence of an NADPH-generating system was taken as a measure of 5α-reductase activity. For the latter two enzyme tests 3-oxo-4-en-steroids were quantitated with isonicotinic acid hydrazide.

Aminopyrine-*N*-demethylase activity was determined according to a slight modification of the method described by Poland and Nebert [14]. Radioactive substrate was purified in the following manner immediately before use. 3 ml [dimethyl-^{14}C]aminopyrine stock solution (2.5 μCi/ml ethanol) was evaporated and the residues redissolved in 6 ml chloroform, which was then extracted twice with water. The organic phase was evaporated and the residues taken up in 3 ml methanol containing 30 mg unlabelled aminopyrine. Finally, the organic solvent was removed under vaccuum and the aminopyrine dissolved in 3 ml water. An aliquot (0.2 ml) of the microsomal fraction (containing the equivalent of 200 mg liver/ml 0.25 mol/l sucrose) was mixed with 0.28 ml 0.1 mol/l citrate buffer, pH 6.0, containing 0.5 μmol NADP, 15.9 μmol glucose-6-phosphate, 2.64 μmol magnesium chloride, 5.27 μmol semicarbazide, 5.27 μmol nicotinamide and 4.7 μg glucose-6-phosphate dehydrogenase, equivalent to 0.66 U. After 5 min preincubation at 37°C the reaction was started by addition of 2.16 μmol [dimethyl-^{14}C]aminopyrine (125 nCi) in 0.05 ml water. Following 30 min incubation at 37°C the reaction was stopped by extraction with 8 ml chloroform and 1 ml 0.1 mol/l sodium hydroxide. 1 ml aqueous phase was transferred to another tube and extracted a second time with a further 8 ml chloroform. Finally 0.5 ml aqueous phase was pipetted into a counting vial, mixed with 0.1 ml 1 mol/l hydrochloric acid and taken up in 10 ml Unisolve I.

All enzyme determinations were performed in triplicate. Blank values were obtained by stopping the reaction immediately after addition of substrate. Protein concentrations were determined according to Lowry *et al.* [15].

Statistical analysis. Results are expressed as mean ± standard deviation. Statistically significant differences ($P < 0.01$ unless otherwise stated) were determined by Duncan's Multiple Range Test.

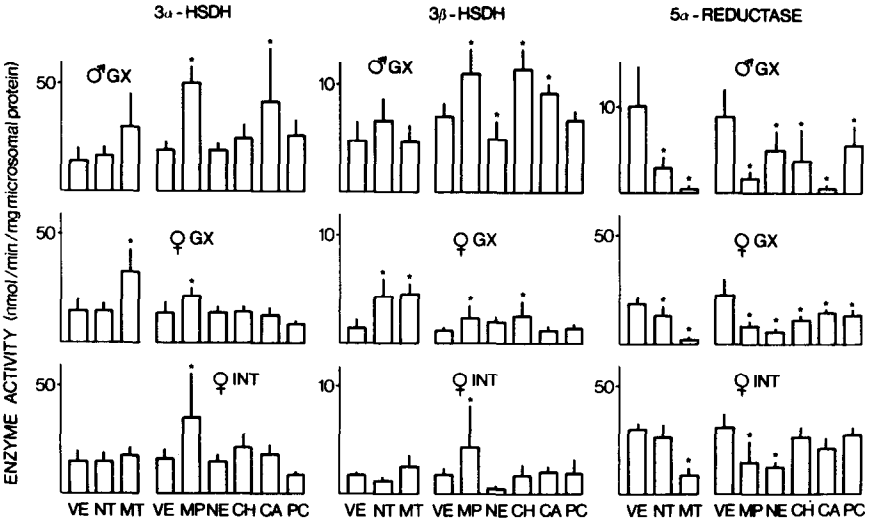

Fig. 1. Effects of various steroids on the activities of microsomal 3α-HSDH, 3β-HSDH and 5α-reductase in the livers of male and female gonadectomized and intact female rats. Details of dosage are given in the text. Abbreviations: GX, gonadectomized; INT, intact; VE, vehicle; NT, 19-nortestosterone; MT, methyltrienolone; MP, medroxyprogesterone acetate; NE, norethisterone; CH, chlormadinone acetate; CA, cyproterone acetate; PC, 16α-cyanopregnenolone. Significant differences ($P < 0.01$; $N \geq 6$) from vehicle-treated rats are denoted by *.

RESULTS

Effect of administration of 5β-dihydrotestosterone, methyltrienolone, 19-nortestosterone, medroxyprogesterone acetate, chlormadinone acetate, cyproterone acetate, norethisterone and 16α-cyanopregnenolone on enzyme activities. A very distinct gradation of effects could be observed both in terms of the individual enzymes and the endocrine status of the animals. In male castrates 5α-reductase activity reacted to the administration of all administered steroids, whereas 3β-HSDH activity reacted only to four and 3α-HSDH activity to three (Fig. 1). A gradation according to endocrine status was also observed with any particular enzyme activity reacting more sensitively to steroid administration in male castrates than in female castrates, and these in turn more sensitively than in intact females. All observed changes in 5α-reductase activity were due to enzyme activity repression, whereas, with one exception, those on 3α- and 3β-HSDH were due to enzyme activity induction. The single exception was the repressive action of norethisterone on 3β-HSDH activity in male castrates. On the basis of all three enzyme activities and all three animal models, medroxyprogesterone acetate and methyltrienolone proved to be the most potent effectors. The response to norethisterone administration was of interest since it was a repressor of 5α-reductase activity (i.e. it elicited an androgen-like response leading to a masculine type of activity level), whereas its effect on 3β-HSDH activity, also repressive, represented a

Table 1. Effect of 5α-dihydrotestosterone, methyltrienolone, medroxyprogesterone acetate and norethisterone administration alone or in combination with flutamide or estradiol on the activity of aminopyrine-*N*-demethylase in intact female and castrate male rats

	Male castrates				Intact females			
	VEH	FLU	E		VEH	FLU	E	
VEH	213 ± 57			VEH	286 ± 23			
DHT	493 ± 119*	264 ± 32†	212 ± 21†	DHT	527 ± 84*	398 ± 61†		
MT	439 ± 161*	204 ± 17†	236 ± 27†	MT	405 ± 57*	294 ± 19†	296 ± 43†	
MPA	356 ± 25*	185 ± 23†	191 ± 25†	MPA	291 ± 27			
NE	247 ± 37			NE	322 ± 47			
	(pmol/min/mg microsomal protein)				(pmol/min/mg microsomal protein)			

Abbreviations: VEH, vehicle; DHT, 5α-dihydrotestosterone; MT, methyltrienolone; MPA, medroxyprogesterone acetate; NE, norethisterone; FLU, flutamide; E, estradiol. Significant differences ($P < 0.01$; $N = 6$) between rats treated with vehicle alone and those treated with 5α-dihydrotestosterone, methyltrienolone, medroxyprogesterone acetate or norethisterone alone are denoted by *, between animals treated with 5α-dihydrotestosterone, methyltrienolone or medroxyprogesterone acetate and those treated with the respective steroids plus flutamide or estradiol are denoted by †.

Table 2. Effect of 19-nortestosterone, methyltrienolone, medroxyprogesterone acetate, norethisterone, chlormadinone acetate, cyproterone acetate and 16α-cyanopregnenolone on seminal vesicle and levator ani weight of orchidectomized males and on uterus weight of ovariectomized females

Animal	Organ	VEH	NT	MT			
mGX	SV	4.66 ± 1.31	8.33 ± 2.20	47.1 ± 8.01*			
mGX	LA	36.8 ± 6.43	75.3 ± 13.9*	77.4 ± 6.51*			
fGX	UT	16.5 ± 2.54	40.6 ± 4.68*	98.3 ± 19.9*			
		VEH	MPA	NE	CHL	CA	PCN
mGX	SV	6.28 ± 1.41	37.5 ± 2.55*	67.5 ± 12.6*	8.76 ± 1.39	7.35 ± 0.53	6.76 ± 1.10
mGX	LA	37.5 ± 5.34	67.5 ± 12.2*	89.1 ± 12.3*	39.3 ± 8.32	32.5 ± 3.84	31.6 ± 3.93
fGX	UT	16.7 ± 3.47	29.2 ± 2.50*	91.9 ± 19.3*	20.9 ± 3.46	20.1 ± 1.60	15.1 ± 0.59
				(mg/100 g body weight)			

Abbreviations: mGX, male castrates; fGX, female castrates; SV, seminal vesicles; LA, levator ani; UT, uterus; NT, 19-nortestosterone; CHL, chlormadinone acetate; CA, cyproterone acetate; PCN, 16α-cyanopregnenolone; for other abbreviations, see legend of Table 1. Significant differences ($P < 0.01$; $N = 6$) from vehicle-treated rats are denoted by *.

change towards a female type activity level. The direction of action of norethisterone on this latter enzyme was directly opposed to that of medroxyprogesterone acetate and methyltrienolone.

The gradation of the response to steroid administration according to endocrine status was also seen in the activity of aminopyrine-*N*-demethylase (Table 1). In male castrates the activity reacted to 5α-dihydrotestosterone, methyltrienolone and medroxyprogesterone acetate treatment, whereas the latter was no longer effective in females.

The biological efficacy of the various treatments was determined by comparing the weights of the seminal vesicles and levator ani of males and uteri of females with those of vehicle-treated rats (Table 2). Methyltrienolone, medroxyprogesterone acetate and norethisterone treatment all increased the weights of the seminal vesicles significantly; these three steroids and 19-nortestosterone also increased the weight of the levator ani in male castrates, and uterus weight in female castrates.

Treatment of animals with 0.5 mg 5β-dihydrotestosterone affected neither enzyme activities nor organ weights in any of the three animal models and the data has not been included.

Slight, but significant increases in microsomal protein concentration occurred after administration of 16α-cyanopregnenolone or cyproterone acetate to male castrates and after cyproterone acetate to ovariectomized females.

Prevention of methyltrienolone, medroxyprogesterone acetate or norethisterone action by either fluta-

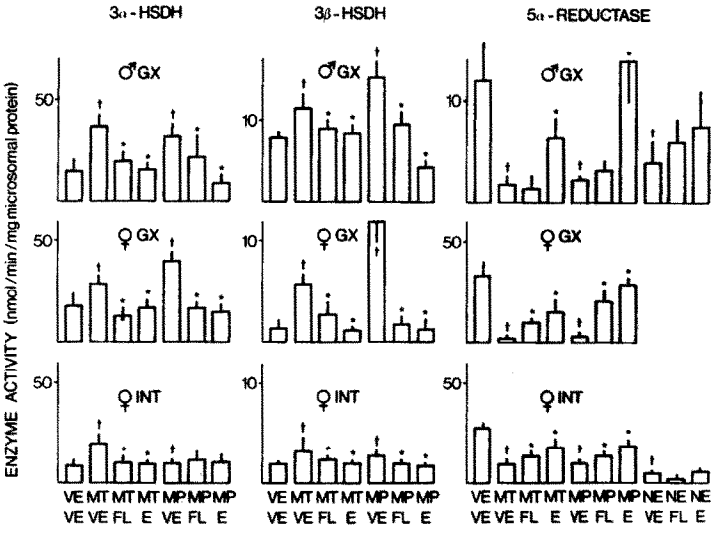

Fig. 2. Effect of administration of flutamide or estradiol on the activities of microsomal 3α-HSDH, 3β-HSDH and 5α-reductase in the livers of male and female gonadectomized and female intact rats receiving either methyltrienolone, medroxyprogesterone acetate or norethisterone. Details of dosage are given in text. Abbreviations: FL, flutamide; E, estradiol; other abbreviations as in legend of Fig. 1. Significant difference ($P < 0.01$; $N = 6$) between data from rats treated with the respective steroid alone (denoted by †) and flutamide- or estradiol-treated rats are denoted by *.

Table 3. Effect of flutamide and estradiol administration on the seminal vesicle, levator ani and uterus weight of methyltrienolone-, medroxyprogesterone acetate- and norethisterone-treated rats

	VEH	FLU	E
Male castrates—seminal vesicle weight			
MT	37.5 ± 5.51	10.3 ± 0.95*	60.3 ± 10.3*
MPA	26.3 ± 2.58	6.65 ± 0.40*	40.2 ± 6.58*
NE	65.1 ± 8.44	19.2 ± 1.31*	51.4 ± 6.11
VEH	3.86 ± 0.57		
Male castrates—levator ani weight			
MT	62.5 ± 13.0	53.5 ± 6.42	72.9 ± 8.74
MPA	49.5 ± 8.38	25.3 ± 4.32*	58.1 ± 8.50
NE	63.2 ± 9.94	32.4 ± 5.29*	71.7 ± 9.32
VEH	26.3 ± 6.31		
Female castrates—uterus weight			
MT	86.1 ± 10.2	74.3 ± 20.2	84.9 ± 8.80
MPA	49.0 ± 13.6	21.3 ± 5.22*	88.3 ± 11.2*
VEH	10.8 ± 1.57		

(mg/100 g body weight)

Abbreviations are as in legends of Tables 1 and 2. Significant differences ($P < 0.01$; $N = 6$) from rats treated with the respective steroids alone are denoted by *.

mide or estradiol. The effects of administration of estradiol or flutamide alone on the three steroid-metabolizing enzyme activities investigated has been reported previously, as has the influence of simultaneous administration of these androgen antagonists with 5α-dihydrotestosterone [4]. Flutamide has no detectable influence on the activities, whereas estradiol causes complete feminization of 3β-HSDH and 5α-reductase in gonadectomized male rats and of the latter enzyme in ovariectomized animals as well. Both flutamide and estradiol counteracted the androgenic effects of 5α-dihydrotestosterone on all enzyme activities in all animal models except for 3β-HSDH in male castrates.

The inductive influence of methyltrienolone and medroxyprogesterone acetate on 3α- and 3β-HSDH activity, and the repressive influence of these steroids on 5α-reductase activity was abolished by simultaneous administration of estradiol (Fig. 2); with the exception of 5α-reductase activity in male castrates the effects of methyltrienolone and medroxyprogesterone acetate were also reversed by flutamide administration. In contrast the influence of norethisterone on 5α-reductase activity was not blocked either by flutamide or estradiol regardless of endocrine status.

The activity of aminopyrine-*N*-demethylase reacted in a similar manner to those of 3α- and 3β-HSDH with both flutamide and estradiol blocking the effects of methyltrienolone and medroxyprogesterone acetate as well as of 5α-dihydrotestosterone (Table 1).

The dose of flutamide administered was sufficient to block the effects of methyltrienolone, medroxyprogesterone acetate and norethisterone on the seminal vesicles (Table 3). It also abolished the effects of the latter two steroids on the levator ani. However, as expected, estradiol had no antagonizing effect on the levator ani and exerted a trophic influence on the seminal vesicles and, in the case of medroxyprogesterone acetate-treated female castrates, on the uterus as well. Flutamide also prevented the trophic action of medroxyprogesterone on the uterus, but not that of methyltrienolone.

DISCUSSION

In untreated intact rats the activities of 3α- and 3β-HSDH are higher than in the female animal, whereas the situation for 5α-reductase is the reverse. These sex differences are almost exclusively due to the imprinting of 3β-HSDH and 5α-reductase levels by androgens in neonatal life and reversible induction (3α-HSDH and 3β-HSDH) or repression (5α-reductase) of the activities in adult life [16]. In many respects these liver enzyme activities react to the loss (after castration) and replacement (exogenous administration) of androgens in the same manner as the growth of an accessory sex organ such as the seminal vesicle or prostate gland. However, there are some important differences—for instance, androgenic effects on these activities can only be demonstrated in animals with intact pituitaries [17–19], the effects of exogenously administered androgen cannot be prevented by the antiandrogen, cyproterone acetate [9], but are extremely efficiently blocked by administration of small amounts of estradiol [4]. Moreover, as explained in the introduction, androgens and estrogens are by no means the only steroids which can affect these activities. In the course of our own previous studies we have demonstrated that the anabolic steroid, 19-nortestosterone, and the antiandrogenic progestin, cyproterone acetate, have androgenic effects on two of these enzyme activities [9, 10] and similar effects have been demonstrated for 5α-reductase activity after administration of the catatoxic steroid, 16α-cyanopregnenolone [11], and norethisterone [7], a progestin with androgenic and estrogenic properties. On the other hand, the administration of the progestin, medroxyprogesterone acetate, which has some androgenic character, is reported to cause induction of 5α-reductase activity [5, 6]; in terms of the physiological sex differences present in this enzyme activity, this effect must be seen as being antiandrogenic or estrogenic.

The studies mentioned above were not performed with homogenous animal material; different strains of rat were used, as well as different ages and sexes. Moreover, no attempt has seriously been undertaken to determine whether the mechanisms involved in the reported activity changes were related to those seen after administration of natural androgens or estrogens. In this study we set out to do this by testing whether the nonsteroidal antiandrogen, flutamide, as well as estradiol, which both block the action of 5α-dihydrotestosterone [4], also prevented the androgen-like effects of other steroids.

In a first series of experiments we screened a number of steroids for their ability to mimic the action of 5α-dihydrotestosterone or estradiol on the three enzyme activities (Fig. 1). Apart from the steroids mentioned above, methyltrienolone (an androgen that binds to progestin receptors [20]), chlormadinone acetate (a progestin with antiandrogenic properties) and 5β-dihydrotestosterone (5β-C_{19}-

steroids are non-androgenic, but stimulate hepatic porphyrin synthesis [21]) were included in this study. The results confirmed our previous finding that 5α-reductase activity reacts more sensitively to exogenous steroid treatment than does that of 3β-HSDH [9]; 3α-HSDH activity is even less sensitive. We were also able to confirm that, for any particular enzyme, the activity in the male castrates reacts more sensitively than that in female castrates which, in turn, reacts more sensitively than that in intact females [4, 9]. Not only those substances which were manifestly androgenic in terms of their effects on the seminal vesicles (methyltrienolone, medroxyprogesterone acetate, norethisterone) caused changes in the enzyme activities. Chlormadinone acetate and cyproterone acetate elicited an androgen-like induction of 3α- and 3β-HSDH as well as repressing 5α-reductase activity; 16α-cyanopregnenolone also had the latter effect. The action of norethisterone deserves special mention because its action on 5α-reductase activity, namely repression, can be considered to be a masculinization or androgen-like effect, whereas its action on 3β-HSDH activity, also repression, represents an estrogen-like feminization of the activity. Of the steroids tested medroxyprogesterone acetate and methyltrienolone had the most prominent androgen-like action and were therefore chosen for further study. Norethisterone whose androgen-like action was limited to 5α-reductase was also investigated.

The fact that flutamide and estradiol block the action of medroxyprogesterone acetate and methyltrienolone on all three enzyme activities in the same manner as they block the action of 5α-dihydrotestosterone [4] is a clear indication that the mechanism of action of all three steroids is the same. In contrast the mechanisms involved in the repression of 5α-reductase activity after norethisterone administration must be different, since neither estradiol nor flutamide can block this effect.

The repression of 5α-reductase activity by norethisterone is probably what we would describe as a "high estrogen dose" effect for it has been shown that the dose response of this enzyme activity to estrogens is biphasic [16], induction occurring at low doses and repression at high doses. The high estrogen dose effect is probably mediated by hepatic estrogen receptors since the lowest doses which can cause repression of the activity [16] correlate well with those necessary to demonstrate *in vivo* translocation of the receptors [22] and exceed those occurring physiologically. This explains why the repressive action of norethisterone is prevented neither by simultaneous estradiol nor flutamide administration. The former only enhances the repression; the latter has no effect because flutamide has no affinity for the estrogen receptor [22]. However, it is not clear whether the high estrogen dose effect of norethisterone is due to direct binding of this steroid to the receptors [23], or whether it binds only after being metabolized to ethynylestradiol [24], which then binds with higher affinity than norethisterone itself. The hypothesis that norethisterone acts as an estrogen would also explain its action on 3β-HSDH activity since estrogens repress this parameter at all effective dosages [16].

The mechanisms by which medroxyprogesterone acetate and methyltrienolone cause their androgen-like "masculinization" of the enzyme activities are complex. From the results it may be inferred that it is the androgen component of these steroids rather than the progestin that is responsible for the changes seen. As outlined elsewhere [16] androgens probably act on the liver indirectly by causing suppression of "feminizing factors" from the pituitary. This androgenic suppression can be alleviated by low estrogen doses, an effect which in turn can be mimicked by administration of human growth hormone [16, 25, 26].

The fact that aminopyrine-*N*-demethylase activity reacts to steroid administration and flutamide and estradiol antagonism in the same manner as the other enzyme activities examined reinforces the widely held view that hepatic drug and steroid metabolizing enzyme activities are regulated in similar manners [26].

These findings do not, however, preclude the possibility that some steroids influence enzyme activities of hepatic steroid metabolism in entirely different manners to those mentioned above. Both chlormadinone acetate and cyproterone acetate, progestins with antiandrogenic components, cause some androgen-like masculinization of enzyme activities without giving any indication of androgenicity or estrogenicity in terms of the investigated sexual organ weights. Progestin receptors have recently been detected in the liver and it is possible that these proteins are involved in a separate progestin response [27, 28]. This might explain why much higher doses of medroxyprogesterone acetate can induce 5α-reductase activity [5, 6], whereas we could only demonstrate an androgen-like repression.

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