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STUDIES ON THE REGULATION OF THE ACTIVITY OF DRUG OXIDATION IN RAT LIVER MICROSOMES BY ANDROGEN AND ESTROGEN

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Abstract—Castration of male rats decreases the activities of aminopyrine *N*-demethylation and hexobarbital hydroxylation, whereas it increases the apparent K_m values. The administration of testosterone or methyltestosterone to castrated male rats increases the hydroxylating activities of the drugs, whereas it decreases the apparent K_m values. The simultaneous injection of estradiol or diethylstilbestrol counteracts the actions of the androgens. Castration of male rats results in a decrease in the magnitude of spectral changes of P-450, which indicate substrate binding with P-450, induced by aminopyrine and hexobarbital and increases the apparent spectral dissociation constant (K_s) values for aminopyrine and hexobarbital. The administration of testosterone or methyltestosterone to castrated male rats increases the magnitude of substrate-induced spectral changes and decreases the apparent K_s values. The simultaneous injection of estradiol or diethylstilbestrol counteracts the actions of the androgens.

These alterations of the apparent K_s values for aminopyrine and hexobarbital-induced spectral changes are parallel to those of the apparent K_m values for the aminopyrine *N*-demethylation and hexobarbital hydroxylation. These results suggest that androgens increase the affinity of P-450 for the binding with substrates and estrogens directly counteract the action of androgen, consequently the alteration of the activities of drug oxidations may occur.

A NUMBER of foreign compounds of high lipid solubility are oxidatively metabolized to more water-soluble compounds by the liver microsomal hydroxylase system, called drug-metabolizing enzymes in the presence of NADPH and oxygen.[1, 2]

Recent studies have established that a hemoprotein called P-450[3] is involved in these monooxygenase reactions as the oxygen-activating enzyme[4–6] and the substrate-binding site.[7, 8]

On the other hand, it is well known that the activities of drug-metabolizing enzymes are higher in microsomes isolated from male rats than those from female.[2, 9–12]

Quinn *et al.*[13] demonstrated that the castration of male rats abolished the sex difference of the hexobarbital hydroxylation by liver microsomes while the treatment with testosterone restored it.

Furthermore, Kato *et al.*[10, 14, 15] demonstrated that the oxidations of carisoprodol and strychnine in castrated rats were stimulated not only by testosterone, but also by a synthetic anabolic steroid, 4-chlortestosterone.

Successively, Booth and Gillette[16] showed that the capacities of androgens to stimulate the activity of drug-metabolizing enzymes were more closely related to their anabolic than the androgenic activities.

The anabolic action of androgen, thus, seems to be a factor responsible for the stimulation of the activity of drug-metabolizing enzymes.

It has recently been reported that apparent K_m value for *N*-demethylation of ethylmorphine of male rats is about one-half that of female rats.[17, 18] On the other hand, Schenkman *et al.*[19] have reported that the hydroxylating activity of hexobarbital and the magnitude of hexobarbital-induced spectral change in liver microsomes isolated from male rats are about three-fold higher than in those from female rats; the castration of male rats abolishes these sex differences. These results suggest that androgen may regulate the capacity of P-450 to interact with the substrate.

In the present communication, we wish to report further evidence that the administration of androgens increases the capacity of P-450 to interact with the substrates; that estrogens directly counteract the action of androgens, and that the activities of drug-metabolizing enzymes are in any case correlated with the capacity of P-450 to interact with the substrates.

MATERIALS AND METHODS

Male and female rats of the Wistar strain, weighing about 180 and 160 g, respectively, were used.

The rats were castrated 23 days before sacrifice and treated with testosterone propionate (5 mg/kg), methyltestosterone (5 mg/kg), estradiol benzoate (500 μg/kg), diethylstilbestrol dibenzoate (500 μg/kg) for 10 days before sacrifice. All the steroids were given subcutaneously in olive oil (1 ml/kg). Control and castrated rats were given only olive oil (1 ml/kg).

Preparation of microsomes. The rats were decapitated and the livers removed, chopped into small pieces, washed well, and homogenized with 3 vol. of 1·15% (isotonic) KCl solution, in a Teflon-glass homogenizer, to minimize the absorption of hemoglobin on microsomes.[3] The homogenate was centrifuged at 10,000 *g* for 20 min and the supernatant fraction then centrifuged at 105,000 *g* for 1 hr, and the microsomes suspended in 1·15% KCl solution.

Assays of aminopyrine N-*demethylation and hexobarbital hydroxylation.* The incubation mixture consisted of 1 ml of microsomal suspension equivalent to 250 mg liver, 20 μmoles of glucose 6-phosphate, 0·8 μmole of NADP, 10 μmoles of AMP, 25 μmoles of nicotinamide, 25 μmoles of $MgCl_2$, 0·7 ml of 0·2 M sodium phosphate buffer (pH 7·4), aminopyrine (5·0 μmoles) or hexobarbital (2·0 μmoles), and water to a final volume of 2·5 ml. The mixtures were incubated at 37° for 20 or 30 min under air. The *N*-demethylation of aminopyrine was determined by the formation of formaldehyde according to the method of Nash.[20] The hydroxylation of hexobarbital was determined by the disappearance of the substrate according to Cooper and Brodie.[21] The hydroxylation of aniline was determined by the formation of *p*-aminophenol according to the method described by Kato and Gillette.[22]

Assay of NADPH-neotetrazolium reductase. The activity of NADPH-neotetrazolium was determined according to the methods described in a previous paper.[23]

Determination of substrate-induced difference spectra. To determine the binding of the substrates with P-450, the spectral changes induced by aminopyrine and hexobarbital were determined according to Schenkman *et al.*[8] The microsomal suspension was diluted with 5 vol. of 0·1 M phosphate buffer (pH 7·4) and 2·8 ml of the sample solution was placed in cuvettes of 1 cm optical path. The various amounts of drugs in

0·1 ml of distilled water were added into the sample cuvette and 0·1 ml of distilled water was added into the reference cuvette. Thirty to sixty sec later the substrate-induced difference spectra were recorded at room temperature in a Hitachi EPS-3T recording spectrophotometer with an integral sphere attachment. The change of absorbance between 420 and 500 mμ on addition of aminopyrine and between 421 and 500 mμ on addition of hexobarbital was expressed as ΔOD per milligram microsomal protein or mμmole P-450 per ml according to Schenkman *et al.*[8] and Imai and Sato.[7]

Assays of microsomal protein and P-450. The microsomal protein was measured according to the method of Lowry *et al.*[24] The content of P-450 was determined by the difference spectrum of the carbon monoxide complex of P-450 according to Omura and Sato.[3] To prepare the carbon monoxide complex of P-450, a 1·5-ml sample of microsomal suspension, equivalent to 375 mg of liver, and 1·5 ml of 0·1 M sodium phosphate buffer (pH 7·4) were placed in cuvettes. The samples were reduced by tipping solid sodium dithionite and allowed for 30 sec. The sample cuvette was then saturated with carbon monoxide by gassing for 30 sec. The difference spectrum between sample cell and reference cell at 450 and 490 mμ was measured with a Hitachi EPS-3T recording spectrophotometer with an integral sphere attachment. The content of P-450 is expressed as mμmoles per milligram microsomal protein by using a value of 91 cm^{-1} mM^{-1} for the molar extinction increment between 450 and 490 mμ.[3]

RESULTS

Effect of androgens and estrogens on liver microsomal protein, seminal vesicle and levator anni muscle

The castration of male rats and the treatments with androgens and estrogens did not result in a significant difference in the liver weight per body weight or the micro-

TABLE 1. EFFECTS OF ANDROGENS AND ESTROGENS ON LIVER MICROSOMAL PROTEIN, SEMINAL VESICLE AND LEVATOR ANNI MUSCLE

Group	Microsomal protein (mg/g wet wt.)	Seminal vesicle (mg/100 g body wt.)	Levator anni m. (mg/100 g body wt.)
1. Control	29·7 ± 0·5	215 ± 12	58 ± 3
2. Castrated	28·1 ± 0·6	20 ± 3	21 ± 2
	−5%	−91%*	−64%*
3. Castrated	28·2 ± 0·4	578 ± 46	94 ± 4
+TS	−5%	+168%*	+62%*
4. Castrated	27·1 ± 0·9	655 ± 57	91 ± 5
+TS+ES	−9% (−4%)	+205%* (+13%)	+57%* (−3%)
5. Castrated	27·9 ± 0·4	625 ± 62	97 ± 5
+TS+DES	−6% (−1%)	+191%* (+8%)	+67%* (+3%)
6. Castrated	29·3 ± 0·7	409 ± 40	78 ± 4
+MT	−1%	+90%*	+34%*
7. Castrated	27·9 ± 0·3	450 ± 48	85 ± 2
+MT+ES	−6% (−5%)	+109%* (+10%)	+47%* (+9%)
8. Castrated	28·5 ± 0·5	395 ± 37	87 ± 3
+MT+DES	−4% (0%)	+84%* (−3%)	+50%* (+12%)

Male rats were castrated 23 days before the experiments. The castrated rats were treated for 10 days with testosterone (TS) (5 mg/kg, s.c.), methyltestosterone (MT) (5 mg/kg, s.c.), estradiol (ES) (500 μg/kg, s.c.) or diethylstilbestrol (DES) (500 μg/kg, s.c.) alone or combined as indicated in the Table. The weights of seminal vesicle and levator anni muscle are expressed as mg wet weight per 100 g body weight. The results are given as averages ± S.E. obtained from seven to eight determinations. The figures with + or − indicate the percentage difference from controls and the figures in the parentheses indicate the percentage difference from the castrated +TS-treated rats or the castrated +MT-treated rats. The stars in the table indicate the significant difference ($P < 0.05$).

somal protein per wet weight of liver (Table 1). The castration markedly decreased the weights of seminal vesicle and levator anni muscle, and the treatment with testosterone or methyltestosterone increased their weights over the control values (Table 1). The simultaneous administration of estradiol or diethylstilbestrol failed to prevent the action of androgens. These results indicate that the amount of estrogens used in the present experiments is not enough for preventing the action of androgens.

Effect of androgens and estrogens on NADPH-neotetrazolium reductase and P-450 content. Since NADPH-neotetrazolium reductase and the content of P-450 showed slight sex difference,[23, 25–27] the effects of androgens and estrogens were investigated.

The castration of male rats decreased the NADPH-neotetrazolium reductase activity and the P-450 content (Table 2). The treatment of the castrated rats with test-

TABLE 2. EFFECTS OF ANDROGENS AND ESTROGENS ON MICROSOMAL P-450 CONTENT AND NADPH-NEOTETRAZOLIUM REDUCTASE

Group	P-450 content (mμmoles/mg protein)	NADPH-neotetrazolium reductase (mμmoles/mg protein/10 min)
1. Control	0·78 ± 0·03	828 ± 75
2. Castrated	0·61 ± 0·06	570 ± 45
	−22%*	−31%*
3. Castrated +TS	0·77 ± 0·04	806 ± 42
	−1%	−3%
4. Castrated +TS+ED	0·64 ± 0·03	677 ± 29
	−18%* (−17%)*	−18%* (−16%)*
5. Castrated +TS+DES	0·65 ± 0·06	620 ± 38
	−17% (−16%)	−25%* (−23%)*
6. Castrated +MT	0·75 ± 0·05	792 ± 50
	−4%	−4%
7. Castrated +MT+ED	0·65 ± 0·04	627 ± 53
	−17%* (−13%)	−24%* (−21%)*
8. Castrated +MT+DES	0·64 ± 0·03	620 ± 62
	−18%* (−15%)*	−25%* (−22%)*

See the legends for Table 1.

osterone or methyltestosterone restored NADPH-neotetrazolium reductase activity and P-450 content.

The administration of estradiol or diethylstilbestrol prevented the action of androgens on NADPH-neotetrazolium reductase activity. The increase in the content of P-450 by androgens was prevented significantly by estradiol.

These results indicate that the content of P-450 is one factor responsible for the sex difference in the activity of drug oxidation as reported in a previous paper.[27]

Effect of androgens and estrogens on the N-*demethylation of aminopyrine and hydroxylation of hexobarbital.* The castration of male rats decreased the *N*-demethylation of aminopyrine and the hydroxylation of hexobarbital and treatment with testosterone or methyltestosterone completely restored these activities (Table 3). The action of androgens was prevented by the simultaneous administration of estradiol or diethylstilbestrol.

Since diethylstilbestrol prevented the action of testosterone or methyltestosterone as well as estradiol did, the structure specificity of androgen and estrogen for the repressor

is assumed to be relatively low. In addition, it is of interest to note that the action of androgens is prevented by the low dose of estrogens which failed to prevent the androgenic action on the target organs.

As shown in Table 3, the activities of aminopyrine *N*-demethylation and hexobarbital hydroxylation per P-450 were decreased by the castration and restored by the treatment with testosterone or methyltestosterone. The action of testosterone or methyltestosterone was antagonized by estradiol or diethylstilbestrol.

These results, thus, suggest that the capacity of P-450 to *N*-demethylate aminopyrine and to hydroxylate hexobarbital is increased by androgens and estrogens block the action of androgens.

TABLE 3. EFFECTS OF ANDROGENS AND ESTROGENS ON THE ACTIVITIES OF AMINOPYRINE *N*-DEMETHYLATION AND HEXOBARBITAL HYDROXYLATION BY LIVER MICROSOMES

Group	Aminopyrine *N*-demethylation* per protein	Aminopyrine *N*-demethylation* per P-450	Hexobarbital hydroxylation† per protein	Hexobarbital hydroxylation† per P-450
1. Control	86·6 ± 5·0	111 ± 7·4	118 ± 3·6	152 ± 4·5
2. Castrated	36·9 ± 2·1 −57%*	60 ± 3·4 −46%*	36 ± 2·3 −69%*	59 ± 4·9 −61%*
3. Castrated +TS	78·7 ± 6·1 −9%	103 ± 8·4 −7%	117 ± 4·8 −1%	152 ± 6·8 0%
4. Castrated +TS+ED	56·2 ± 5·0 −35%* (−29%)*	87 ± 5·8 −22%* (−26%)*	62 ± 4·3 −47%* (−47%)*	95 ± 6·6 −37%* (−37%)*
5. Castrated +TS+DES	45·9 ± 4·7 −47%* (−42%)*	72 ± 4·9 −35%* (−30%)*	61 ± 3·8 −48%* (−48%)*	94 ± 4·8 −38%* (−38%)*
6. Castrated +MT	92·0 ± 8·7 +6%	121 ± 10·3 +9%	117 ± 5·2 −1%	156 ± 6·4 +3%
7. Castrated +MT+ED	58·3 ± 5·4 −33%* (−37%)*	88 ± 6·4 −21%* (−27%)*	58 ± 5·0 −51%* (−50%)*	88 ± 9·1 −42%* (−44%)*
8. Castrated +MT+DES	43·2 ± 4·8 −50%* (−53%)*	68 ± 5·6 −39%* (−44%)*	49 ± 3·2 −58%* (−58%)*	91 ± 6·2 −40%* (−42%)*

See the legends for Table 1.

* The activity was expressed by mμmole formaldehyde formed per mg microsomal protein or per mμmole P-450 for 30 min.

† The activity was expressed by mμmole hexobarbital disappeared per mg microsomal protein or per mμmole P-450 for 30 min.

Effect of androgens and estrogens on the magnitude of spectral changes of P-450 induced by aminopyrine and hexobarbital. The castration decreased the magnitude of spectral change of P-450 induced by aminopyrine (Table 4). The administration of testosterone or methyltestosterone to the castrated rats restored the substrate-induced spectral change. The action of androgens on the substrate-induced spectral change was blocked by estradiol or diethylstilbestrol. Furthermore, similar results were obtained with hexobarbital (Table 4).

As shown in Table 4, the magnitude of the aminopyrine-induced spectral change per P-450 was decreased by castration. The administration of testosterone or methyltestosterone to the castrated rats restored the magnitude of the aminopyrine-induced spectral change per P-450. In addition, the increase in the aminopyrine-induced spectral change by androgens was blocked by the treatment with estradiol or diethylstilbestrol. Furthermore, similar results were obtained with hexobarbital.

These results, therefore, suggest that the capacity of P-450 to interact with the substrates is increased by androgens, and estrogens block this action.

The relationship between the activity of drug oxidations and the magnitude of the drug-induced spectral changes in the rats treated with androgens and estrogens. The ratio of *N*-demethylation of aminopyrine to the magnitude of aminopyrine-induced spectral change was not significantly affected by castration and the treatment with androgens and estrogens (Table 5). Similarly, the ratio of hydroxylation of hexo-

TABLE 4. EFFECTS OF ANDROGENS AND ESTROGENS ON THE DIFFERENCE SPECTRUM OF P-450 INDUCED BY AMINOPYRINE OR HEXOBARBITAL

Group	Aminopyrine-induced spectral change†		Hexobarbital-induced spectral change†	
	per protein	per P-450	per protein	per P-450
1. Control	9·84 ± 0·32	12·7 ± 0·4	13·6 ± 0·8	17·4 ± 1·0
2. Castrated	4·89 ± 0·54 −50%*	9·8 ± 1·0 −23%*	4·5 ± 0·3 −67%*	7·4 ± 0·7 −57%*
3. Castrated +TS	9·84 ± 0·61 0%	12·8 ± 0·8 +1%	14·2 ± 1·2 +4%	18·4 ± 1·4 +6%
4. Castrated +TS+ED	6·30 ± 0·45 −36%* (−36%)*	9·8 ± 0·8 −23%* (−24%)*	6·5 ± 0·7 −52%* (−54%)*	10·2 ± 1·3 −41%* (−45%)*
5. Castrated +TS+DES	5·22 ± 0·42 −47%* (−47%)*	8·0 ± 0·8 −37%* (−38%)*	8·1 ± 0·5 −40%* (−43%)*	12·4 ± 1·0 −29%* (−33%)*
6. Castrated +MT	9·48 ± 0·67 −4%	12·7 ± 0·9 0%	13·4 ± 1·1 −1%	18·0 ± 1·5 +3%
7. Castrated +MT+ED	6·25 ± 0·33 −36%* (−34%)*	9·6 ± 0·4 −24%* (−24%)*	7·1 ± 0·3 −48%* (−47%)*	10·9 ± 0·5 −37%* (−40%)*
8. Castrated +MT+DES	5·08 ± 0·36 −48%* (−46%)*	7·9 ± 0·6 −38%* (−38%)*	6·0 ± 0·5 −56%* (−55%)*	9·4 ± 1·0 −46%* (−48%)*

See the legends for Table 1. The change of absorbance between 420 and 500 mμ on the addition of aminopyrine (2 mM) was calculated from the aminopyrine-difference spectrum. The change of absorbance between 421 and 500 mμ on the addition of hexobarbital (1·6 mM) was calculated from the hexobarbital-difference spectrum.

† The magnitudes of spectral changes are expressed by $\Delta OD \times 10^3$ per mg microsomal protein or mμmole P-450 per ml.

TABLE 5. EFFECTS OF ANDROGENS AND ESTROGENS ON THE RATIO OF THE ACTIVITIES OF AMINOPYRINE *N*-DEMETHYLATION AND HEXOBARBITAL HYDROXYLATION TO THE MAGNITUDE OF THE SPECTRAL CHANGES

Group	Aminopyrine *N*-demethylation (mμmoles/$\Delta OD \times 10^3$/30 min)	Hexobarbital hydroxylation (mμmoles/$\Delta OD \times 10^3$/30 min)
1. Control	8·80 ± 0·91	8·23 ± 0·63
2. Castrated	7·58 ± 0·53 −14%	8·03 ± 0·51 −2%
3. Castrated +TS	8·02 ± 1·22 −9%	8·25 ± 0·81 0%
4. Castrated +TS+ED	8·93 ± 0·88 +1% (+11%)	9·53 ± 0·93 +16% (+16%)
5. Castrated +TS+DES	8·80 ± 0·59 0% (+10%)	7·57 ± 0·55 −8% (−8%)
6. Castrated +MT	9·68 ± 1·45 +10%	8·73 ± 0·89 +6%
7. Castrated +MT+ED	9·33 ± 1·03 +6% (−4%)	8·18 ± 0·91 −1% (−6%)
8. Castrated +MT+DES	8·63 ± 0·73 −2% (−11%)	8·19 ± 0·58 0% (−6%)

See the legends for Tables 1 and 4. The ratios are expressed by the hydroxylating activities/mg protein/30 min per $\Delta OD \times 10^3$/mg protein/ml.

barbital to the magnitude of hexobarbital-induced spectral change was not significantly altered by castration and treatment with androgens and estrogens. These results, thus suggest that the activities of drug oxidations by liver microsomes are closely related to the amount of binding of the substrates with P-450 (Table 5). Therefore, it is clear that the activities of aminopyrine *N*-demethylation and hexobarbital hydroxylation are regulated by androgens and estrogens partly through the content of P-450 and principally through the capacity of P-450 to bind with aminopyrine and hexobarbital.

In further experiments, it has been observed that as low as 25 μg/kg of estrogen prevent the effects of the androgens on the microsomal hydroxylase systems.

Kinetic studies on the effect of estrogens and androgens on the drug oxidations by liver microsomes

As shown in Figs. 1 and 2, and Table 6, there were clear sex differences in the

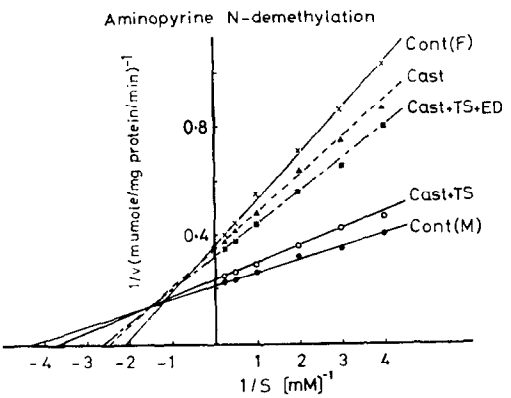

FIG. 1. Effects of testosterone and estradiol on the reciprocal plots of the aminopyrine *N*-demethylation. Male rats were castrated 23 days before sacrifice and treated with testosterone propionate (5 mg/kg, s.c.) and estradiol benzoate (500 μg/kg, s.c.) for 10 days before sacrifice. The activity of aminopyrine *N*-demethylation was measured under conditions described in Materials and Methods. TS: Testosterone treatment, ED: Estradiol treatment.

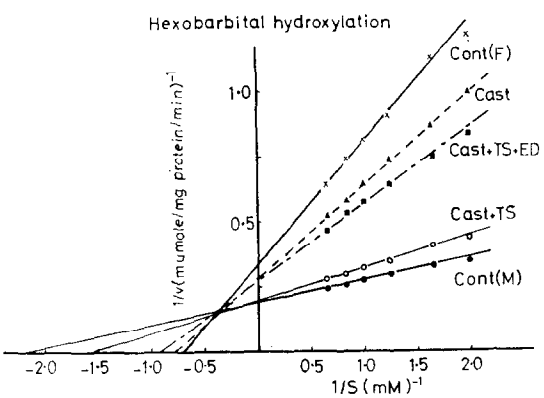

FIG. 2. Effects of testosterone and estradiol on the reciprocal plots of the hexobarbital hydroxylation. See the legends for Fig. 1. Hexobarbital hydroxylation was measured under conditions described in Materials and Methods. TS: Testosterone treatment, ED: Estradiol treatment.

V_{max} values for the *N*-demethylation of aminopyrine and hexobarbital hydroxylation in liver microsomes. Both V_{max} values were decreased in microsomes from castrated rats. The administration of testosterone to the castrated rats restored the V_{max} values and the simultaneous injection of estradiol prevented the action of testosterone.

On the other hand, the apparent K_m values for the *N*-demethylation of aminopyrine and hexobarbital hydroxylation were greater in microsomes from female rats than in those from male rats and castration increased the apparent K_m values in male rats (Figs. 1 and 2, Table 6). The administration of testosterone to the castrated rats

TABLE 6. EFFECTS OF TESTOSTERONE AND ESTROGEN ON THE MICHAELIS CONSTANTS (K_m) MAXIMUM VELOCITY (V_{max}), SPECTRAL DISSOCIATION CONSTANTS (K_s) AND MAXIMUM SPECTRAL CHANGE (ΔOD_{max}) FOR AMINOPYRINE AND HEXOBARBITAL

Group	K_m (mM)	V_{max} (mμmoles/mg protein/min)	K_s (mM)	ΔOD_{max} ($\Delta OD \times 10^3$/mg protein/ml)
A. Aminopyrine				
Male control	0·23	4·42	0·113	13·9
Castrated	0·40(+ 74%)	2·83(−39%)	0·324(+187%)	8·4(−40%)
Castrated+TS	0·28(+ 22%)	4·15(− 6%)	0·155(+ 37%)	13·5(− 3%)
Castrated+TS+ED	0·42(+ 83%)	3·12(−29%)	0·317(+181%)	9·1(−35%)
Female control	0·46(+100%)	2·85(−36%)	0·420(+272%)	8·0(−42%)
B. Hexobarbital				
Male control	0·49	7·25	0·068	19·4
Castrated	1·42(+190%)	4·83(−33%)	0·163(+140%)	12·3(−39%)
Castrated+TS	0·70(+ 43%)	7·02(− 3%)	0·090(+ 32%)	17·9(− 8%)
Castrated+TS+ED	1·28(+161%)	4·97(−32%)	0·169(+149%)	11·4(−41%)
Female control	1·59(+224%)	4·11(−43%)	0·187(+175%)	10·8(−44%)

The treatments of rats are the same as given in Table 1. The results are expressed as averages from four to seven experiments. Pooled livers from four to five rats were used for each experiment. The figures in the parentheses indicate the percentage difference from control values. K_m (Michaelis constants) and V_{max} (maximum velocity) were calculated from Figs. 1 and 2. K_s (spectral dissociation constant) and ΔOD_{max} (maximum spectral change) were calculated from Figs. 3 and 4 according to Schenkman *et al.*[8]

decreased the K_m values and the simultaneous injection of estrogen counteracted the action of testosterone. These results indicate that the androgen increases not only the V_{max} values for the aminopyrine *N*-demethylation and hexobarbital hydroxylation, but also decreases the apparent K_m values for the hydroxylating activities in castrated rats.

Kinetic studies on the effect of estrogens and androgens on the drug-induced spectral changes of cytochrome P-450

There was clear sex difference in the ΔOD_{max} (maximum spectral change) values for the *N*-demethylation of aminopyrine and hexobarbital hydroxylation. Both the ΔOD_{max} values were decreased in the castrated rats (Figs. 3 and 4, and Table 6). The administration of testosterone to the castrated rats restored the ΔOD_{max} values and the simultaneous injection of estrogen prevented the action of testosterone.

Moreover, the apparent K_s (spectral dissociation constant) values[7, 8] for the *N*-demethylation of aminopyrine and hydroxylation of hexobarbital were greater in microsomes isolated from female rats than in those from male and the castration of

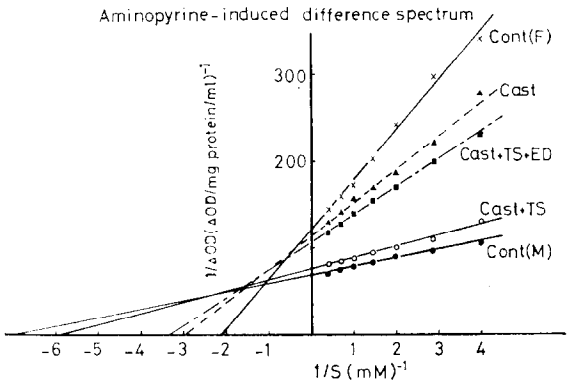

FIG. 3. Effects of testosterone and estradiol on the reciprocal plots of the spectral change induced by aminopyrine. Male rats were castrated 23 days before sacrifice and treated with testosterone propionate (5 mg/kg, s.c.) and estradiol benzoate (500 μg/kg, s.c.) for 10 days before sacrifice. The decrease in absorbance between 420 and 500 mμ on the addition of various concentrations of aminopyrine was calculated from the aminopyrine-induced difference spectrum. The results are expressed by the change of optical density per mg protein per ml. From these results the K_s (spectral dissociation constant) values were calculated according to Schenkman *et al.*[8] TS: Testosterone treatment, ED: Estradiol treatment.

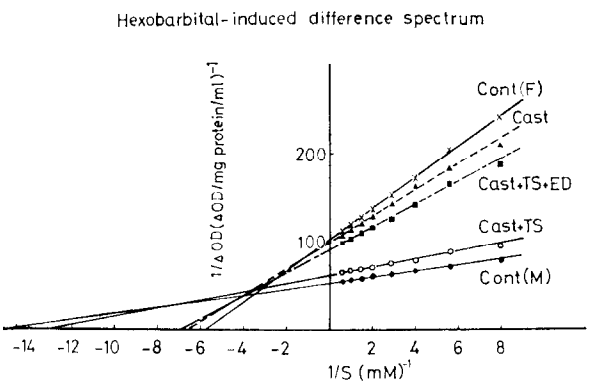

FIG. 4. Effects of testosterone and estradiol on the reciprocal plots of the spectral change induced by hexobarbital. See the legends for Fig. 3. The decrement of absorbance between 421 and 500 mμ on the addition of various concentrations of hexobarbital was calculated from the hexobarbital-induced difference spectrum. The results are expressed by the change of optical density per mg protein per ml. From these results the K_s (spectral dissociation constant) values were calculated according to Schenkman *et al.*[8] TS: Testosterone treatment, ED: Estradiol treatment.

male rats increased the apparent K_s values. The administration of testosterone to the castrated rats decreased the apparent K_s values and the simultaneous injection of estrogen prevented the action of testosterone. These results indicate that the androgen increases not only the ΔOD_{max} values for the aminopyrine and hexobarbital-induced spectral changes, but also decreases the apparent K_s values.

It is of interest that the alterations of the apparent K_s values for the spectral changes

induced by aminopyrine and hexobarbital by castration and treatment with testosterone and estradiol are parallel to those of the apparent K_m values for the aminopyrine *N*-demethylation and hexobarbital hydroxylation.

DISCUSSION

It is well known that there is clear sex difference in the activities of drug-metabolizing enzymes of rat liver microsomes.[9–11, 25] Since these activities are dependent upon the anabolic action of androgen, the castration of male rats markedly decreases these activities while the treatments with testosterone and other anabolic hormones restore them.[13, 14–16] In addition, slight sex difference is observed in the content of P-450 and activities of NADPH-linked electron transport system of rat liver microsomes.[23, 25–27]

On the other hand, there is no sex difference in the activities of drug-metabolizing enzymes, P-450 content and NADPH-linked electron transport system of liver microsomes of rabbits, mice, guinea-pigs and hamsters, and the administration of androgenic and anabolic steroids did not cause any significant increase.[27]

These results suggest that there is an androgen-dependent regulation mechanism of the activities of drug-metabolizing enzyme systems in rats, but this mechanism seems to be genetically lacked in other species of animals.[26] At the present time, we do not yet know about the nature of androgen-dependent regulation mechanism, but according to the operon theory of Jacob and Monod,[28] it might be assumed that androgen may act as an inactivator of an active endogenous repressor, which may repress the action of a regulator gene responsible for the activities of microsomal drug-metabolizing enzyme systems.[27]

On the other hand, Quinn *et al.* reported that the administration of estradiol to male rats decreases the activity of hexobarbital hydroxylation.[13] These results suggest that the estrogen may counteract the action of androgen. However, the administration of estrogen markedly decreases the rate of biosynthesis of androgen by testis,[29, 30] therefore it is not clear whether estrogen counteracts the action of androgen directly or influences through the action on the rate of biosynthesis of androgen. In the present studies, the treatment with estradiol or diethylstilbestrol counteracts the stimulatory action of testosterone or methyltestosterone on the activities of drug-metabolizing enzymes in castrated male rats (Tables 3 and 4). These results clearly indicate that the action of estrogen to counteract the action of androgen is not mediated through the testis and probably acts directly at the site of the action of androgen.

It might be possible to assume that if androgen plays a role as an inactivator of the repressor, estrogen may interfere the binding of androgen with the repressor. Since estradiol and diethylstilbestrol counteract the stimulatory action of testosterone and methyltestosterone equally, these actions of androgens and estrogens do not seem limited to the natural androgen and estrogen.

Androgen increases not only activities of drug hydroxylation, but also slightly increases the content of P-450 and the activities of NADPH-linked electron transport system, such as NADPH oxidase, NADPH-cytochrome c reductase and NADPH-neotetrazolium reductase.[26, 27] It has been observed in the present investigation that estrogens block the stimulatory action of androgens on the content of P-450 and the activity of NADPH-neotetrazolium reductase (Table 2).

In agreement with the observation of Schenkman *et al.*[19] the magnitudes of spectral changes induced by hexobarbital and aminopyrine were higher in microsomes from

male rats than in those from female rats and the magnitudes of the spectral changes were markedly decreased by castration. Moreover, the administration of testosterone or methyltestosterone to castrated male rats increased the magnitudes of the spectral changes induced by hexobarbital and aminopyrine and the simultaneous injection of estradiol or diethylstilbestrol counteracts the action of androgens.

As shown in Table 5, there is no difference in the ratio of the hydroxylating activities to the magnitudes of spectral changes induced by aminopyrine and hexobarbital. These results indicate that the principal site of the androgen action to stimulate the drug-oxidating activities of liver microsomes is the level of the substrate interaction with P-450 and this action of androgen is counteracted by estrogen.

In agreements with the result of Schenkman *et al.*,[19] the apparent K_m values for the hexobarbital hydroxylation and aminopyrine *N*-demethylation and the apparent K_s values for the spectral changes induced by hexobarbital and aminopyrine were lower in microsomes from female rats than those from male rats (Figs. 1–4 and Table 6). The lower K_m values observed in microsomes isolated from male rats were increased by the castration. The administration of testosterone to the castrated rats again decreased the increased K_m values and the simultaneous injection of estradiol counteracted the action of testosterone. These alterations of the apparent K_s values for the spectral changes induced by aminopyrine and hexobarbital are parallel to those of the K_m values for the aminopyrine *N*-demethylation and hexobarbital hydroxylation. In contrast to the present results, Davies *et al.* reported that the apparent K_m values for the *N*-demethylation of ethylmorphine by liver microsomes of male rats were not altered by castration.[18] The reason for the difference between the present results and those of Davies *et al.* is not clear and will require further investigation.

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