Journal of Neurochemistry
37(3):640–648, September. Raven Press, New York

0022-3042/81/0901-0640/$02.75/0

Gonadal Influences on the Sexual Differentiation of Monoamine Oxidase Type A and B Activities in the Rat Brain

A. Vaccari, A. Caviglia, A. Sparatore, and R. Biassoni

Institute of Pharmacology, School of Pharmacy, Genova, Italy

Abstract: The sex-dependent differentiation of monoamine oxidase (MAO) in the hypothalamus of 60-day-old, Charles River rats was found to involve only type A (MAO-A), and not type B (MAO-B) enzyme. *In vivo* inhibition of type A by clorgyline, and type B by (−)deprenyl, however, tended to decrease the specific activity of both types of MAO to a smaller extent in the female than in the male hypothalamus. When masculinization was prevented by neonatal administration of estradiol (E) to males, hypothalamic MAO-A and MAO-B activities increased in both control and MAO-inhibited rats. Androgenization of females, however, had little effect on the MAO activity. Whereas the effects of neonatal estrogenization were attributable neither to a direct influence of E nor to a sexual difference in the peripheral clearance of the MAO-inhibitor used, single, high doses of steroids to adult, but not to newborn rats, did acutely affect the kinetics of MAO-A. The activity of MAO-A was also decreased by high concentrations of E or TS *in vitro*. The imprinting for patterns of hypothalamic MAO-A and MAO-B in the two sexes results, probably, from genetic predetermination. Neonatal changes in the homeostasis of gonadal hormones may result in type-MAO nonspecific effects in adulthood, whereas the short-term effects of high concentrations of steroids may be selective for the A form. **Key Words:** Development—Monoamine oxidase—Gonadal hormones—MAO inhibitors—Hypothalamus. **Vaccari A. et al.** Gonadal influences on the sexual differentiation of monoamine oxidase type A and B activities in the rat brain. *J. Neurochem.* 37, 640–648 (1981).

The central and peripheral nervous systems follow different maturational schedules in male and female mammals. Sex-related differences extend to the brain biochemistry, including the monoaminergic pathways (Vaccari et al., 1977; Vaccari, 1980). The enzyme monoamine oxidase (MAO), which inactivates neuronal and platelet catecholamines, 5-hydroxytryptamine (5-HT; serotonin), and minor amines, has been repeatedly shown to act differently in the two sexes. This difference ranges from rats to humans, and occurs in brain, platelets, and some peripheral tissues, where the overall tendency for MAO is to be more active in female than in male subjects (see Vaccari, 1980 for references).

Although a genetic component in the control of intersexual differences has been advocated, at least for human platelet MAO (Nies et al., 1973; Murphy and Buchsbaum, 1978), perinatal gonadal influences appear to play a major priming role in the sexual differentiation of brain MAO. It is well known that the "imprinting" for brain maturation is the consequence, at least in the rat, of the perinatal exposure to gonadal hormones that organize the development of the sexual "type" of brain (Gorski et al.,

Received February 4, 1981; accepted March 19, 1981.
Address correspondence and reprint requests to Dr. Andrea Vaccari, Institute of Pharmacology, School of Pharmacy, Via Capo S. Chiara 5, 16146 Genova.

Abbreviations used: BEA, Benzylamine; E, Estradiol valerate; 5-HT, 5-Hydroxytryptamine (serotonin); MAO-A, Monoamine oxidase type A; MAO-B, Monoamine oxidase type B; SDH, Succinic dehydrogenase; TS, Testosterone enanthate.

1977; McEwen, 1978; Döhler and Hancke, 1978). Further, hormonal influences on platelet MAO in women (Belmaker et al., 1974; Feine et al., 1977) and in adult rat brain MAO (Zolovick et al., 1966; Kamberi and Kobayashi, 1970; Holzbauer and Youdim, 1973; Kueng et al., 1976) during the sexual cycle have been shown. Finally, treatment of normal adult, pregnant, or newborn rats with sex hormones can affect brain MAO (Collins et al., 1970; Luine and McEwen, 1977; Breuer et al., 1978; Franz et al., 1978; Wilson et al., 1979).

The major object of the present work was to ascertain through kinetic analysis whether sexual differentiation involves both types of MAO in the hypothalamus. Further, we sought to determine whether disruption of neonatal endocrine patterns by estrogenization of young males or androgenization of female rats might specifically influence MAO. The final purpose of this work was to discover whether inborn sex differences and the effects of endocrine manipulations are reflected in the kinetics of MAO inhibition.

MATERIALS AND METHODS

Chemicals

5-Hydroxy [2-^{14}C]tryptamine creatinine sulfate (58 mCi/mmol specific activity), and benzylamine [methylene-^{14}C]hydrochloride (56 mCi/mmol specific activity) were obtained from the Radiochemical Centre, Amersham, United Kingdom. Clorgyline was a gift of May & Baker Ltd., Dagenham, United Kingdom; (−)deprenyl hydrochloride was a gift from Prof. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary. Iodonitrotetrazolium violet was purchased from Serva Feinbiochemica, Heidelberg. Insta-Fluor was from Packard Ltd.

Animals and Hormone Treatments

Charles River albino rats were housed under constant conditions of temperature, humidity, and a 12-h lighting cycle. Food and water were freely available. The offspring of rats mated at this institute were sexed at birth, and separated from their mothers at 30 days, when males and females were also caged separately. The pups had been assigned at birth so that, whenever possible, control and hormone-treated rats were from the same litter.

Administration of sex hormones started 24 h from birth, and was stopped at 50 days of age, 10 days before sacrifice. The rationale for the dosage schedule was to start with doses high enough either to obtain an early and persistent masculinization of females or to inhibit the masculinization of males, and to end with doses low enough to approach the physiological levels in normal rats of the other sex at that age. The long-acting steroids (Murad and Gilman, 1975) testosterone enanthate (TS) and estradiol valerate (E) were injected into females and males, respectively, via a subcutaneous route in 0.05 ml corn oil; leakage of the liquid from the newborn skin was counteracted with collodion.

Female pups were given 270 μg TS at birth, then 100 μg at days 5, 10, and 17; 10 μg on day 24; 1 μg at days 34 and 44; and 0.5 μg at 50 days of age. Male newborns received 500 μg E at birth, then 200 μg on days 5, 10, and 17; 100 μg at 24 days; 1 μg on days 34 and 44, and 0.5 μg at 50 days postpartum. Six male and six female rats received 1 μg and 0.5 μg E or TS at days 44 and 50 only, to assess the influence of the last hormone treatment by itself.

Control pups received the vehicle on the same days as the steroid-treated rats. All rats were weighed through development; the testes and the ovary plus uterine tubes were weighed after sacrifice. Visual inspection of the uterus and vaginal smears indicated that our 60-day-old females were in late metestrus or diestrus (Short and Woodnott, 1969).

Preparation of Tissues

The hypothalamus was dissected over ice, then frozen over Dry Ice, and stored at −80°C until assayed. Dissection of the entire hypothalamus was limited anteriorly by the margin of the optic chiasma, laterally by the lateral fissures, and posteriorly by the margin of the mammillary body. The block was about 2.5 mm deep from the basal surface of the hypothalamus. The average weight of the hypothalamic region in control male and female rats, and in estrogenized males or androgenized females at 60 days of age was of 69.0 ± 1.0 mg, 63.0 ± 0.9 mg, 65.3 ± 0.9 mg, and 65.3 ± 1.0 mg (N = 18), respectively. Single hypothalami were homogenized (ground-glass homogenizer) in 50 vol. (w/v) of 0.3 M-sucrose, sonicated for 30 s, and then centrifuged at 1000 g for 10 min. The supernatants were used for enzyme assays and protein determination (Lowry et al., 1951).

Enzyme Assays

All assays were performed in duplicate immediately after the preparation of the supernatants; the linearity of the reactions with time and tissue concentration was checked for each enzyme assay.

Monoamine oxidase

The monoamine oxidase assay was carried out basically according to McCaman et al. (1965), with minor adaptations (Gabay et al., 1976). Aliquots (25-μl for MAO-A or 50-μl for MAO-B; approx. 50 or 100 μg proteins) of the supernatant were incubated at 37°C in air for 30 min in a total volume of 200 μl of 0.1 M-Na-K phosphate buffer, pH 7.4 (MAO-A) or 7.8 (MAO-B). Final concentration of [^{14}C]5-HT plus unlabeled 5-HT for kinetic analysis of MAO-A ranged from 7×10^{-6} M to 1×10^{-3} M. Final concentration of [^{14}C]BEA plus unlabeled BEA (MAO-B) ranged from 1×10^{-6} M to 1×10^{-3} M. The reaction products were extracted with diethyl ether (MAO-A) or toluene (MAO-B) and counted in 10 ml Insta-Fluor.

The incubation medium for the *in vitro* inhibition of MAO activity included 50 μl of clorgyline (MAO-A inhibitor), from 10^{-12} M to 10^{-7} M, and 3×10^{-4} M ^{14}C plus unlabeled 5-HT (approx. twice as high as the K_m for 5-HT in control rats). MAO-B was inhibited with 50 μl of (−)-deprenyl, from 10^{-9} M to 10^{-5} M; final concentration of the

substrate was 1×10^{-3}M ^{14}C plus unlabeled BEA (approx. 10-fold as high as the K_m for BEA in normal rats). The supernatants and the buffers were preincubated 15 min at 20°C with the MAO inhibitor used.

In vivo inhibition of MAO was provoked by pretreating rats with either 1 mg/kg i.p. of clorgyline (Fuentes and Neff, 1975) or 2 mg/kg deprenyl at 5 h before sacrifice of the rats. The hypothalami from MAO-inhibited rats were assayed for MAO activity as described previously.

Succinic dehydrogenase

Succinic dehydrogenase (SDH) was assayed according to Adams et al. (1963), as described by Achee et al. (1974); 50 μl of the sonicated (1 min) supernatant (approx. 100 μg proteins) were incubated at 37°C for 15 min. Final concentrations of succinic acid for the kinetic analysis ranged from 5×10^{-5}M to 1×10^{-3}M. Blanks included succinic acid, and iodonitrotetrazolium violet (INT) was substituted by its solvent acetone.

Activity was expressed as μmol INT reduced/mg protein.

Statistics

One male and one female control, one estrogenized male and one androgenized female, whenever possible obtained from the same litter, were assayed simultaneously in each experiment. Results were analyzed with the paired *t*-test; the one-way analysis of variance was otherwise used. Lineweaver-Burk plots for kinetic analysis were fitted by linear regression analysis.

RESULTS

Effects of Endocrine Manipulations on Body Growth and Sexual Organs

Neither estrogenization nor androgenization through development affected the survival of rats. The body weight of 50-day-old females was 10% ($P < 0.01$) lower than in male counterparts; estrogenization of males inhibited it by 18%, and androgenization of females increased it by 10%, compared with controls. Significant alterations in the body growth persisted 10 days after interruption of the treatments (219 ± 5.3 g, $N = 62$, and 172 ± 3.9 g, $N = 67$, in control males and females, respectively; 149 ± 4.2 g, $N = 58$, $P < 0.001$, and 186 ± 4.3 g, $N = 55$, $P < 0.02$, in estrogenized males, and androgenized females, respectively).

The same treatments displayed dramatic effects on the weight of sexual organs at 60 days of age; the testes and the ovary plus uterine tubes were decreased 93% ($P < 0.001$) and 26% ($P < 0.001$) in estrogenized males and androgenized females, respectively, compared with controls.

MAO Activity in Normal and Hormone-Exposed Rats

The V_{max} of MAO-A tended to be 11% higher in female hypothalami than in male littermates; the difference was, however, nonsignificant (Table 1). When present results and those from the experiment on the effects of the last injections of steroids were pooled, the V_{max} in males and females was 107.5 ± 8.6 nmol/h/mg protein, $N = 9$, and 130.2 ± 11.7 nmol/h/mg protein, $N = 9$, respectively. The intersex difference of 21%, however, was still not significant. The kinetic parameters of MAO-B were similar in both sexes (Table 1).

Estrogenization of males stimulated by 45% and 11% the V_{max} of MAO-A and MAO-B, whereas androgenization of females had no effect, compared with control littermates. The K_m for both substrates of MAO was similar in all groups (Table 1).

In Vivo Inhibition of MAO

Pretreatment of 60-day-old rats with 1 mg/kg i.p. of clorgyline, or 2 mg/kg i.p. deprenyl, 5 h before sacrifice, inhibited by 84% and 90%, on average, the V_{max} of MAO-A and MAO-B, respectively, in male and female controls (Table 2). The V_{max} values for MAO-A and MAO-B after MAO inhibition were

TABLE 1. *Kinetic parameters of MAO-A and MAO-B activities in the hypothalami of normal and sex hormone-treated, male and female, 60-day-old rats*

		MAO-A		MAO-B	
Sex	Treatment	K_m	V_{max}	K_m	V_{max}
Male	Vehicle	128 ± 18	114.0 ± 11.2 (6)	103 ± 8	84.4 ± 6.6 (5)
Female	Vehicle	128 ± 17	127.0 ± 14.7 (6)	107 ± 10	87.7 ± 4.7 (6)
Male	Estradiol	201 ± 42	165.8 ± 15.0 (6)[b]	105 ± 7	93.8 ± 5.9 (5)[a]
Female	Testosterone	107 ± 8	102.4 ± 8.4 (6)	91 ± 10	82.3 ± 6.9 (6)

Mean values ± S.E.M. from (N) experiments. K_m = μM; V_{max} = nmol/h/mg protein. Newborn rats received the vehicle or gonadal steroids from birth up to 50 days of age (see Materials and Methods). One control male and one control female, one estrogenized male, and one androgenized female were assayed simultaneously in each experiment. Duplicate determinations were run for each concentration of 5-HT (MAO-A) or BEA (MAO-B). Lineweaver-Burk plots were calculated by linear regression analysis.
[a] $P < 0.02$; [b] $P < 0.01$, estrogenized versus control males, paired *t*-test.

TABLE 2. In vivo *inhibition of MAO-A and MAO-B in the hypothalami of normal and sex hormone-treated, male and female, 60-day-old rats*

Sex	Treatment	MAO-A		MAO-B	
		K_m	V_{max}	K_m	V_{max}
Male	Vehicle	197 ± 37	15.3 ± 4.7 (6)	54 ± 0.4	4.8 ± 0.8 (6)
Female	Vehicle	203 ± 28	25.4 ± 5.8 (6)[a]	70 ± 0.5	9.9 ± 1.6 (6)[a]
Male	Estradiol	185 ± 49	23.7 ± 5.3 (5)	78 ± 0.8[b]	7.4 ± 0.5 (6)[b]
Female	Testosterone	150 ± 25	14.7 ± 2.7 (6)	55 ± 0.6[c]	6.9 ± 0.8 (6)

Mean values ± S.E.M. from (N) experiments. K_m = μM; V_{max} = nmol/h/mg protein. Newborn rats received the vehicle or gonadal steroids from birth up to 50 days of age (see Materials and Methods). Clorgyline (for MAO-A), 1 mg/kg, and deprenyl (for MAO-B), 2 mg/kg were injected i.p. 5 h before sacrifice, at 60 days of age. For additional details see footnote to Table 1.

[a] $P < 0.02$, female versus male controls; [b] $P < 0.02$, estrogenized versus control males; [c] $P < 0.05$, masculinized versus control females, paired t-test.

66% and 105% as high in female as in male littermates or, in other words, clorgyline and deprenyl inhibited hypothalamic MAO less efficiently in females.

The V_{max} after inhibition of MAO-A and MAO-B was 55% and 53% as high in estrogenized as in control males, whereas it was similar in both control and androgenized females after MAO inhibition (Table 2). The K_m for 5-HT was not affected, whereas the K_m for BEA was increased (46%) or decreased (22%) in estrogenized males or androgenized females, respectively (Table 2).

Time Course of MAO-A Inhibition in the Hypothalamus of Male and Female, 60-Day-old Rats

To ascertain whether the time course of MAO inhibition differed with respect to sex, MAO-A activity was assayed in the male and female hypothalami at various times after intraperitoneal injection of 1 mg/kg clorgyline. The steady-state activity of MAO-A in normal, female rats was here 50% higher than in male littermates (Table 3), thus supporting the tendency of female MAO to exceed MAO activity in male counterparts. MAO-A was constantly more active (145% on average) in female supernatants through 5 h after clorgyline injection. The same picture was obtained when MAO-A was expressed as a percent of its original activity (Table 3).

In Vitro Inhibition of MAO

The dose of clorgyline reducing MAO-A activity by 50% (I_{50}), as calculated from plots of percent MAO inhibition versus concentration of the inhibitor, was decreased 85% in androgenized females compared with controls (Table 4). The other treatments did not provoke significant effects. The I_{50} values for deprenyl against MAO-B activity were similar in all experimental groups; they ranged from $4.95 \pm 0.7 \times 10^{-8}$M, $N = 4$, to $3.80 \pm 0.4 \times 10^{-8}$M, $N = 5$, in control and estrogenized males, respectively.

Effects of Gonadal Steroids in Prepubertal Rats

To ascertain whether the last injections of steroids 10 days before sacrifice, *per se*, had the capacity to affect MAO activity and its inhibition, young male and female rats were given 1 μg and 0.5 μg of E or TS, respectively, on days 44 and 50 after birth. Thus, they received only the two final injections scheduled in the main experiment.

Neither estrogenization of males nor androgenization of females, in the absence or presence of MAO inhibitors, significantly affected the kinetics of hypothalamic MAO-A and MAO-B.

Acute Effects of Sex Hormones on MAO and Its Inhibition in Newborn and Adult Rats

The possibility that gonadal steroids exerted direct effects on MAO activity and its inhibition was assessed as follows.

(1) E, 100 μg and 200 μg, or TS, 10 μg and 200 μg,

TABLE 3. *Time course of MAO-A inhibition in the hypothalami of 60-day-old, normal male and female rats*

Time after clorgyline (min)	Males		Females	
	nmol/h/mg protein	%	nmol/h/mg protein	%
Controls	81.8 ± 1.3	100	123.1 ± 3.3[b]	100
60	21.8 ± 2.7	27	31.8 ± 4.0	26
150	15.0 ± 1.6	18	42.3 ± 7.4[a]	34
300	14.2 ± 5.5	17	43.8 ± 17.1	36

Mean values ± S.E.M. from three experiments. Male and female rats were given clorgyline, 1 mg/kg i.p.; controls received saline. Duplicate determinations were run with a fixed amount of 5-HT (3×10^{-4}M final concentration).

[a] $P < 0.05$; [b] $P < 0.01$, females versus males, one-way analysis of variance.

TABLE 4. *In vitro inhibition of MAO-A in the hypothalami of normal and sex hormone-treated, male and female, 60-day-old rats*

Sex	Treatment	I_{50}
Male	Vehicle	$5.62 (\pm 1.0) \times 10^{-11}$ M
Female	Vehicle	$6.37 (\pm 1.3) \times 10^{-11}$ M
Male	Estradiol	$3.62 (\pm 1.2) \times 10^{-11}$ M
Female	Testosterone	$0.97 (\pm 0.2) \times 10^{-11}$ M[a]

Mean values ± S.E.M. from four experiments. I_{50} = molar dose of clorgyline that inhibited by 50% the specific MAO-A activity. Newborn rats received the vehicle or gonadal steroids from birth up to 50 days of age (see Materials and Methods). Duplicate determinations were run for each concentration of clorgyline (10^{-12}M–10^{-7}M), in the presence of 3×10^{-4}M 5-HT. Clorgyline was preincubated 15 min at 20°C with supernatants obtained from 60-day-old rats.

[a] $P < 0.05$, androgenized versus control females, one-way analysis of variance.

TABLE 5. *Acute effects of gonadal steroids on MAO-A activity in the hypothalami of 60-day-old, male and female rats*

Sex	Treatment	K_m	V_{max}
Male	Vehicle	174 ± 11	181.6 ± 14.2
	E, 20 μg	147 ± 9	163.2 ± 4.2
	E, 200 μg	150 ± 11	159.6 ± 11.9
Female	Vehicle	171 ± 9	160.0 ± 3.2
	TS, 20 μg	172 ± 6	162.4 ± 1.1
	TS, 200 μg	140 ± 3[a]	144.5 ± 1.2[b]

Mean values ± S.E.M. from four experiments. Duplicate determinations were run for each concentration of 5-HT. Linear regression analysis of Lineweaver-Burk plots was used to calculate the K_m (μM) and the V_{max} (nmol/h/mg protein). Either estradiol valerate (E) or testosterone enanthate (TS) were injected i.p. to 60 day-old rats; sacrifice was 5 h later.

[a] $P < 0.05$; [b] $P < 0.01$, versus controls of the same sex, one-way analysis of variance.

were injected intraperitoneally into 24-day-old, control male and female pups. (2) E or TS, 20 μg and 200 μg, were given intraperitoneally to 60-day-old, control and clorgyline or deprenyl-pretreated male and female rats, respectively. Hypothalamic MAO was assayed 5 h later (7 h after administration of the MAO inhibitors).

Acute gonadal manipulations on groups of three 24-day-old rats did not affect the pattern of MAO-A and MAO-B activity.

A single injection of 20 μg or 200 μg E to 60-day-old males (doses 40- to 400-fold as high as the final ones given to chronically treated newborns) did not significantly affect the kinetics of MAO-A and MAO-B. There was a slight tendency of both V_{max} and K_m to decrease, compared with controls (Table 5). The higher dose of TS to females did slightly depress the V_{max} (by 10%) and the K_m (by 18%) of MAO-A compared with controls (Table 5). MAO-B was not affected by androgenization of females.

The enzyme kinetics in acutely estrogenized, 60-day-old males were similar in the absence or presence of MAO-inhibitors. Both doses of TS to females elicited a nonsignificant tendency in the V_{max} of MAO-A to decrease; this trend was confirmed by a 46% significant decrease in the steady-state activity of MAO-A, after 200 μg TS (Table 6).

Peripheral Clearance of Clorgyline in the Two Sexes

The occurrence of a lower MAO inhibition in the female hypothalamus led us to ascertain whether it might result from a faster peripheral clearance of the inhibitor in adult females, compared with male counterparts. Since clorgyline and deprenyl have a similar structure, we limited our research to clorgyline. Hypothalamic supernatants were incubated with increasing volumes of liver homogenates obtained from clorgyline-pretreated, male and female rats. Liver MAO had been previously heat-inactivated (Kastl et al., 1970). Liver homogenates taken from clorgyline-administered females inhibited hypothalamic MAO-A at any time after clorgyline injection, more strongly than male homogenates (Fig. 1). It thus appears that female livers contained more nonmetabolized clorgyline than male tissues, as a consequence of a slower clearance.

Succinic Dehydrogenase Activity

Neither sex-related differences in control hypothalami nor hormone-provoked alterations occurred in the kinetics of SDH (V_{max}: 1.03 ± 0.05 and 1.09 ± 0.03 μmol/h/mg protein, $N = 6$ in male and female controls, respectively; K_m: 112.2 and 113.5 μM, respectively).

DISCUSSION

A major purpose of this work was to characterize more effectively the sexual differentiation of the two types of MAO in the central nervous system. The choice of the hypothalamus was justified, first, on the basis of its strict involvement in the sexual differentiation of the brain (Barraclough, 1966; Gorski et al., 1977; Gorski et al., 1978). Second, hypothalamic MAO differentiates as early (Gaziri and Ladosky, 1973; Wilson et al., 1979) as other brain regions (Vaccari et al., 1977), thus being sensitive to the "imprinting" influence of the neonatal, gonadal secretions (Gorski et al., 1977; McEwen, 1978; Dörner, 1978).

Previous studies using tyramine, a common substrate for type A and B enzymes had shown that hypothalamic MAO was more active in the adult female, compared with male rats (Kamberi and Kobayashi, 1970; Rosecrans and Schechter, 1972),

TABLE 6. *Acute effects of testosterone on the* in vivo *inhibition of hypothalamic MAO-A in 60-day-old female rats*

Sex	Treatment	K_m	V_{max}	Steady-state activity[a]
Female	Vehicle	147 ± 28	31.1 ± 8.6	32.2 ± 5.5
	TS, 20 μg	93 ± 4	18.2 ± 1.7	21.0 ± 5.6
	TS, 200 μg	119 ± 24	14.7 ± 4.5	17.5 ± 0.7[b]

Mean values ± S.E.M. from three experiments. Duplicate determinations were run for each concentration of 5-HT. Linear regression analysis of Lineweaver-Burk plots was used to calculate the K_m (μM) and the V_{max} (nmol/h/mg protein). Female, 60-day-old rats were injected i.p. clorgyline, 1 mg/kg; 2 h later they received i.p. testosterone enanthate (TS); sacrifice was 5 h after the steroid treatment.

[a] Final concentration of 5-HT was 1×10^{-3}M.

[b] $P < 0.05$, versus controls, one-way analysis of variance.

whereas the opposite was true in 12-day-old pups, when using tyramine (Wilson et al., 1979), or the type A substrate 5-HT (Gaziri and Ladosky, 1973). In the present investigation, kinetic analysis showed, however, that hypothalamic MAO-A at postpuberty tended to be slightly (by approx. 20%) more active in the female than in male littermates; MAO-B was not sexually differentiated (Table 1).

One might, thus, infer that genetic imprinting and early gonadal influences, when demonstrated, do differently "regulate" the development of the two types of MAO. This would be consistent with recent statements that MAO-A and MAO-B activities correspond to two distinct enzyme molecules (Cawthon and Breakefield, 1979); it contrasts, however, with the present evidence that neonatal administration of gonadal steroids had no effects specific to type of MAO. Both types of MAO were, indeed, similarly responsive to the same endocrine manipulations: their specific activity was stimulated in rats whose masculinization had been prevented by E given at birth, and then through development. This finding further supports the statement that the net effect of female sex hormones "seems to be to sustain the level of MAO above some minimal activity" (Sourkes, 1979). Castration, another model of disruption of male patterns, was shown to increase similarly brain, liver, and platelet MAO activity in adult male rats (Klaiber et al., 1967; Illsley and Lamartiniere, 1980) or Rhesus monkeys (Redmond et al., 1976). Androgen administration to females provoked less clear-cut effects. It is unfortunate that data were not available on the effects of E to females, to ascertain whether estrogen-provoked alterations of MAO were sex-specific.

The apparent V_{max} values in the female hypothalami after MAO-A and -B *in vivo* inhibition were 66% and 105% as high as in male littermates, respectively. In other words, there was a lower inhibition by clorgyline and deprenyl in females. Estrogenization of newborn males raised the V_{max} values above their control levels, in clorgyline- and, to a lesser extent, deprenyl-treated rats (Table 2). The same trend occurring in rats given E in the absence

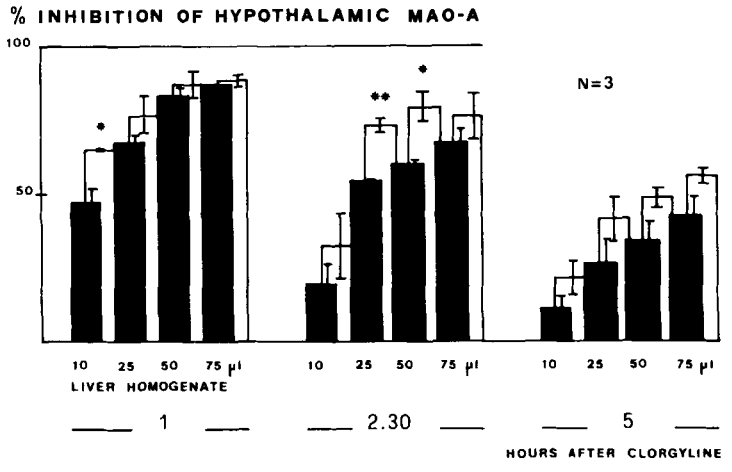

FIG. 1. Peripheral clearance of clorgyline (1 mg/kg i.p.) in 60-day-old, male ■ and female □ rats. The livers were taken 60, 150, and 300 min after clorgyline administration, then homogenized (10% w/v). Endogenous MAO in the liver homogenates was heat-inactivated by 1-min exposure to boiling water. Liver MAO-A activity in control rats was not sex-specific (124.4 ± 5.4 and 122.9 ± 8.7 nmol/h/mg protein, $N = 3$, in male and female tissues, respectively). Twenty-five microliters of hypothalamic supernatant obtained from 60-day-old males were preincubated for 15 min at 20°C with increasing volumes of liver homogenate; the reaction was started by addition of 3×10^{-4}M-5-HT (final concentration) to the incubation medium (see Materials and Methods). The extent of inhibition in hypothalamic MAO-A was intended as a consequence of the amount of nonmetabolized clorgyline left in liver homogenates, and thus an indirect measure of its metabolism. *$P < 0.05$; **$P < 0.01$, males versus females, one-way analysis of variance.

of MAO inhibitors (Table 1) thus persisted here. When MAO was inhibited *in vitro*, the results did not support the outcome from the *in vivo* inhibition. The lack of a complete correlation between *in vitro* and *in vivo* inhibition of MAO is well known (see Achee et al., 1977; Murphy, 1978 for references). A reason might be a faster peripheral clearance of the inhibitor in females, resulting in less circulating and, consequently, less clorgyline and deprenyl locally available to inhibit hypothalamic MAO, compared with male counterparts. However, this hypothesis was not supported here. In fact, there was indirect evidence for a slower liver metabolism of clorgyline in females (Fig. 1). Liver metabolism and MAO inhibition in the hypothalamus, therefore, appear to be two independent events; one cannot exclude, however, that the local clearance of clorgyline and deprenyl may differ in the two sexes.

Precocity and/or chronicity in the exposure to estrogen was a prerequisite for influencing MAO activity. Treatment of prepubertal rats with the two last, quasi-physiological doses (Döhler and Wuttke, 1975) of steroids did not affect MAO activity at 60 days of age. Single doses (up to 400-fold higher than those ending the chronic treatment) of estrogen or androgen given to 60-day-old rats provoked moderate effects, which were opposite to those occurring in chronically treated rats. The tendency in the kinetic parameters of MAO-A to decrease slightly after acute estrogen administration to males, or androgen administration to females, might result from a direct, type-MAO-selective effect on the enzyme. This suggestion is supported by experiments where hypothalamic supernatants obtained from two male and two female, 60-day-old rats were preincubated with a wide range of concentrations of E or TS. The steady-state activity of MAO-A was decreased (−29%) by 500 ng E, or 50 ng−500 ng TS (−23% and −38%, respectively), compared with buffer- or solvent-preincubated controls (Fig. 2). MAO-B activity was not affected by gonadal steroids *in vitro* (Fig. 2).

If one assumes that there was a quantitatively similar distribution of steroids in the body compartments after the last injection scheduled in the main experiment (500 ng E or TS), a rough estimate would indicate a content of approx. 0.2 ng per hypothalamus (average weight 65 mg) in 50-day-old rats (average body weight 170g). This concentration is far below the ones that could affect MAO-A *in vitro*. The stimulation of MAO-A and MAO-B activity induced by neonatal estrogenization may be, thus, the consequence of disruption of the normal "organizational" activity of gonadal secretions on the structural development of the hypothalamus. The number of mitochondria did not appear to be involved, since SDH activity, a marker for the inner mitochondrial membrane, was not affected by hormonal manipulations.

In conclusion, MAO in the rat hypothalamus undergoes a moderate sexual differentiation that is enzyme type-specific and, probably, genetically predetermined. There is no specificity, however, in the response of MAO-A and MAO-B to changes in the homeostasis of estrogen and, to a lesser extent, androgen throughout perinatal age. In fact, alterations in the enzyme activity probably result from gonadally driven modifications in the structure of the hypothalamus (Gorski et al., 1978), and do not appear to involve the enzyme characteristics. On the other hand, the effects of high concentrations of steroids *in vivo* or *in vitro* may be directly selective for type-A MAO. A similar selectivity reportedly occurs in rat liver MAO-A also (Illsley and Lamartiniere, 1980).

There is a sexual difference also in the selective enzyme inhibition by clorgyline and deprenyl, the inhibition being significantly less intense in the

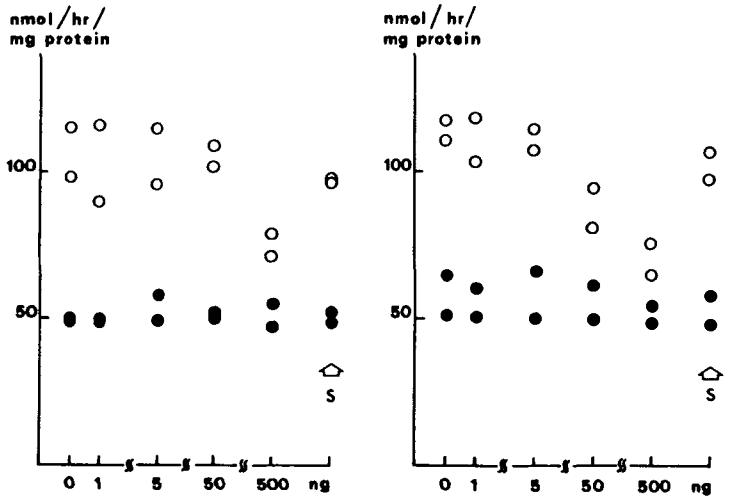

FIG. 2. Direct effects of estradiol (left) and testosterone (right) on MAO-A (○) and MAO-B (●) activity *in vitro*. Aliquots of the hypothalamic supernatant obtained from two male (left) and two female (right), 60-day-old rats, were preincubated for 15 min at 20°C with increasing concentrations of either E or TS, dissolved in 1:20 (v/v) ethanol-water solution (S). Ten microliters of S did not affect by itself MAO activity. Final concentration of 5-HT (MAO-A) or BEA (MAO-B) was 3×10^{-4}M. For further details see Materials and Methods.

female, than in male hypothalamus. The patterns of inhibition can be modified by estrogen administration.

Biochemical differences have fairly evident pharmacological correlates after MAO-B (Vaccari, in preparation) and, to a lesser extent, MAO-A inhibition (Vaccari et al., 1980) in the two sexes. The occurrence of a direct influence of high doses of gonadal steroids on MAO-A activity and on MAO inhibition deserves further investigation, since it might be of a practical importance when steroid and anti-depressant or anti-Parkinson therapies are co-administered.

ACKNOWLEDGMENTS

We are grateful to Dr. Bruce McEwen and Dr. Victoria Luine, The Rockefeller University, New York, and Dr. Paola S. Timiras, University of California, Berkeley, for their helpful criticism and suggestions. This work was supported by grants from the C. N. R. (CT 78.01457.04) and from M. P. I. (1978) to A. V.

REFERENCES

Achee F. M., Togulga G., and Gabay S. (1974) Studies on monoamine oxidases: Properties of the enzyme in bovine and rabbit brain mitochondria. *J. Neurochem.* **22**, 651–661.

Achee F. M., Gabay S., and Tipton K. F. (1977) Some aspects of monoamine oxidase activity in brain. *Prog. Neurobiol.* **8**, 325–348.

Adams C. W., Davison A. N., and Gregson N. A. (1963) Enzyme inactivity of myelin: Histochemical and biochemical evidence. *J. Neurochem.* **10**, 383–395.

Barraclough C. A. (1966) Modification in the CNS regulation of reproduction after exposure of prepubertal rats to steroid hormones. *Recent Prog. Hormone Res.* **22**, 503–539.

Belmaker R. H., Murphy D. L., Wyatt R. J., and Loriaux D. L. (1974) Human platelet monoamine oxidase changes during the menstrual cycle. *Arch. Gen. Psychiatry* **31**, 553–556.

Breuer H., Schneider H. T., Wandscheer D. E., and Ladosky W. (1978) Regulation of catecholamine metabolism in the brain by oestrogens, in *Hormones and Brain Development* (Dörner G. and Kawakami M., eds), pp. 167–174. Elsevier/North-Holland, Amsterdam.

Cawthon R. M. and Breakefield X. O. (1979) Differences in A and B forms of monoamine oxidase revealed by limited proteolysis and peptide mapping. *Nature* **281**, 692–694.

Collins G. G., Pryse-Davies J., Sandler M., and Southgate J. (1970) Effect of pretreatment with oestradiol, progesterone and Dopa on monoamine oxidase activity in the rat. *Nature* **226**, 642–643.

Döhler K. D., and Hancke J. L. (1978) Thoughts on the mechanism of sexual brain differentiation, in *Hormones and Brain Development* (Dörner G. and Kawakami M., eds), pp. 153–158. Elsevier/North-Holland, Amsterdam.

Döhler K. D., and Wuttke W. (1975) Changes with age in levels of serum gonadotropins, prolactin, and gonadal steroids in prepubertal male and female rats. *Endocrinology* **97**, 898–907.

Dörner G. (1978) Hormones, brain development and fundamental processes of life, in *Hormones and Brain Development* (Dörner G. and Kawakami M., eds), pp. 13–25. Elsevier/North-Holland, Amsterdam.

Feine R., Belmaker R. H., Rimon R., and Ebstein R. P. (1977) Platelet monoamine oxidase in women with premenstrual syndrome. *Neuropsychobiol.* **3**, 105–110.

Franz J. R., Hull E. M., Snyder A. M., and Roth J. A. (1978) The effect of maternal progesterone on brain monoamine oxidase activity of neonatal rats. *Brain Res.* **158**, 397–406.

Fuentes J. A. and Neff N. H. (1975) Selective monoamine oxidase inhibitor drugs as aids in evaluating the role of type A and B enzymes. *Neuropharmacology* **14**, 819–825.

Gabay S., Achee F. M., and Mentes G. (1976) Some parameters affecting the activity of monoamine oxidase in purified brain mitochondria. *J. Neurochem.* **27**, 415–424.

Gaziri L. C. and Ladosky W. (1973) Monoamine oxidase variation during sexual differentiation. *Neuroendocrinology* **12**, 249–256.

Gorski R. A., Harlan R. E., and Christensen L. W. (1977) Perinatal hormonal exposure and the development of neuroendocrine regulatory processes. *J. Toxicol. Environ. Health*, **3**, 97–121.

Gorski R. A., Gordon J. H., Shryne J. E., and Southam A. M. (1978) Evidence for a morphological sex difference within the medial preoptic area of the rat brain. *Brain Res.* **148**, 333–346.

Holzbauer M. and Youdim M. B. (1973) The oestrus cycle and monoamine oxidase activity. *Br. J. Pharmacol.* **48**, 600–608.

Illsley N. P. and Lamartiniere C. A. (1980) The imprinting of adult hepatic monoamine oxidase levels and androgen responsiveness by neonatal androgen. *Endocrinology* **107**, 551–556.

Kamberi I. A. and Kobayashi Y. (1970) Monoamine oxidase activity in the hypothalamus and various other brain areas and in some endocrine glands of the rat during the estrus cycle. *J. Neurochem.* **17**, 261–268.

Kastl L., De Ropp R. S., and Furst A. (1970) Heat sensitivity of monoamine oxidase, comparing rodent brain- and liver-derived enzyme. *Proc. West. Pharmacol. Soc.* **13**, 14–15.

Klaiber E. L., Broverman D. M., and Kobayashi Y. (1967) The automatization cognitive style, androgens, and monoamine oxidase. *Psychopharmacologia* **11**, 320–336.

Kueng W., Wirz-Justice A., Menzi R., and Chappuis-Arndt E. (1976) Regional brain variations of tryptophan, monoamines, monoamine oxidase activity, plasma free and total tryptophan during the estrus cycle of the rat. *Neuroendocrinology* **21**, 289–296.

Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Luine V. N. and McEwen B. S. (1977) Effect of oestradiol on turnover of type A monoamine oxidase in brain. *J. Neurochem.* **28**, 1221–1227.

McCaman R. E., McCaman M. W., Hunt J. M., and Smith M. S. (1965) Micro determination of monoamine oxidase and 5-hydroxytryptophan decarboxylase activities in nervous tissues. *J. Neurochem.* **12**, 15–23.

McEwen B. S. (1978) Sexual maturation and differentiation: the role of the gonadal steroids, in *Maturation of the Nervous System* (Corner M. A. et al., eds), pp. 291–307. Elsevier/North-Holland, Amsterdam.

Murad F. and Gilman A. G. (1975) In *The Pharmacological Basis of Therapeutics* (Goodman L. S. and Gilman A., eds), pp. 1423–1471, Macmillan, New York.

Murphy D. L. (1978) Substrate-selective monoamine oxidase-inhibitor, tissue, species and functional differences. *Biochem. Pharmacol.* **27**, 1889–1893.

Murphy D. L. and Buchsbaum M. S. (1978) Neurotransmitter-related enzymes and psychiatric diagnostic entities, in *Critical Issues in Psychiatric Diagnosis* (Spitzer R. L, and Klein D. F., eds), pp. 305–321. Raven Press, New York.

Nies A., Robinson D. S., Lamborn K. R., and Lampert R. P. (1973) Genetic control of platelet and plasma monoamine oxidase activity. *Arch. Gen. Psychiatry* **28**, 834–838.

Redmond D. E., Baulu J., Murphy D. L., Loriaux D. L., Zeigler M. G., and Lake C. R. (1976) The effects of testosterone on plasma and platelet monoamine oxidase (MAO) and plasma dopamine-β-hydroxylase (DBH) activities in the male Rhesus monkey. *Psychosomat. Med.* **38**, 315–326.

Rosecrans J. A. and Schechter M. D. (1972) Brain 5-hydroxytryptamine correlates of behavior in rats: Strain and sex variability. *Physiol. Behav.* **8**, 503–510.

Short D. J. and Woodnott D. P. (1969) In *The I.A.T. Manual of Laboratory Animal Practice and Technique* (Short D. J. and Woodnott D. P., eds), p. 366. Crosby Lockwood & Son, Ltd., London.

Sourkes T. L. (1979) Influence of hormones, vitamins and metals on monoamine oxidase activity. In *Monoamine Oxidase: Structure, Function, and Altered Functions* (Singer T. P., Von Korff R. W., and Murphy D. L., eds), pp. 291–307. Academic Press, New York.

Vaccari A. (1980) Sexual differentiation of monoamine neurotransmitters, in *Biogenic Amines in Development* (Parvez S., and Parvez H., eds), pp. 327–352. Elsevier/North-Holland, Amsterdam.

Vaccari A., Brotman S., Cimino J., and Timiras P. S. (1977) Sex differentiation of neurotransmitter enzymes in central and peripheral nervous systems. *Brain Res.* **132**, 176–185.

Vaccari A., Caviglia A., Gemignani A., and Biassoni R. (1980) Sex-hormones in development: Influence on 5-HT/monoamine oxidase and its inhibition, in *Progress in Psychoneuroendocrinology* (Brambilla F., Racagni G., and de Wied D., eds), pp. 579–590. Elsevier/North-Holland, Amsterdam.

Wilson W. E., Agrawal A. K., and Zeller E. A. (1979) Androgen modulation of brain monoamine oxidase types A and B in the preweanling rat, in *Monoamine Oxidase: Structure, Function, and Altered Functions* (Singer, T. P., Von Korff R. W., and Murphy D. L., eds), pp. 309–315. Academic Press, New York.

Zolovick A. J., Pearse R., Boehlke K. W., and Eleftheriou B. E. (1966) Monoamine oxidase activity in various parts of the rat brain during the oestrus cycle. *Science* **154**, 649.