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Gonadal Hormone Regulation of MAO and Other Enzymes in Hypothalamic Areas

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Abstract. Activities of type A monoamine oxidase (MAO), acetylcholine esterase (AChE), and glucose-6-phosphate dehydrogenase (G6PDH) were differentially altered in hormone-sensitive areas of the preoptic-hypothalamic continuum after administration of estrogen and progesterone. Estrogen increased activity of AChE in the bed nucleus of the stria terminalis and activity of G6PDH in the periventricular area (PVE) of the preoptic area, arcuate-median eminence (Ar-ME) and pituitary. Estrogen decreased activity of MAO in the PVE of the anterior hypothalamus, pars lateralis of the ventromedial nucleus and in the Ar-ME. Acute administration of progesterone (1 h) to estrogen-treated females did not further alter estrogen-dependent changes in AChE or G6PDH; however, MAO activity in the ventromedial nucleus and Ar-ME was rapidly increased after progesterone. Without prior estrogen administration, progesterone did not affect MAO activity. Administration of the protein synthesis inhibitor anisomycin prior to progesterone did not antagonize progesterone-dependent increases in MAO. Progesterone added in vitro to homogenates from estrogen-treated but not from untreated females increased MAO activity. The hormonal specificity, time course of effects and anatomical location of the enzymatic changes suggest that some of them may participate in the mediation of gonadal hormone action in the CNS. In particular, changes in MAO activity in the ventromedial nucleus and Ar-ME are consistent with reported effects of these hormones on monoamine turnover which in turn have been suggested to contribute to hormonal regulation of feminine sexual behavior and gonadotropin secretion.

The investigation of neural events which may underlie the central regulation of neuroendocrine function by gonadal hormones have led to recent studies directed at discrete areas within the preoptic-hypothalamic continuum. The heterogeneity of the hypothalamus demands such anatomically defined sampling since closely aligned cell groupings within this area have been shown to exert inhibitory, facilitative or minimal effects on hormone-dependent sexual behavior and gonadotropin secretion [22]. We and others have reported that the activity of a number of enzymes involved in neurotransmitter and energy metabolism are affected in the hypothalamus or preoptic area by ovariectomy, estrogen treatment or estrous state [21]. These enzymatic changes may be involved in reported gonadal hormone effects on neurotransmitter and energy metabolism [5, 6, 31–33, 36].

In this study, we have sought a finer anatomical resolution for reported gonadal hormone-dependent changes in activities of type A monoamine oxidase (MAO) and acetylcholine esterase (AChE), enzymes responsible for monoamine and acetylcholine degradation, respectively, and in glucose-6-phosphate dehydrogenase (G6PDH), regulatory enzyme of the pentose phosphate pathway of glucose oxidation. Results indicate that enzymatic responses exhibit hormonal and anatomical specificity. In addition, hormone-dependent changes of MAO in the arcuate-median eminence and ventromedial nucleus may contribute to reported effects of hormones on neurotransmitter levels and turnover which in turn may contribute to hormonal effects on gonadotropin secretion and sexual behavior.

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Materials and Methods

Animals and Treatments

Adult, female rats weighing approximately 220 g (Charles River, Wilmington, Mass.) were ovariectomized or ovariectomized-adrenalectomized under Metofane anesthesia. 1 week later they received either daily s.c. injections of estradiol benzoate (Steraloids, Pawling, N.Y.) dissolved in sesame oil at doses and durations indi-

cated or s.c. silastic capsules containing 17β-estradiol (Sigma, St. Louis, Mo.). Capsules were constructed to contain a 1-cm column of packed 17β-estradiol or remained empty to serve as control [18]. In order to produce different levels of circulating estrogen (fig. 1), 17β-estradiol containing Silastics were also made at 0.25, 0.50 and 1.5 cm in length, and to achieve circulating levels less than 40 pg/ml, capsules were made which contained a 1-cm column of 100 μg/ml of estradiol benzoate dissolved in peanut oil. For in vivo experiments, progesterone (Sigma) was dissolved in propylene glycol and 200 μg was administered i.v. (200 μl) via a lateral tail vein. For in vitro experiments, basomedial hypothalamic and pituitary homogenates prepared according to *Luine* et al. [16] received progesterone dissolved in ethanol to equal 10^{-7} and 10^{-8} *M*. Anisomycin, dissolved in acidified saline, was obtained from General Diagnostics (Morris Plains, N.J.).

Tissue Sampling Biochemical Measurements

Brains were removed and mounted on cryostat chucks, sectioned serially at 300 μm beginning at 300 Å [25], 7470 in *König and Klippel* [12], and stored overnight in airtight boxes at -40 °C. Preoptic-hypothalamic nuclei were indentified using atlases [12, 25] and removed with 300 or 500 μm cannulae under a dissecting microscope as described by *Palkovits* [24, 26]. When the arcuate nucleus and median eminence was assayed together, it was obtained by cutting a wedge-shaped area from the ventral aspect fo the hypothalamus. Nuclei sampled and abbreviations used: St, bed nucleus of the stria terminalis; POA, medial preoptic nucleus; AH, anterior hypothalamic nucleus; PVE, periventricular area of the anterior hypothalamus; VML, pars lateralis of ventromedial nucleus; VMN, pars medialis of ventromedial nucleus; A-ME, arcuate nucleus, and median eminence.

Nuclei were expelled onto Teflon homogenizers, homogenized in 60 μl of 50 m*M* sodium phosphate buffer, pH 7, with a motor-driven shaft. Aliquots of 10 μl were removed for protein measurement by the method of *Lowry* et al. [14] (fig. 1; table I, IV) or that of Bradford [2] (fig. 2, 3; table II, III).

Whole pituitaries were removed and stored at - 40 °C. Within a week they were homogenized in 200 μl of 50 m*M*, Tris-HCl, pH 8, and glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49) activity measured fluorometrically as previously described [16].

Monoamine oxidase activity (MAO type A) (EC 1.4.3.4) was measured in 15-μl homogenate aliquots using ^{3}H-5-hydroxytryptamine as previously described [19]. 5-Hydroxytryptamine (serotonin) and norepinephrine are reported to be metabolized by type A MAO while dopamine is metabolized by both type A and B MAO [see ref. 20]. Acetylcholine esterase activity (AChE) (EC 3.1.1.7) was measured radiochemically by the method of *Johnson and Russell* [11] utilizing incubation of 2 μl of homogenate at room temperature. G6PDH activity was measured fluorometrically in 4-μl homogenate aliquots (2 μl for PVE and ME) using the micromethod described by *Luine and Kauffman* [15]. Utilizing these methods, duplicates of MAO and AChE and a single determination of G6PDH could be made in each nucleus from 1 animal. MAO and AChE were measured on day of sampling. G6PDH activity was measured one day after sampling in table I and on day of sampling in table II; activity is higher in table II. For the in vivo treatment studies (tables I–III; fig. 1, 2), groups of 6–8 rats which contained all treatment groups were sacrificed and all enzymes assayed. This procedure was then repeated until the indicated n was achieved.

Estradiol in sera was kindly measured in the laboratory of Dr. *Linda Atkinson* at The Population Council, New York. N.Y. The RIA technique of *Hotchkiss* et al. [10] was utilized with antisera raised against the 6-O-carboxy-methyloximebovine serum albumin conjugate of estradiol. Ovariectomized females gave values of estradiol less than 20 pg/ml serum.

Statistical Analysis

Differences between data consisting of two groups were tested by Student's t test. Data consisting of more than 2 groups were analyzed by analysis of variance and differences between groups were tested by Newman-Keuls procedure (fig. 2, 3; table II) or Dunnet Multiple comparison t statistic (table IV) [35].

Table I. Effect of estradiol benzoate (EB) injections on enzyme activities in preoptic-hypothalamic areas and pituitary of ovariectomized rats

Sample	MAO		G6PDH		AChE	
	SO	EB	SO	EB	SO	EB
Bed nucleus (stria terminalis)	107.0 ± 7	91.2 ± 8	111 ± 24	184 ± 22	1,140 ± 140	1,660 ± 110*
Periventricular area (POA)	79.1 ± 8	60.2 ± 9	328 ± 19	545 ± 44*	1,700 ± 120	1,830 ± 205
Anterior hypothalamic nucleus	95.6 ± 12	124 ± 16	225 ± 49	231 ± 35	1,580 ± 220	1,740 ± 270
Arcuate nucleus	67.0 ± 3	47.8 ± 3*	222 ± 52	392 ± 80	1,400 ± 82	1,270 ± 147
Median eminence	93.0 ± 13	57.5 ± 4.5*	458 ± 75	841 ± 66*	1,280 ± 380	1,170 ± 220
Dorsomedial nucleus	72.2 ± 7.6	99.6 ± 27.1	231 ± 55	229 ± 39	2,050 ± 420	1,940 ± 340
Ventromedial nucleus (lateral)	120.0 ± 21	60.5 ± 9.5*	345 ± 65	318 ± 72	-	-
Pituitary	-	-	634 ± 19	1,100 ± 123**	-	-

Enzyme activities are expressed as nmoles substrate consumed/h/mg protein and are average ± SEM for determinations in 6–8 animals. Female rats were ovariectomized for 1 week and then received 3 days of sesame oil (SO) or 30 μg/220 g body weight of estradiol benzoate (EB). Data analyzed by Student's t test where * $p < 0.05$, ** $p < 0.01$.

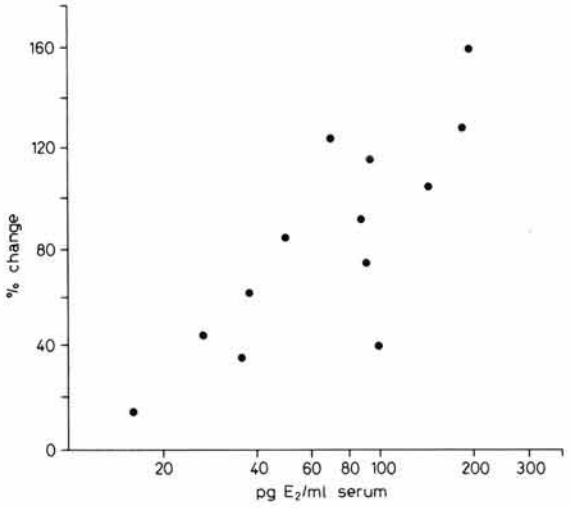

Fig. 1. Pituitary G6PDH activity as a function of circulating estradiol (E_2) 1 week after ovariectomy, females received empty Silastics of Silastics containing varying amounts of estradiol (see 'Methods'). 1 week later, they were killed and serum estradiol and pituitary G6PDH activity were measured. Each point represents values obtained in 13 individual females who received various estrogen-containing Silastics. The percent change in G6PDH activity was calculated as compared to the average value of 6 ovariectomized controls (control value, 430 ± 45 n*M* NADP consumed/h/mg protein).

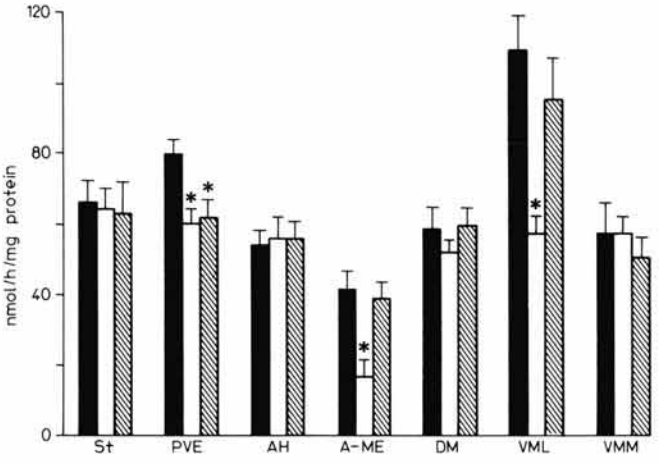

Fig. 2. Effect of estradiol and progesterone on MAO activity in preoptic-hypothalamic nuclei of ovariectomized-adrenalectomized rats. Entries are the mean ± SEM for determinations in 7–8 females. Solid bars are ovariectomized-adrenalectomized (OVX-ADX) females; open bars are estradiol-treated females; striped bars are estradiol and progesterone-treated females. The same animals and treatments were used in table II. Abbreviations can be found in 'Methods'. Data analyzed by Anova, and differences between groups tested by Newman-Keuls procedure. In PVE, estradiol and estradiol + progesterone were significantly different from OVX-ADX but not from each other, $p < 0.05$. In A-ME and VML estradiol was significantly different from OVX-ADX and from estradiol + progesterone, $p < 0.05$.

Results

Effects of Estrogen

Estradiol benzoate (EB) administered for 3 days to ovariectomized rats resulted in increased AChE and G6PDH and decreased MAO activity in some but not all nuclei in the preoptic-hypothalamic area (table I). MAO activity was decreased 30–50% in the arcuate nucleus, median eminence and pars lateralis of the ventromedial nucleus. G6PDH activity increased approximately 60% after EB in the periventricular area of the preoptic area and in the median eminence. Pituitary G6PDH activity also increased approximately 75% after EB administration. Changes in AChE were confined to the bed nucleus of the stria terminalis, where EB administration resulted in a 45% increase in activity.

While results presented in table I have provided a finer anatomical resolution to previously reported estrogen-dependent changes in hypothalamic G6PDH and MAO, EB injections result in variable levels of circulating estrogen. Silastic capsules containing estradiol have been used in many recent neuroendocrine experiments because they give continuous and sustained estrogen release [13, 18, 36]. In a preliminary experiment, the efficacy and reliability of silastics were tested utilizing pituitary G6PDH as a marker for estrogen action. Silastic capsules of various composition and length were implanted in ovariectomized females. 1 week later, animals were sacrificed and plasma estradiol and pituitary G6PDH activity measured. Figure 1 shows that the magnitude of increase in G6PDH was related to levels of circulating estradiol (linear regression, $r = 0.84$, $p < 0.05$). Silastics containing 0.5 and 1 cm of packed estradiol gave circulating values of approximately 90 and 130 pg/ml serum, respectively, values similar to those reported by *Legan* et al. [13]. Silastics containing 1 cm of pure estradiol were used in all subsequent experiments.

Effects of Estrogen and Progesterone

Since not only estrogen but also progesterone influences neuroendocrine-mediated events, the effect of progesterone on the enzymes was also assessed. Female sexual behavior and the LH surge is activated in ovariectomized rats by chronic estrogen and acute progesterone administration [5, 6, 23, 28]. Therefore, to assess effects of progesterone, estrogen silastics were implanted in ovariectomized-adrenalectomized (ADX-OVX) rats for 3 days, on the morning of the 4th day progesterone (200 μg) was given i.v., and all groups were killed 1 h later. Figure 2 shows the effects of this hormone regimen on MAO activity. As was found after EB injections, activity in the arcuate-median eminence and lateral ventromedial nucleus was decreased by estradiol in silas-

Table II. Effect of estradiol (E_2) and progesterone (P) on enzyme activities in preoptic-hypothalamic areas and pituitary of ovariectomized-adrenalectomized (O-A) rats

Sample	G6PDH			AChE		
	O-A	+E_2	+E_2+P	O-A	+E_2	+E_2+P
Bed nucleus (stria terminalis)	585 ± 57	776 ± 69	623 ± 58	1,120 ± 125	1,560 ± 118*	1,550 ± 96*
Periventricular area (anterior hypothalamus)	1,077 ± 89	818 ± 57	937 ± 65	1,451 ± 27	1,303 ± 234	1,540 ± 110
Anterior hypothalamic nucleus	830 ± 58	727 ± 56	750 ± 63	1,240 ± 126	1,470 ± 260	1,110 ± 150
Dorsomedial nucleus	858 ± 129	975 ± 107	832 ± 99	1,430 ± 250	1,540 ± 110	1,470 ± 220
Ventromedial nucleus (lateral)	868 ± 109	758 ± 50	879 ± 123	710 ± 72	621 ± 60	655 ± 55
Ventromedial nucleus (medial)	924 ± 122	891 ± 67	950 ± 140	1,200 ± 240	1,220 ± 338	1,310 ± 251
Arcuate-median eminence	1,360 ± 130	1,990 ± 186*	2,040 ± 205*	543 ± 62	590 ± 32	576 ± 50
Pituitary	589 ± 32	1,115 ± 237**	1,007 ± 66**	-	-	-

Enzyme activities are expressed as nmoles substrate consumed/h/mg protein and are average ± SEM for determinations in 6 animals. 1 week after ovariectomy-adrenalectomy rats received s.c. empty silastic capsules or silastic capsules containing 1 cm of packed estradiol (E_2) for 3 days. On day 4, one E_2 group received 200 µg of progesterone (P) i.v. (others vehicle). 1 h after P, all groups were sacrificed. Data analyzed by Anova and differences between groups tested by Newman-Keuls procedure where * different from OVX-ADX but not from each other, * $p < 0.05$, ** $p < 0.01$.

Table III. Effect of progesterone on MAO activity in preoptic-hypothalamic areas of ovariectomized-adrenalectomized (OVX-ADX) rats

Group	St[1]	POA	AH	PVE	VML	VMM	A-ME
OVX-ADX	81.7 ± 20	61.0 ± 13	58.2 ± 6	88.4 ± 9	163 ± 13	94.1 ± 13	118 ± 7
OVX-ADX + progesterone	76.9 ± 14	54.3 ± 9	54.7 ± 5	82.7 ± 9	155 ± 21	82.1 ± 8	131 ± 27

Entries are the mean ± SEM for determinations in 5–6 females. 1 week after OVX-ADX, rats received 200 µg of progesterone or vehicle i.v. They were sacrificed 1 h later and MAO activity measured. No significant differences by Student's t test. See 'Methods' for abbreviations.

[1] Expressed as nmol 5HT consumed/h/mg protein.

tic capsules. In addition, activity of MAO in the periventricular area (PVE) of the anterior hypothalamus was measured, and estrogen administration decreased it. Progesterone administration resulted in a reversal of the estrogen effect in the arcuate-median eminence and the ventromedial nucleus. Activity of MAO in estrogen-progesterone treated rats was rapidly increased within 1 h to values found in OVX-ADX females. Activity in the PVE was not affected by progesterone, values in estrogen-progesterone-treated females were the same as in estrogen-treated females.

Activities of G6PDH and AChE in estrogen and estrogen-progesterone treated females are shown in table II. Estrogen-dependent increases in G6PDH and AChE obtained with estradiol-containing silastics occurred in the same hypothalamic areas at approximately the same magnitude as changes found after EB injections. Unlike MAO, activity of these enzymes was unaffected by progesterone treatment. G6PDH activity increased approximately 45% in the arcuate-median eminence and 90% in the pituitary of estradiol-treated females, and progesterone did not further alter activity. AChE in the bed nucleus of the stria terminalis was increased by approximately 40% with estradiol treatment, and values for estradiol-progesterone treatment were not different from estrogen treatment alone.

Mechanisms of Estrogen-Progesterone Effect on MAO

Effects of gonadal hormones on MAO activity were futher investigated in terms of estrogen dependency and mechanism for the progesterone effect. Table III shows the effect on MAO activity of acute progesterone administration to OVX-ADX rats. Without prior estrogen administration, progesterone did not alter MAO activity. Thus, estrogen pretreatment appears necessary for progesterone to alter MAO activity.

The role of protein synthesis in the MAO response was next investigated since progesterone has been shown to translocate neural progesterone receptors to the cell nucleus within 15 min [23]. Thus, increased MAO activity might oc-

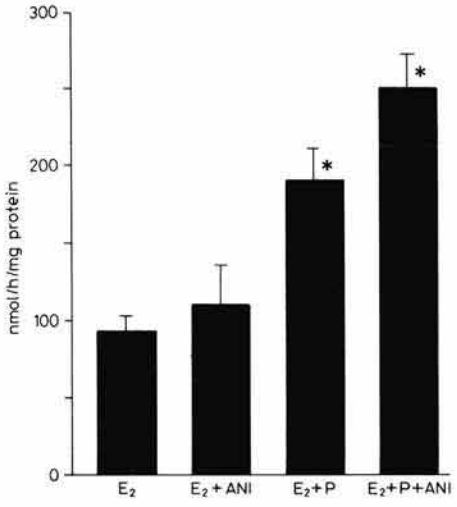

Fig. 3. Effect of anisomycin on progesterone-dependent increase in lateral ventromedial nucleus MAO activity. 1 week after ovariectomy-adrenalectomy rats received s.c. Silastic capsules containing 1 cm of packed estradiol (E_2) for 3 days. On day 4, rats were divided into 4 groups, two of which received 100 mg/kg s.c. of anisomycin (ANI) and the others saline. Half an hour after ANI, progesterone (P) or propylene glycol was given i.v. Rats were killed 1 h after progesterone administration. Entries are the mean ± SEM for determinations in lateral ventromedial nucleus from 5 animals. Data analyzed by Anova and differences between groups tested by Newman-Keuls procedure where * different than other groups, but not from each other, $p < 0.05$.

cur through increased synthesis of new MAO enzyme by progesterone. This mechanism was tested utilizing the protein synthesis inhibitor, anisomycin, which inhibits protein synthesis in the basomedial hypothalamus by approximately 90% from 30 min to 2 h after a s.c. 100 mg/kg dose [28]. Estrogen-treated females were given anisomycin, followed ½ h later by progesterone and then killed 1 h after progesterone administration. Figure 3 shows that anisomycin did not antagonize the progesterone-dependent increase in MAO activity in estrogen-treated females. Thus, under these conditions, increased MAO activity in the ventromedial nucleus after progesterone administration does not appear to be mediated by protein synthesis.

Progesterone-dependent effects on MAO activity were also investigated by adding progesterone to homogenates of the basomedial hypothalamus from OVX-ADX females and from OVX-ADX females who received 3 days of estrogen treatment. Table IV shows that, similar to in vivo results, progesterone at 1×10^{-7} and 10^{-8} *M* increases MAO activity in estrogen-treated females but not in untreated females. The in vitro effect in basomedial hypothalamic homogenates was smaller than the in vivo effect in the arcuate-median eminence or ventromedial nucleus. Pituitary G6PDH activity is unaltered after progesterone addition to homogenates from estrogen-treated females, a result similar to in vivo findings.

Table IV. Effect of in vitro progesterone (P) on MAO and G6PDH activity

Group	BM-HYP MAO	Pit G6PDH
OVX-ADX	64.6 ± 3.2	-
+P (10^{-8} *M*)	69.6 ± 3.2	-
+P (10^{-7} *M*	65.9 ± 1.8	-
OVX-ADX+EB	50.0 ± 2.5	1,075 ± 79
+P (10^{-8} *M*	60.8 ± 3.3*	1,114 ± 49
+P (10^{-7} *M*)	61.5 ± 2.7*	976 ± 43

Enzyme activity is expressed as nmoles 5HT or NADP consumed/h/mg protein. Entries are the average ± SEM for determinations in 8 animals for MAO and 4 animals for G6PDH. 1 week after surgery, rats received 3 days of sesame oil or EB (15 µg/day). They were sacrified and homogenates made of basomedial hypothalamus (BM-Hyp) and pituitary (Pit). Three aliquots of each homogenate were taken: one received ethanol vehicle, and the others received progesterone to equal 1×10^{-8} *M* and 1×10^{-7} *M*. 30 min later enzyme activities were measured. Differences between groups were tested by Dunnett's multiple t test where * $p < 0.05$.

Discussion

Results of this study demonstrate that the activities of MAO, G6PDH and AChE are altered by gonadal hormones in estrogen-sensitive areas of the preoptic-hypothalamic continuum. G6PDH activity was increased by estrogen in the periventricular area of the preoptic area, the arcuate-median eminence and in the pituitary. AChE was increased by estrogen in the bed nucleus of the stria terminalis. MAO activity was decreased in the periventricular area of the anterior hypothalamus, pars lateralis of the ventromedial nucleus and in the arcuate-median eminence after estrogen administration to ovariectomized rats. Areas showing enzymatic responses share the common property of containing high levels of receptor proteins for estradiol or receive direct projections from receptor containing areas (median eminence) [30]. These results have provided a finer anatomical resolution to previous studies which showed that injection of larger doses of estradiol for extended periods would alter activity of these enzymes in the wohle hypothalamus. While such studies utilized large, heterogeneous brain areas, they showed that changes in MAO and G6PDH after estrogen were directly related to estrogen since they were blocked by the estrogen antagonist MER-25, showed a dose-response relationship to estrogen, hormonal specificity and occured in hypophysectomized and adrenalectomized females [16–18].

Administration to OVX-ADX females of progesterone for 1 h after estrogen priming for 3 days, a paradigm similar to those used previously to initiate LH surges and facilitate female sexual behavior [5, 6, 23, 28, 36], also produced en-

zyme-specific responses. Increases in G6PDH and AChE activity after estrogen administration were not altered by subsequent progesterone administration, but activity of MAO, which was lowered by estrogen administration in some nuclei was rapidly increased after progesterone treatment to levels in untreated OVX-ADX females. This synergistic effect of estrogen plus progesterone on MAO activity, which was confined to the arcuate-median eminence and pars lateralis of the ventromedial nucleus, is of interest in relation to monoaminergic regulation of neuroendocrine function since these two hypothalamic areas are of critical importance in mediating hormone effects on gonadotropin secretion and feminine sexual behavior, respectively.

Monoaminergic activity within the arcuate-median eminence has been implicated in release of LH, and recent studies show that progesterone enhances turnover of norepinephrine in the median eminence of estrogen-treated ovariectomized females [5, 36]. Increased MAO activity in the median eminence after progesterone administration may participate in this enhanced norepinephrine turnover. Monoamines have also been implicated in regulation by gonadal hormones of female sexual behavior [25]. Based on studies from several neuroscience subdisciplines, the ventromedial nucleus is a major locus for central regulation of hormone-dependent female sexual behavior [27]. Thus, estrogen-progesterone-dependent changes in MAO activity in pars lateralis of this nucleus may provide a nexus for integration of these two observations.

Specific sites in the brain where norepinephrine and dopamine may modulate sexual behavior have not been localized [22], but results of pharmacological studies suggest that 5HT present in the area of the ventromedial nucleus may exert tonic inhibition on the display of lordosis behavior [7, 9, 19, 20]. Infusion of serotonin into the area of the ventromedial nucleus but not the lateral hypothalamus significantly reduces lordotic responding [9], and pargyline, a MAO inhibitor which elevates serotonin levels [20], rapidly inhibits lordotic responding when implanted in the ventromedial but not the dorsomedial nucleus of estrogen-progesterone-primed females [19]. Thus, the rapid increase in MAO activity in the ventromedial nucleus which we find after progesterone administration may serve as one of many possible mechanisms for lowering 5HT levels and releasing behavior from tonic inhibition. However, the role, if any, of hormone-dependent changes in MAO activity in the regulation of LH secretion and feminine sexual behavior awaits results of further studies utilizing more physiological levels of hormones and application of drugs which show greater specificity among monoaminergic neurons.

Changes in the degradatory enzyme for acetylcholine, AChE, occured in only one of the nuclei sampled, the bed nucleus of the stria terminalis. The role of cholinergic systems in hormone-dependent neuroendocrine events is not as well characterized as monoaminergic components; however, changes in Nist AChE activity are consistent with the observation that stereotaxic application of cholinergic agents into the Nist or dorsal POA alters female sexual behavior [4].

The precise mechanism whereby gonadal hormones alter MAO activity is largely unknown. Previous work indicated that estrogen treatment decreases activity of MAO A but not MAO B, and the turnover rate of MAO A is enhanced by estrogen [18]. Enhanced turnover of the enzyme was due to an increased rate constant for degradation without a change in the rate constant for synthesis. Estrogen-dependent decreases in MAO A, unlike progesterone-dependent changes in MAO, require some hours to develop. Direct addition of estrogen to homogenates does not alter MAO activity [17], nor does 1 h of estrogen i.v. [18]. However, 12 h after estradiol benzoate administration, MAO activity is decreased in some brain areas [3]. The ability of progesterone to rapidly increase MAO activity in the ventromedial nucleus does not appear to depend on enzyme synthesis since the protein synthesis inhibitor, anisomycin, did not block the progesterone-dependent increase. The observation that progesterone addition to hypothalamic homogenates from estrogen-treated females increases MAO activity suggests a direct activation of MAO enzyme protein by progesterone, a progesterone receptor complex or a second messenger.

Previous studies of MAO have shown the lipid microenvironment of the enzyme to be critical for activity [8] and recently *White and Stine* [34] reported that MAO A and B are distinct enzymes embedded in the phospholipid matrix of the mitochondrial membrane. Further, the activity of MAO A depends critically on the phospholipid microenvironment whereas that of MAO B does not. *White and Stine* [34] speculated that 'activity of MAO A may be easily influenced by endogenous factors to allow for a subtle metabolic regulation of certain biogenic amines in vivo'. At present, effects of gonadal hormones on MAO A and B activity are consistent with their findings. Estradiol, either through a direct or a genomically mediated action on mitochondrial membranes may alter the lipoprotein microenvironment of MAO A resulting in faster degradation or loss of catalytic efficiency. Alterations in the membrane may also provide a milieu suitable for progesterone to alter MAO activity. While effects of estradiol have been traditionally viewed as occuring through genomic mediation, direct membrane effects of estradiol have also been noted, e.g., a lowering of the B_{max} for 5HT-1 receptors in brain [1]. The rapid effects of progesterone measured electrophysiologically or behaviorally have always led to speculation for nongenomic effects of this hormone in the CNS [7]. It should be noted that other estrogen- and progesterone-mediated events occur in the ventromedial nucleus which involve protein synthesis, and these events appear to be crucial for activation of feminine sexual behavior [28, 29].

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