Original  EFFECTS OF ESTRADIOL AND PROGESTERONE
ON MONOAMINE OXIDASE ACTIVITY
IN VARIOUS REGIONS OF RAT BRAIN AND
ENDOMETRIUM

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Abstract: The effects of 17β-estradiol and progesterone on MAO activity in seven regions of the brain and in their target organ, the endometrium, were examined. In intact animals in diestus, (confirmed by vaginal smears), MAO activity in various regions of the brain decreased in the following order: hypothalamus > hippocampus > cortex-striatum > cerebellum > midbrain > medulla oblongata. The activity in the medulla oblongata was about half that in the hypothalamus. In ovariectomized rats 14 days after operation the activities in the various regions of the brain were not significantly different from those in normal rats. The MAO activity in the endometrium was 2.6 times higher in ovariectomized rats than in intact animals. Injection of 17β-estradiol for three consecutive days decreased MAO activity in the brain, the decrease being greater in ovariectomized rats. The decrease was greatest (13-15%) in the cortex of intact rats and the cerebellum, hypothalamus and cortex of ovariectomized rats. Injection of progesterone (4 mg/kg) for three consecutive days caused a significant increase in MAO activity in the cortex of intact rats; injection of progesterone (2 mg or 4 mg/kg) for three consecutive days caused a slight, but not significant, increase in MAO activity in the hypothalamus and medulla oblongata. Injection of 19-norethisterone (0.4 mg/kg) for three consecutive days caused a slight increase in MAO activity in the hypothalamus only. A single injection of 17β-estradiol (0.2 mg/kg) increased MAO activity significantly in the midbrain after two hours, but did not affect the activity in other areas. A single injection of progesterone (4 mg/kg) caused no changes in MAO activity. Injection of 17β-estradiol (0.2 mg/kg) for three consecutive days decreased MAO activity significantly in the endometrium, especially in ovariectomized rats. Progesterone increased endometrium MAO activity significantly in intact rats, but not in ovariectomized rats. No significant differences were observed in the weight or protein content of the brain of intact or ovariectomized rats after repeated injections of 17β-estradiol (0.2 mg/kg, s.c.), progesterone (2 mg or 4 mg/kg, s.c.) or 19-norethisterone (0.4 mg/kg, s.c.) or single injection of 17β-estradiol (0.2 mg/kg, i.p.) or progesterone (4 mg/kg, i.p.) compared with the values of the controls. Injection of 17β-estradiol (0.2 mg/kg) for three consecutive days caused a significant increase in tissue weight of the endometrium of ovariectomized rats. It also increased the uterine weight of intact rats and the protein content of intact and ovariectomized rats, but the changes from the control values were not statistically significant. Repeated injections of progesterone did not affect the uterine weight or the protein content of intact or ovariectomized rats.

Pugh and Quastel first demonstrated the existence of monoamine oxidase (MAO) (EC 1.4.3.4) in rat brain in 1937. Since then there have been many studies on the enzymological properties and distribution of the enzyme in various organs and tissues.

Noradrenaline was identified as a neurochemical transmitter released from peripheral adrenergic nerve endings, and catecholamine and serotonin were suggested to be putative neurochemical transmitters in the brain. The dynamic changes and inhibition of
MAO, which is involved in the metabolism of these biogenic amines, have been investigated in relation to mental functions, and the pathogenesis and therapy of various types of psychosis.

Extensive studies have shown that many factors are involved in the actions of hormones on their target organs, but details of the mechanisms of their actions and their involvement in the mechanisms of metabolic regulation by enzymes have not been fully clarified.

With regard to the effect of sex hormones on MAO activity in brain, it has been reported that MAO activity is decreased in the brain of ovariectomized rats by administration of estradiol, but not by progesterone. However, there are no reports on hormonal effects on MAO activity in various regions of the brain.

The present paper reports the effects of 17β-estradiol and progesterone on MAO activity in the cerebellum, medulla oblongata, hypothalamus, striatum, hippocampus, midbrain and cortex of intact and ovariectomized rats, and in comparison with their effects on the endometrium, which is the target organ of these two hormones.

**Materials and Methods**

*Experimental animals and enzyme preparations*

The animals used were normal female Sprague-Dawley strain rats of 10-weeks-old (weighing about 200 g) and ovariectomy was performed 14 days before used. The effects of single or repeated injections of steroids on MAO activity in the two groups were investigated. Intact rats were given repeated subcutaneous injections of 0.2 mg/kg of 17β-estradiol or 4 mg/kg of progesterone in olive oil once a day (at 10:00 A.M.) for 3 consecutive days. Ovariectomized rats were injected with 0.2 mg/kg of 17β-estradiol, 2 mg/kg or 4 mg/kg of progesterone or 0.4 mg/kg of 19-norethisterone in the same way. Animals were killed by decapitation 24 hr after the last injection. In other experiments, ovariectomized rats were given a single intraperitoneal injection of 0.2 mg/kg of 17β-estradiol or 4 mg/kg of progesterone suspended in saline and killed by decapitation 2 hr later. Control animals were given repeated injections of an equal volume of olive oil or a single injection of saline.

The brain and uterus were rapidly removed, and the cerebellum, medulla oblongata, hypothalamus, striatum, hippocampus, midbrain and cortex were separated as described by Glowinski and Iversen. The tissues were weighed and homogenized in 0.1 M Tris-HCl buffer (pH 7.5) to give a 2% (v/w) homogenate. Samples of 0.25 ml of homogenates of various regions of the brain and 0.75 ml for the homogenate of endometrium were used for enzyme assay.

*Assay of MAO activity*

Tyramine hydrochloride, at a final concentration of 1 mM containing 4×10^{-10} M of tyramine-2,14C-hydrochloride (specific activity 50 mCi/mmol, New England Nuclear Co.) was used as substrate and the incubation was carried out under air at 37°C for 30 min. MAO activity was expressed as nmoles of product formed/mg of protein in 30 min.

*Measurement of protein content*

In the presence of 0.1 N sodium cholate, protein concentrations were measured by the method of Lowry et al. using bovine serum albumin as a standard.

**Results**

*MAO activity in various regions of rat brain and effect of ovariectomy on MAO activity*

The MAO activities in 7 regions of the brain of rats in diestrus were determined with tyramine as substrate and compared with those of rats 14 days after ovariectomy. As shown in Fig. 1, MAO activity was highest in the hypothalamus and decreased in the following order: hypothalamus > hippocampus > cortex > striatum > cerebellum > midbrain > medulla oblongata. MAO activity in the medulla oblongata was about
Fig. 1 MAO activity in seven regions of the brain of intact and ovariectomized rats. MAO activity is expressed as nmoles of reaction product formed from tyramine/mg of protein in 30 min. Columns and vertical bars represent means ± S. E. (N=3). : intact, : ovariectomized rat (14 days after operation)

50% of that in the hypothalamus. MAO activity was slightly but not significantly, higher in these regions in ovariectomized rats than in intact rats (Fig. 1).

Effects of 17β-estradiol and progesterone on MAO activity in brain

The effects of repeated injections of 17β-estradiol and progesterone on MAO activity in the brain of intact rats are shown in Fig. 2. At 24 hr after the last injection of 17β-estradiol (0.2 mg/kg) for 3 consecutive days, the activity in the cortex was decreased significantly, but the decreases in activity in hypothalamus, striatum, hippocampus and midbrain (6-12% of the control) were not significant. Thus estradiol had no significant effect.

Injection of progesterone (4 mg/kg) for 3 consecutive days increased MAO activity in the cortex significantly, and increased the activity in the cerebellum, medulla oblongata, hypothalamus and midbrain of the progesterone treated group slightly, but not significantly (Fig. 2).

Fig. 3 shows the effects of repeated injections of these steroids once a day for 3 consecutive days, beginning 14 days after ovariectomy. Injection of 17β-estradiol (0.2 mg/kg) decreased MAO activity in all regions of the brain, and especially the cerebellum,
hypothalamus and cortex, where the decrease was about 13 to 15%. Repeated subcutaneous injections of progesterone (2 or 4 mg/kg) caused a slight, but not significant or dose-dependent, increase in MAO activity in the hypothalamus and medulla oblongata (Fig. 3). It did not affect the activity in the other 5 areas of the brain. This confirms previous reports that the injection of progesterone into rats did not affect MAO activity in the brain\(^3\). Progesterone (2 or 4 mg/kg) also had no significant effect on MAO activity in the 7 regions of the brain of ovariectomized rats. The synthetic steroid 19-norethisterone (0.4 mg/kg) caused a slight increase in MAO activity in the hypothalamus, but had no significant effect on the activity in other regions of the brain.

The changes in MAO activity 2 hr of single injections of steroids into ovariectomized rats are shown in Fig. 4. Intraperitoneal injection of 17\(\beta\)-estradiol (0.2 mg/kg) caused a significant decrease in MAO activity in the midbrain, a slight (4 to 7%), but not significant, decrease in the activity in the medulla oblongata and cortex, no change in the striatum and hippocampus, and slight but not significant increases in other regions.

Progesterone (4 mg/kg) caused no significant change in MAO activity in any region of the brain.

**Effects of 17\(\beta\)-estradiol and progesterone on MAO activity in the endometrium**

Steroids were injected into intact and ovariectomized rats once a day for 3 consecutive days. Results on MAO activity with tyramine as substrate are shown in Fig. 5. In intact animals, 17\(\beta\)-estradiol (0.2 mg/kg) decreased the MAO activity in the endometrium to 64% of the control activity, and progesterone (4 mg/kg) increased the activity to about 1.9 times that of the control. Injections were started when the rats were in diestrus and animals were killed in the stage of estrus II (17\(\beta\)-estradiol group), diestrus (progesterone group) or estrus I (control group).

![Fig. 5 Effects of estradiol and progesterone on MAO activity in rat endometrium.](image)

**Fig. 5** Effects of estradiol and progesterone on MAO activity in rat endometrium. 

- □: control (olive oil), □: 17\(\beta\)-estradiol (0.2 mg/kg), □: progesterone (4 mg/kg).

The procedure was as for Fig. 2. Results are expressed as nmoles of reaction product formed from tyramine/mg of protein in 30 min and are shown as means±S.E. (N=3). * and **: Significantly different from the control at p≤0.05 and p≤0.01, respectively. ***: Significantly different from the ovariectomized control (p≤0.01).
In ovariectomized rats, MAO activity in the endometrium was about 2.6 times that in the controls. Repeated injections of 17β-estradiol (0.2 mg/kg) significantly decreased MAO activity to about 29.6% that of the controls, but injection of progesterone (4 mg/kg) resulted in no significant increase in the activity compared with that in the controls. At the time of sacrifice, the 17β-estradiol treated group were in estrus II, and the control and progesterone treated groups were in diestrus, judging from vaginal smears.

Effects of 17β-estradiol and progesterone on the weight and protein content of the endometrium

In intact rats 24 hr after injection of 17β-estradiol (0.2 mg/kg) for 3 consecutive days there was a slight, but not significant, increase in the weight of the endometrium (estrus I). Injection of progesterone (4 mg/kg) also had no effect on the weight of the endometrium.

The weight of the endometrium was about 35% less in ovariectomized rats than in intact animals. In ovariectomized rats, injection of 17β-estradiol (0.2 mg/kg) for 3 consecutive days increased the tissue weight to about 4 times that of the controls. Injection of progesterone (4 mg/kg) for 3 consecutive days had no significant effect (Fig. 6).

As shown in Fig. 6, in intact rats the protein content of the endometrium increased slightly after injection of 17β-estradiol, and decreased slightly after injection of progesterone, but the changes were not significant.

In ovariectomized rats, the protein content of the endometrium was 28.1% less than that in intact animals. Injection of 17β-estradiol increased the protein content to the level in intact animals, but this increase was not significant. Injection of progesterone had no significant effect on the protein content of the endometrium in ovariectomized animals.

Discussion

In the present study the effects of 17β-estradiol, progesterone and 19-norethisterone...
on MAO activity in the seven regions of brain and the endometrium of intact and ovariectomized rats were examined. In the brain of intact rats, MAO activity was found to be highest in the hypothalamus, followed in order by the hippocampus, cortex, striatum, midbrain cerebellum and medulla oblongata. These results are in agreement with the results of histochemical studies reported by Shimizu et al. The enzyme activities in these regions of the brain were increased slightly, but not significantly, 14 days after ovariectomy. Vaginal smears showed that the ovariectomized rats were in diestrus. Kobayashi et al. reported that MAO activity in the anterior and posterior hypothalamus increased between 21 to 60 days after ovariectomy and then returned to normal. They observed no significant change in the activity in the frontal cortex from 5 to 150 days after ovariectomy. There are also reports that administration of estradiol to rats decreased the MAO activity in whole brain, but that administration of progesterone did not affect the activity. However, in these previous experiments, since MAO activity was measured in whole brain, the exact site of action of estradiol was not clarified and the effect of progesterone on different regions of the brain was not examined. Therefore, in the present study, changes in activity in seven separate regions of the brain were examined.

In intact rats a single intraperitoneal injection of 17β-estradiol (0.2 mg/kg) caused a significant decrease in MAO activity in the midbrain, but not in the other regions, and a single injection of progesterone (4 mg/kg) caused no significant change of MAO activity in any region of the brain. Repeated injections of estradiol (0.2 mg/kg) caused a significant decrease in MAO activity in the cortex of intact and ovariectomized rats, and slight decreases in activity in the hypothalamus, striatum, hippocampus and midbrain of intact animals, and in all regions of ovariectomized rats. These decreases in activity were greater in ovariectomized rats than in intact rats. Progesterone caused a significant increase in MAO activity in the cortex of intact animals, and a slight increase in activity in the hypothalamus of intact and ovariectomized rats. 19-Norethisterone also caused only a slight increase in the activity in the hypothalamus of ovariectomized animals. Histochemical studies have demonstrated that the hypothalamus is rich in monoamine containing neurons and the monoamine content of this region varies during the estrous cycle. MAO activity in this area increased in proestrus and decreased in metoestrus. The decrease in MAO activity in this area observed in the present study after treatment with estradiol seems to be compatible with the decrease in enzyme activity during the metoestrous phase reported by Kamberi.

Increase in MAO activity in the endometrium, a target organ of these steroids, was about 2.6 times greater in ovariectomized rats than in intact animals. Repeated injection of estradiol decreased the activity in the endometrium of both intact and ovariectomized rats, the decrease being greater in ovariectomized rats, as in the case of brain. Repeated injections of progesterone resulted in a significant increase in MAO activity only in intact animals. Thus the effect of estradiol on MAO activity was greater in ovariectomized rats, whereas that of progesterone was greater in intact animals where hormonal regulation is normal. This difference between the actions of estradiol and progesterone may be due to various factors including the dose and duration of the hormonal action and the endocrinological conditions of the animals; namely, progesterone stimulates release of the luteinizing hormone in the presence of estrogen in ovariectomized rats. It was reported that MAO activity in the uterus was not changed by a single injection of norethynodrel, a synthetic progestin with a strong progestational action, but was increased by a small amount of estrogen. These reports, suggesting that progesterone exerts its effect
only under certain endocrinological conditions, support the present findings that progesterone was less effective in ovariectomized rats than in intact rats. Fuxe and Hökfelt\(^5\) reported that dopamine in the hypothalamus had a strong regulatory influence on secretion of gonadotropic hormones. From this finding and the present results indicating that brain MAO activity did not change in ovariectomized rats and that the effects of steroids differ in ovariectomized and intact animals, it is conceivable that the effects of hormones on MAO activity, which may have a role in metabolism of neurotransmitters, may not be direct effects, but secondary effects through the action of catecholamines. However, Yoshihara et al.\(^17\) recently reported evidence that sex steroids have no direct action on tyrosine hydroxylase activity, a rate-limiting enzyme in the biosynthesis of catecholamines. The mechanism of the action of steroids on MAO activity has not been fully clarified, though it has been suggested\(^14\) that they may 1) act as allosteric effectors 2) to change the permeability of the outer membrane of mitochondria in which MAO is located.

There are reports that injection of estradiol induced increase in weight and protein synthesis in the uterus\(^18,19\). In the present study, repeated injection of estradiol also caused a significant increase in endometrial weight in ovariectomized rats, a slight increase in weight in intact rats and a slight increase in protein content in intact and ovariectomized rats. On the other hand, injection of progesterone did not have any effect on the endometrium. Thus, the significant increase in MAO activity in progesterone-treated intact rats and decrease in activity in 17β-estradiol-treated ovariectomized rats do not seem to be due to changes in protein content.

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References

ラット脳各部位および子宮内膜中の monoamine oxidase に対する estradiol および progesterone の影響

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従来の MAO 活性に対する性ホルモンの研究では全脳を使用して mashed されている可能性がある。そこで脳を 7 部位に分け脳内 MAO 活性に対する性ホルモン（estradiol および progesterone）の影響を無処置および卵巣摘出ラットを用い詳細に検討し、同時にこれらを含む標的器官である子宮についても内膜を用い同様に検討した。ラットの脳を Glowinski & Iversen の方法に従い 7 部位に分け 0.1 M-Tris-HCl buffer (pH 7.5) にて homogenate を作製した。子宮内膜についても同様にして homogenate を作製し、MAO 活性はこれら homogenate を酵素材料とし 14C-tyramine (50 mCi/m mole) を基質として測定した。MAO 活性に対する steroids の影響は反復あるいは 1 回投与について観察した。反復投与では estradiol 0.2 mg/kg, progesterone 2, 4 mg/kg, 19-norethisterone 0.4 mg/kg を 1 日 3 回間隔で皮下注射し、1 回投与では estradiol 0.2 mg/kg, progesterone 4 mg/kg を腹腔内注射し次の結果を得た。

無処置ラットの脳内 MAO 活性は hypothalamus で最も高く、以下 hippocampus ＞ cortex ≧ striatum ＞ cerebellum ＞ midbrain ＞ medulla oblongata であり medulla oblongata の活性値は hypothalamus の約 1/2 であった。この無処置ラットの各部位の MAO 活性値と卵巣摘出 14 日後のラットの活性値との間には有意な差は認められなかった。Steroids の反復投与による脳内 MAO 活性に対する影響は estradiol 投与では無処置ラットの cortex, 卵巣摘出ラットの cerebellum, hypothalamus, cortex で対照の約 13〜15％の低下が認められた。他の部位でも低下傾向がみられ、その程度は卵巣摘出ラットで著しかった。Progesterone の反復投与では無処置ラット cortex で活性の上昇がみられた。他にやや高値を示す部位もあったが有意差、投与量との関係は認められなかった。Progestational 作用のより強力な 19-norethisterone でも同様であり MAO 活性に殆ど影響は認められなかった。Steroids の 1 回投与による脳内 MAO 活性への影響は estradiol により midbrain に活性の低下が認められたが他の部位では有意な差は認められず、progesterone 投与ではいずれの部位でも変動はなかった。これら steroids の標的器官である子宮内膜における MAO 活性については卵巣摘出により無処置ラットの約 2.6 倍にまで上昇し、steroids の反復投与による影響は estradiol 投与では著明な活性の低下が認められ、特に卵巣摘出ラットではその傾向が強く無処置対照群のレベル以下の値にまで低下した。Progesterone の反復投与は estradiol の場合とは異なり卵巣摘出ラットにおいては影響は認められなかったが無処置ラットでは子宮内膜 MAO 活性を対照の約 1.9 倍にまで上昇させた。

すなわち estradiol による脳内 MAO 活性への影響は特定の部位でなく広範囲にみられ、progesterone による影響は殆ど認められず、またその標的器官である子宮内膜の MAO 活性に対しても内分泌環境が normal な無処置動物では活性を増加させるが卵巣摘出動物では変動しないことなどからこれら steroids の MAO 活性に対する影響は直接的なものではないように考えられる。

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