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Total monoamine oxidase activity in the hypothalamus, ovary and uterus of rats with an extreme number of ovarian corpora lutea

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The activity of total monoamine oxidase (MAO) in the rat ovary and uterus fluctuates significantly under various physiological conditions. We analyzed total MAO activity in the hypothalamus, uterus and ovary in adult rats, having an extreme number of corpora lutea (hyperluteinized ovaries) resulting from the mechanical lesions in the posterior hypothalamic region of neonatal rats. Total MAO activity in the hypothalamus (30.21±1.53 pmol/mg tissue/min) and uterus (3.16±0.61 pmol/mg tissue/min) of rats with hyperluteinized ovaries did not show a significant difference as compared to that of intact controls (31.09±1.72 and 2.90 ± 0.40 pmol/mg tissue/min, respectively). In contrast, in the ovaries of hyperluteinized rats, total MAO activity (21.16±1.70 pmol/mg tissue/min) was significantly higher ($p<0.01$) when compared to that of intact controls (13.61±1.30 pmol/mg tissue/min). The increased MAO activity in the hyperluteinized ovaries may be attributed to the increased number of transformed and accumulated corpora lutea as a consequence of diminished luteolysis.

Keywords: MAO activity, ovarian hyperluteinization, rats

In previous reports (16, 18), it was demonstrated that mechanical lesions, placed in the posterior hypothalamic region encompassing the posterior mamillary body of neonatal rats, induced the accumulation of an extreme number of ovarian corpora lutea, far above their usual number. In intact rats of our Wistar strain, the average number of corpora lutea per ovary is 7, with a maximum of 20, but in the hyperluteinized ovary, there may be as many as 50 corpora lutea per ovary.

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It has been reported that the mechanical lesion in the hypothalamus of rats did not disturb the normal estrus cycle. However, in rats with hyperluteinized ovaries, the circulatory preovulatory prolactin (PRL) level was significantly increased but the preovulatory circulatory level of the luteinizing hormone (LH) was reduced as compared to intact controls. No significant changes in the preovulatory follicular stimulating hormone (FSH) content have been detected in animals with hyperluteinized ovaries compared to intact controls (9, 10). The level of preovulatory circulatory estradiol in these rats was significantly higher, while progesterone production did not differ significantly in comparison with the control rats (9, 19).

Our previous results show that norepinephrine (NE) and epinephrine (E) levels in the ovarian and uterine tissues of animals with hyperluteinized ovaries were significantly lower as compared to those of intact controls (11, 19). This clearly indicates that NE containing neurons have different biochemical characteristics and that catecholamines have a high ranking position among the agents participating in the regulation of reproductive function (13). It has been further shown that MAO activity changes in ovarian tissue during the estrus cycle (3), as well as in ovarian follicles, corpora lutea and the ovarian interstitial tissue (24). It has also been reported that total MAO activity significantly varies in the pig uterine myometrium during the estrous cycle (5).

The aim of the present report is to further elucidate the mechanism by which the hyperluteinized ovary is maintained and the origin of the previously described changes in catecholamine (NE and E) levels. Thus, we measured the total MAO activity in the hypothalamus, uterus and ovaries of animals with hyperluteinized ovaries, having in mind the importance of MAO in the regulation of catecholamine metabolism in the brain and peripheral tissues.

Materials and methods

Female rats of Wistar strain were used in the experiment. Hyperluteinized ovaries were obtained as described by Martinovitch et al. (16). Briefly, animals not older than 12 hours were subjected to mechanical lesions in the posterior hypothalamic region, with a fine steel knife. The lesions encompassed the region of the posterior mamillary body, including the anterior-posterior planes between $A_{3.4}$–$A_{3.8}$ according to the De Groot rat brain stereotaxic atlas (4). After the operation, the pups were returned to their mothers. The number of pups per mother was 6–8. The animals were kept under control conditions (temperature, 22±2 °C and with lights on from 7:00–19:00 h). The animals had access to water *ad lib* and were fed with standard rat pellets. The young animals were weaned at one month, separated from the mothers and caged in groups up to 6. At the age of 4 months, the animals were laparatomized to confirm the presence of hyperluteinized ovaries (HL) and sacrificed by decapitation with a small animal guillotine at the age of 7 months in the estrus phase of the estrous cycle. Control animals were intact females of the same age kept under the same conditions and also sacrificed in the estrus phase. After sacrifice, the hypothalamus was dissected from the

brain by the method of Glowinski and Iversen (7), the optic chiasma delimiting the anterior part of the hypothalamus, the anterior commissure being the horizontal reference and the mamillary bodies delimiting the posterior part of the hypothalamus. After removing the ovaries and uterus, they were placed on an ice-cold microscope slide to free them of surrounding tissue. The excised tissues were immediately frozen and kept at –70 °C less than one month until assayed for total MAO activity.

Total MAO activity was determined as described by Wurtman and Axelrod (23). The tissues were homogenized in 10 ml of chilled potassium phosphate buffer, 0.2M, pH 7.4, containing 0.9% KCl. The homogenates were centrifuged for 30 min at 12,000 g. The supernatant was incubated at 37 °C for 20 min with mild shaking in the presence of ^{14}C-tryptamine bisuccinate (specific activity 2 μCi/μmol) as a substrate (purchased from NEN Great Britain). The reaction was stopped by immersion in an ice-cold water bath and adding 2N HCl. Extraction of the formed ^{14}C-indol acetic acid was achieved by shaking the samples with 6 ml of toluol (Fluka, Germany). The samples were centrifuged for 3 min at 3000 rpm and the organic phase was removed and placed in vials with 10 ml of scintillation solution, omnifluor (NEN, USA) for counting on an LKB 1219 scintillation counter. The obtained values are expressed as pmol of ^{14}C-indole-acetic acid per mg of tissue per minute of incubation.

The two-tailed Student's t-test was used for statistical analysis and comparison of tissue masses. The one way ANOVA followed by the Scheffe test was used for comparing the means of total MAO concentrations. The level of statistical significance was set at $p<0.05$.

Results

Body, ovary and uterus mass

The body mass of rats with hyperluteinized ovaries (261.50±9.70 g) is insignificantly higher (5.6%) than that of intact controls. (246.86±3.14 g). However, the hyperluteinized ovary mass (179.95±18.26 mg) is 68% greater ($p<0.001$) as compared to that of intact controls (58.31±2.70 mg). The uterine mass of animals with hyperluteinized ovaries (437.12±78.21 mg) is insignificantly lower (14%) as compared to intact controls (509.53±55.68 mg) (Table I).

Total MAO activity in the hypothalamus, ovary and uterus

As presented in Figure 1, the total MAO activity in the hypothalamus of rats with hyperluteinized ovaries (30.21±1.53 pmol/mg tissue/min, n=6) is not significantly different (ANOVA; $F_{(1,10)}=0.1446$, $p>0.05$) from that in intact control rats (31.09±1.72 pmol/mg tissue/min, n=6). In contrast to the hypothalamus, significantly

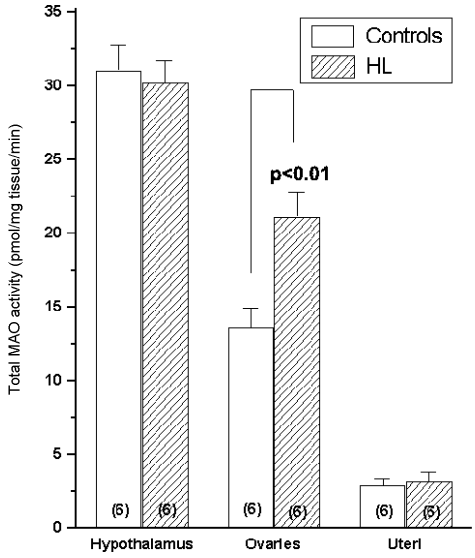

Fig. 1. Total monoamine oxidase activity in the hypothalamus, ovaries and uteri
C – Control animals
HL – Animals with hyperluteinized ovaries
( ) No of animals
p – Level of significance

Table I

The mean values of body, ovary and uterus mass in intact controls (C) and rats with hyperluteinized (HL) ovaries (sacrified at the age of 7 months)

	C	HL	P
Body mass (g)	246.86 ± 3.14* (236 – 259)** (7)***	261.50 ± 9.70 (237 – 300) (6)	>0.05
Ovary (mg)	58.31 ± 2.70 (49.7 – 70.0) (7)	179.95 ± 18.26 (104.05 – 242.25) (6)	<0.001
Uterus (mg)	509.53 ± 55.68 (363.0 – 778.0) (7)	437.12 ± 78.21 (271.25 – 816.75) (6)	>0.05

* Mean ± S.E.M.
** Range
*** Number of animals
P – Level of significance

higher (ANOVA; $F_{(1,10)}$=12.6308, $p<0.01$) total MAO activity has been detected in the hyperluteinized ovary (21.16±1.70 pmol/mg tissue/min, n=6) as compared to that in the ovary of corresponding intact controls (13.61±1.30 pmol/mg tissue/min, n=6). No significant difference (ANOVA; $F_{(1,10)}$=0.0589, $p>0.05$) has been detected in total MAO activity between the uterus of rats with hyperluteinized ovaries (3.16±0.61 pmol/mg tissue/min, n=6) and uterus of their corresponding intact controls (2.90±0.40 pmol/mg tissue/min, n=6).

Discussion

The physiological role of the sympathetic innervation of the ovary and uterus has not been completely explained, although it has been suggested that this innervation modulates steroidogenesis. Total MAO activity in the rat reproductive organs, the ovary and uterus, fluctuates significantly in correlation with steroid levels and tissue catecholamine contents during pregnancy (15). A number of reports have shown that catecholamines played a significant role in ovarian regulation (14, 20). In this report, aimed at contributing to a better understanding of the mechanism of ovarian hyperluteinization after lesions in the posterior hypothalamus, we bore in mind the complexity of ovarian regulation. In rats with hyperluteinized ovaries, the development of ovarian follicles and formation of corpora lutea proceeds continuously throughout the estrus cycles resulting in the accumulation of corpora lutea. The factors responsible for the accumulation of corpora lutea after mechanical lesions in the posterior hypothalamus are still unknown. Our previous results, showing the higher circulatory levels of estradiol in the rats with hyperluteinized ovaries, may also indicate that hyperovulation is one of the reasons for the formation of hyperluteinized ovaries (9). It has been demonstrated that estrogen has a tissue specific role in the regulation of MAO-A and MAO-B (8), but that at the hypothalamic level, estrogen does not influence total MAO activity (21). In this context, it may be explained that the higher circulatory levels of estradiol in rats with hyperluteinized ovaries, as compared to that in intact controls (24), did not result in significant differences in the hypothalamic total MAO activity between the two animal groups (Fig. 1). Injecting of female rats with progesterone induces an increased uterine MAO activity (2, 17). In human females, however, an increased uterine MAO activity was demonstrated in the second half of the menstrual cycle when the circulatory progesterone concentration rises (22). The absence of difference in uterine MAO activity between the animals with hyperluteinized ovaries and their intact controls, observed in the present experiments (Fig. 1), is in agreement with the findings that no differences in circulatory progesterone concentrations were detected between the experimental animals with hyperluteinized ovaries and intact controls (18). The histological picture of the uterus, however, from animals with hyperluteinized ovaries, showing a reduced thickness of *tunica mucosa* and *muscularis* and poorly developed uterine glands (9), may explain the lower uterine mass in the

animals with hyperluteinized ovaries as compared to the uteri of intact controls (Table I).

The noradrenergic innervation of the ovary acts tonically inhibitory during the selection of follicles triggered for the process of development and ripening (1, 6, 12). The decreased NE concentrations in the hyperluteinized ovaries as compared to those of intact controls as demonstrated previously (19), may explain the increased ovarian MAO activity in the hyperluteinized ovaries. This reduced ovarian NE concentration in the hyperluteinized ovaries (19) may be responsible for triggering off the development and ripening of an increased number of follicles confirmed by the higher circulatory estradiol levels during the preovulatory peak in the animals with hyperluteinized ovaries as compared to the intact controls. The reduced NE concentration in the hyperluteinized ovaries as compared to that in control animals (10, 19) may be the result of the increased NE deamination due to the increased MAO activity in the hyperluteinized ovaries (Fig. 1). Since the activity of MAO in the ovaries increases during corpora lutea transformation or aging (24), the increased activity of MAO in the hyperluteinized ovaries may result from the accumulated old corpora lutea.

In conclusion, increased total MAO activity observed in ovaries of hyperluteinized rats correlated with the previously reported reduction of catecholamine levels in ovarian tissue of these animals (10, 19). This also suggests that MAO activity has a significant physiological role in the maintenance of the hyperluteinized ovary syndrome.

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