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The oestrous cycle and monoamine oxidase activity

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Summary

1. During the oestrous cycle of the rat, monoamine oxidase (MAO) activity was studied in the uterus, the ovaries and the adrenal glands and also in 4 brain regions which are rich in monoamines. In all these structures the activity was lowest during the night between oestrus and metoestrus. High values were found in di-oestrus, with the exception of the uterus in which the MAO activity reached its peak during metoestrus.

2. A stimulating effect of progesterone on uterine, ovarian and adrenal MAO activity was observed.

3. An index for the cyclic variations in progesterone production was obtained by measuring the adrenal and ovarian progesterone content at the same stages of the cycle as MAO activity.

4. The sum of the amounts of progesterone contained in the ovaries and adrenal glands was low in early oestrus but increased during late oestrus when MAO activity was at its minimum. Progesterone contents were high in met- and di-oestrus when MAO activity was also high.

Introduction

Evidence is accumulating that the activity of the enzyme monoamine oxidase ((MAO) EC 1.4.3.4) can be influenced by endocrine glands. For example, in the human endometrium up to ten-fold increases in MAO activity were observed between the 19th and 21st day of the menstrual cycle (Southgate, Grant, Pollard, Pryse-Davies & Sandler, 1968), the maxima coinciding with the peaks in blood progesterone concentrations. In the rat, injections of progesterone were found to increase the MAO activity of the uterus (Collins & Southgate, 1970) whereas oestradiol inhibited the enzyme *in vivo* and *in vitro* (Collins, Pryse-Davies, Sandler & Southgate, 1970 ; Collins & Southgate, 1970).

In order to investigate whether the naturally occurring changes in hormone concentrations are large enough to influence MAO activity in tissues other than the uterus several groups of workers (e.g., Zolovick, Pearse, Boehlke & Eleftherious, 1966 ; Salseduc, Jofre & Izquierdo, 1966 ; Cavanaugh & Zeller, 1967 ; Kamberi & Kobayashi, 1970 ; Holzbauer & Youdim, 1972) have studied the activity of the enzyme in the brain of the rat during the oestrous cycle. The results obtained varied and led to different conclusions.

In the present work this problem was reinvestigated in rats which were kept under carefully controlled conditions. MAO activity was measured during the different phases of the oestrous cycle at times of sleep and wakefulness. The tissues analyzed were the ovaries and the adrenal glands, in which progesterone is

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synthesized, the uterus, which is the main target organ for progesterone, and four brain regions which are rich in monoamines. Simultaneously, the progesterone concentration in the ovaries and the adrenal glands of rats from the same colony were measured as an index of the production rate of progesterone during the cycle.

Methods

Unless otherwise stated, the experiments were carried out on Wistar rats (100–160 g body weight) obtained from A. Tuck & Son, Laboratory Breeding Station, Rayleigh, Essex and fed on Oxoid 41B rat pellets. They were kept in a temperature controlled room (20° C) with white light between 02.00–14.00 h and red light between 14.00 and 02.00 h. Vaginal smears were taken daily to monitor the oestrous cycles. Only rats which exhibited at least 3 regular cycles of 4 day length were used. They were decapitated either at 10.00 h or at 17.00 h and the relevant tissues removed and immediately frozen. The brain structures dissected out were the septum, both caudate nuclei, the hypothalamus and the posterior portion of the telencephalon. The latter included part of the hippocampal formation and several nuclei of the amygdaloid complex. The cranial half of the right uterus, the apex of the heart (approx. 3 mm including part of the left and right ventricle and septum), a piece of liver weighing about 200 mg and the ovaries and adrenal glands were also analysed.

Ovariectomy was performed under ether anaesthesia through two flank incisions.

The effect of progesterone (Sigma, 1 (mg/kg body weight)/day i.p., for 8 days) and 17β-oestradiol (Sigma, 0·5 (mg/kg body weight)/day i.p., for 8 days) was studied in Charles-River female rats (150–200 g). The steroids were injected as a suspension of 1 mg/ml in 0·9% w/v NaCl solution. Control rats were given i.p. injections of appropriate volumes of 0·9% w/v NaCl solution. The uterus, ovaries and adrenal glands were analysed and also liver, heart and kidney.

The tissues were homogenized by hand in a glass homogenizer with 0·1 M phosphate buffer, pH 7·4 to give a tissue to buffer ratio of about 1:10 (wet weight of tissue: volume). Monoamine oxidase was assayed with kynuramine as substrate by the method of Kraml (1965). To 0·1 ml of tissue homogenate were added, 0·8 ml 0·1 M phosphate buffer pH 7·4, and 0·1 ml of a 1 mM kynuramine solution. The samples were incubated at 37° C in a metabolic shaker for 30 min and the reaction was stopped with 1 ml 10% trichloroacetic acid. The amount of 4-hydroxyquinoline formed was measured in an Aminco Bowman Spectrophotofluorimeter. The proteins were estimated by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard.

The progesterone in the adrenal glands and ovaries was estimated by gas-liquid chromatography as described previously (Holzbauer & Newport, 1967; Fajer, Holzbauer & Newport, 1971).

Results

Monoamine oxidase activity during the oestrous cycle

The changes in the MAO activity which occur during the oestrous cycle in different tissues of the rat are shown in Figures 1 and 2. In all tissues analysed the lowest activities were found during the night between oestrus and metoestrus.

In the ovaries and adrenal glands as well as in the four brain regions studied the enzyme activity was highest between di-oestrus and pro-oestrus. In contrast, the uterus showed the highest activity during metoestrus. MAO activity in the posterior telencephalon was similar to that found in the septum. For the sake of clarity

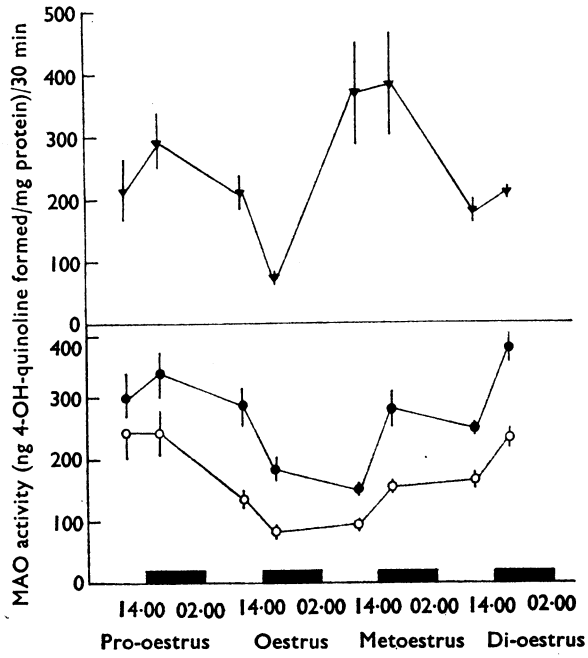

FIG. 1. Monoamine oxidase activity in the uterus (▼——▼), the ovary (●——●) and the adrenal gland (○——○) of rats during different phases of the oestrous cycle. Dark bars on abscissae indicate periods of red light (dark period). Rats were killed either after 8 h in white light (light period) or 3 h in red light. (Mean values and standard errors of the mean; $n=5$ or 6).

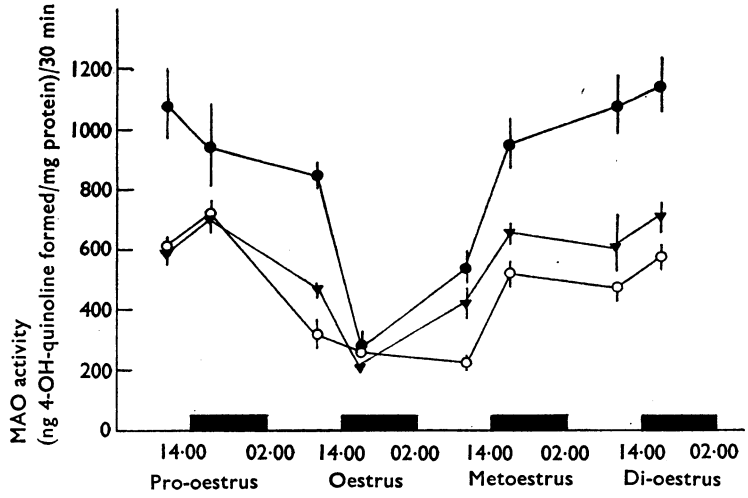

FIG. 2. Monoamine oxidase activity in the hypothalamus (●——●), the caudate nucleus (▼——▼) and the septum (○——○) of rats during different phases of the oestrous cycle. Dark bars indicate periods of red light. Killing schedule as described for Figure 1. (Mean values and standard errors of the mean; $n=5$ or 6).

the values were not included in Figure 2. Among the structures analysed the highest MAO activity per mg protein was found in the hypothalamus.

Progesterone during the oestrus cycle

As it is well established that the hormone content in a steroid-producing gland reflects the rate at which it is secreted (Holzbauer, 1957; Van Goch, De Wied & Schönbaum, 1963; Vernicos-Danellis, Anderson & Trigg, 1966; Holzbauer & Newport, 1969; Fajer *et al.*, 1971; Holzbauer, 1971) the progesterone contents of the ovaries and the adrenal glands were also measured during the oestrous cycle. This was done in another group of rats from the same breeding stock of similar body weight and kept under the same conditions as the rats in which MAO activity was measured.

In Fig. 3 the cyclic variations in the MAO activity of the hypothalamus are compared with the variations in the progesterone content of ovaries and adrenal glands. In individual rats, the quantities of progesterone found in these two tissues were added and the mean values from 9 rats (early and late pro-oestrus, early

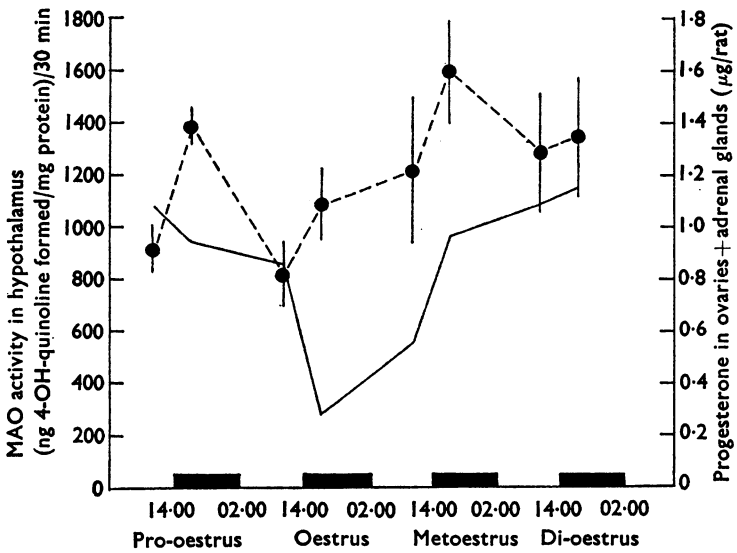

FIG. 3. Comparison of the amount of progesterone contained in the adrenal glands plus the ovaries with MAO activity in the hypothalamus during the oestrus cycle. Progesterone (●– – –●): mean values and standard errors of the mean; pro-oestrus and early oestrus: $n=9$; late oestrus: $n=6$; metoestrus and di-oestrus: $n=3$ or 4. Continuous line: monoamine oxidase activity (compare Fig. 1). Killing schedule as in Figure 1.

oestrus), 6 rats (late oestrus), 4 rats (early metoestrus) or 3 rats (late metoestrus, early and late di-oestrus) were calculated. These values will be an indication for the total amount of progesterone secreted into the circulation at the different phases of the cycle. Whereas MAO activity was at its lowest during the red-light period between oestrus and metoestrus, progesterone was low in early oestrus but already high in late oestrus and in early metoestrus when MAO activity was still low.

In Fig. 4 the cyclic changes in the MAO activity of the adrenal glands are compared with progesterone contained only in the adrenal glands. In this tissue,

where most of the progesterone is located at the same intracellular site as the MAO activity (Holzbauer, Bull, Youdim, Wooding & Godden, 1973) the progesterone concentration remained low in late oestrus, when MAO activity was at its lowest.

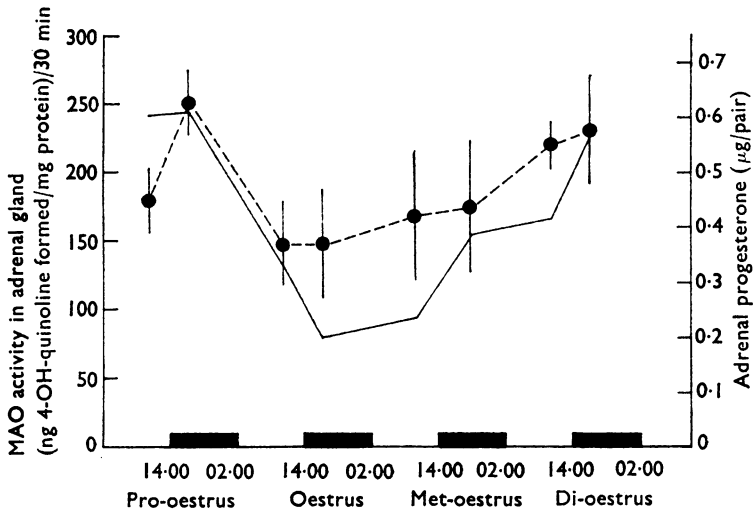

FIG. 4. Comparison between the changes of the adrenal progesterone content during the oestrous cycle and the changes of the monoamine oxidase activity in the adrenal gland. Progesterone (●- - -●): mean values and standard errors of the mean; pro-oestrus: $n=12$; early oestrus: $n=10$; late oestrus: $n=8$; metoestrus and di-oestrus: $n=4$–8. Continuous line: adrenal monoamine oxidase (compare Figure 2). Dark bars indicate periods of red light. Killing schedule as described for Figure 1.

Effect of exogenous progesterone and oestradiol on monoamine oxidase activity

Table 1 shows results of experiments in which the effects of progesterone and oestradiol on MAO activity in the ovaries, the uterus and the adrenal glands were studied. The results confirm that, in the quantities employed, progesterone can increase and oestradiol can decrease MAO activity. Liver, heart and kidney did not show any significant changes in enzyme activity with either of these steroids.

TABLE 1. *Effect of oestradiol and progesterone on monoamine oxidase (MAO) activity*

	MAO activity*		
Steroid	Ovary	Uterus	Adrenal gland
Control	3·64±0·12	4·96±0·15	3·44±0·10
Oestradiol	2·36±0·21	3·62±0·21	2·68±0·12
% change from control	−35% P<0·001	−27% P<0·001	−21% P<0·01
Control	3·40±0·21	4·52±0·19	3·10±0·15
Progesterone	4·52±0·23	7·86±0·17	6·36±0·27
% change from control	+33% P<0·001	+74% P<0·001	+105% P<0·001

*MAO activity is expressed as fluorescence intensity of (4-OH-quinoline formed/mg protein)/30 minutes. Oestradiol 17β (Sigma (0·5 mg/kg body weight)/day) or progesterone (Sigma (1 mg/kg body weight)/day) were suspended in 0·9% w/v NaCl solution (1 mg/ml) and injected intraperitoneally for 8 days. The controls were injected with comparable volumes of 0·9% w/v NaCl solution only. The rats used were intact females of 150–200 g, five rats per group.

Ovariectomy

Table 2 shows the effect of ovariectomy on MAO activity. The MAO activity in ovariectomized rats was similar to the lowest values seen during the oestrous cycle in all tissues studied.

TABLE 2. *Effect of ovariectomy on monoamine oxidase (MAO) activity in female rats*

Group	No. of rats	MAO activity (ng 4-OH-quinoline formed/mg protein)/30 min Septum	Hypo-thalamus	Caudate nucleus	Telen-cephalon	Adrenal gland	Uterus
Rats	10	210±19	452±20	246±12	225±12	90±9	116±20
Intact rats, killed at 17.00 h:							
between oestrus and metoestrus	5	240±27	286±57	222±62	313±64	78±13	70±9
between di-oestrus and pro-oestrus	5	593±51	1177±92	676±44	640±60	232±17	214±15

The rats were kept under controlled lighting (12 h white light, 12 h red light) and temperature (20°C) conditions for at least 2 weeks. The ovariectomized rats were killed 12 days after the operation, 5 rats during the white light period (10.00 h), 5 rats during red light (17.00 h). As both groups gave similar results, the results were pooled.

Monoamine oxidase activity in male rats

A study was also carried out in male rats in order to see whether changes in MAO activity occur during the periods of sleep or wakefulness. In experiments on 6 different groups of rats performed between April 1971 and July 1971 (Holzbauer & Youdim, 1972) MAO activity was found to be significantly higher in rats killed during the period of red light (17.00 h) than in rats killed during white-light period (10.00 h). These experiments were resumed in November 1971 and continued till July 1972 on another 18 groups of rats each consisting of 4–6 animals. Every month one group of rats was killed during the white-light (10.00 h) and, on the same day, a second group during the red-light (17.00 h) period. The MAO activity in all tissues of these rats was significantly lower than in the rats examined between April and July 1971 and no changes between the light and dark periods occurred.

Discussion

In the present work MAO activity was measured in a carefully controlled group of rats throughout the oestrous cycle not only during periods of light but also during darkness when the rats were awake and active. This allowed us to detect the significant fall in MAO activity during the night between oestrus and metoestrus which was especially pronounced in the hypothalamus and in the uterus.

In an attempt to test the suggested link between MAO activity and endocrine function an indirect assessment of progesterone production was made by measuring the progesterone content of the ovaries and the adrenal glands which can synthesize and secrete about equal amounts of progesterone in the rat (Feder & Ruf, 1969; Fajer *et al.*, 1971).

A comparison between the cyclic changes of MAO activity in the brain and the changes in the combined progesterone content of these glands (Fig. 4) showed that in late oestrus, when MAO activity was at its minimum, the progesterone production was already rising. A similar relation existed between progesterone

production and uterine MAO activity. If the rises in MAO activity in these organs were chiefly caused by progesterone, there would be a time lag between the steroid reaching the target organ and its effect on MAO becoming apparent. No assessment of the oestrogen production in these rats was made. Yoshinaga, Hawkins & Stocker (1969) reported a peak in oestrogen secretion in early pro-oestrus. If the amounts produced *in vivo* were sufficient to cause the fall in MAO activity during late oestrus, a time lag would also exist between oestrogen secretion and its effect on MAO.

The effects of exogenous steroids on MAO activity have so far only been studied individually whereas under physiological conditions they are present simultaneously and will be able to compete. Furthermore, progesterone and oestrogens are not the only steroids which are secreted at different rates during the different phases of the oestrous cycle. The ovary of the rat secretes, e.g., several pregnane derivatives with strong central depressant actions (Holzbauer, 1971). They are produced in smallest quantities during early oestrus and in large quantities during metoestrus.

An earlier observation on 6 groups of male rats, which showed a rise in MAO activity during periods of wakefulness (Holzbauer & Youdim, 1972) could not be confirmed at a later date. The enzyme activities observed in the later experiments were significantly lower than in the earlier ones. The reason for this discrepancy remains obscure. It is possible that the rats in the earlier experiments showed different activity patterns from the rats in the later experiments. Continuous activity records of the rats have not been made.

Although numerous studies have been carried out on the physico-chemical properties of MAO (see Sandler & Youdim, 1972; Tipton, 1972) little is known about the mechanisms by which it can be influenced. The activity of the enzyme could be increased by increasing the permeability of the outer mitochondrial membrane, the site of MAO (Schnaitman, Erwin & Greenawalt, 1967), thus providing easier access for the substrate to the enzyme. Or, the enzyme could have been released from the action of an inhibitory substance. Facilitation of MAO protein synthesis seems an unlikely cause for the increased enzyme activity because of the slow rate of protein synthesis. The half life of MAO was reported to be 14–17 days (Callingham & Della Corte, 1972) and it took 6–8 weeks for the return of MAO activity after the enzyme had been irreversibly inhibited by phenelzine (Clineschmidt & Horita, 1969).

Disagreement between results on cyclic variations of MAO activity obtained in different laboratories may often be due to methodological differences, e.g., strain and age of rats, housing, exact time of killing or the substrate used for measuring MAO activity. In Fig. 5 a comparison is attempted between our observations on cyclic fluctuations of MAO activity in the hypothalamus and those of Kamberi & Kobayashi (1970). These authors exposed their rats for longer periods to white light and did not measure MAO activity during the dark periods. Thus, they did not measure MAO activity in late oestrus when we found it lowest. Furthermore they used tyramine as substrate, whereas we used kynuramine.

The importance of the choice of the substrate has only recently become apparent, when multiple forms of MAO have been described possessing different substrate specificities (Youdim, 1972, 1973; Youdim, Collins & Sandler, 1969). Southgate (1972) found that after progesterone treatment uterine MAO activity was more

enhanced when dopamine was used as the substrate than when benzylamine or tyramine were used. In contrast, oestradiol seemed without effect using dopamine as substrate, whereas the activity towards benzylamine was inhibited.

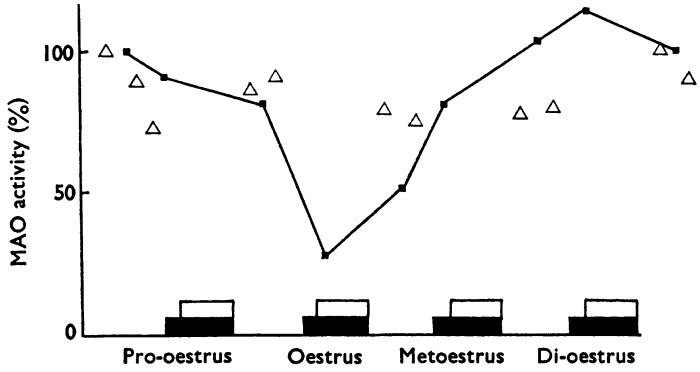

FIG. 5. Monoamine oxidase activity in the hypothalamus of rats during the oestrous cycle. Comparison between results reported by Kamberi & Kobayashi (1970) using [^{14}C]-tyramine (△) as substrate and the results obtained in our experiments, using kynuramine (■——■). Dark bars on abscissae: periods of red light in the present experiments; open bars: dark periods in the experiments of Kamberi & Kobayashi. All results are expressed as a percentage of the activity found during the white light period on the day of pro-oestrus (=100%).

The observed variations in MAO activity support the suggestion that the catabolism of monoamines undergoes changes during the oestrous cycle. Cyclic variations in the tissue concentrations of catecholamines and 5-hydroxytryptamine have been looked for and were reported on several occasions (e.g., Stefano & Donoso, 1967; Sandler, 1968; Kurachi & Hirota, 1969; Greengrass & Tonge, 1971). It has been speculated that such variations might be linked with a possible interaction between brain amines and the release of pituitary hormones or with behavioural changes occurring during the oestrous cycle. The information available at present is far from complete and does not yet allow any general conclusions to be drawn.

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