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CYCLICAL VARIATIONS IN ENDOMETRIAL MONOAMINE OXIDASE: CORRELATION OF HISTOCHEMICAL AND QUANTITATIVE BIOCHEMICAL ASSAYS

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Abstract—Parallel histochemical and biochemical microassay procedures have been used to confirm that a large increase in endometrial monoamine oxidase occurs in the late secretory phase of the human menstrual cycle. No such variation was observed during the rat oestrus cycle. The value of biochemical microassay procedures in supplementing information obtained by histochemical methods is pointed out.

THE ENZYME, monoamine oxidase (monoamine: O_2 oxido-reductase (deaminating) EC 1.4.3.4) (MAO), which is probably involved in the *in vivo* inactivation of the catecholamines, 5-hydroxytryptamine (5-HT), tyramine and other biologically active monoamines of importance, has latterly been the subject of intense interest.[1, 2]

Whilst there have been few convincing studies of its location and significance in the female genital tract, Cohen *et al.*[3, 4] have recently noted marked histochemical variations in MAO staining pattern in human endometrium during the menstrual cycle. They found its activity to be weak and confined to particles early in the proliferative phase but becoming progressively more intense and more diffuse later in the cycle; shortly before the onset of menstruation, the particulate appearance is superseded by a diffuse staining of the whole cell. Cohen *et al.* speculated that this second staining pattern represents an inactive form of the enzyme which would allow amine substrates of MAO to accumulate in the endometrium, perhaps in sufficient concentration to initiate menstruation by causing spasm of the spiral arteries.[5]

Measurement of enzyme activity by histochemical staining can at best be semi-quantitative. It therefore seemed important to try to correlate such visual assessments with quantitative MAO assay both during the human menstrual cycle and the rat oestrus cycle.

MATERIALS AND METHODS

In an attempt to obtain relatively normal endometrium, biopsy specimens were examined from 17 women aged between 24 and 40 yr attending the central clinic of the Council for the Investigation of Fertility Control and from 13 aged between 21 and 47 yr attending Chelsea Hospital for Women for a variety of minor disorders (listed below). All subjects had regular menstrual cycles and normal endometrial morphology; none was on hormonal treatment at the time of curettage. Of the first

group, 7 had received no oral contraceptive and 10 were changing their regime, with gaps of one to four cycles before that during which the biopsy was taken. Of the second group, 7 were under investigation for secondary infertility, 2 for heavy but regular periods, 1 for dysmenorrhoea and 2 were curetted incidentally during treatment of a vulval cyst and a cervical erosion.

Whole uteri were obtained from virgin white 'Wistar' rats.

Biochemical assay of MAO

MAO was assayed by the method of Wurtman and Axelrod.[6] Tissues were homogenised in chilled isotonic potassium chloride. In a typical assay, 0·5 ml. phosphate buffer (0·5 M, pH 7·4), 0·05 ml. homogenate, 0·1 ml tryptamine-2-^{14}C (10,000 cpm, 25 mμ mole) were mixed in a glass stoppered tube and incubated for 20 min at 37°. The reaction was stopped by the addition of 0·4 ml 2N HCl and the deaminated radioactive material was extracted into 6 ml toluene. After centrifugation, a 4 ml aliquot of the organic layer was transferred to a vial containing 10 ml phosphor (300 mg 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)-benzene and 5 g 2,5-diphenyloxazole in 1 l. toluene) and counted in a Tri-Carb liquid scintillation spectrometer. A correction for the small amount of substrate extracted during this procedure was obtained by incubating ^{14}C-tryptamine with a control enzyme preparation previously inhibited by 30 min incubation at 37° with 0·2 ml iproniazid (0·01 M in phosphate buffer) followed by acidification and extraction as in the test.

Histochemical assessment of monoamine oxidase

Human endometrial fragments were frozen to −70° immediately after curettage by placing them on solid carbon dioxide. Portions of rat uteri including both myometrium and endometrium were similarly frozen. Tissues were stored at this temperature and transferred to a microtome chuck within 24 hr for cryostat sectioning at −20°: all sections were cut at 15 μ and mounted on coverslips. The histological structure was assessed after haematoxylin and eosin staining. The method of Glenner *et al.*[7] was used to demonstrate MAO, sections being incubated at 37° for 45 min: an enzyme-inhibited control was prepared by pre-incubating one section in 0·01 M iproniazid for 30 min. The same substrate, tryptamine hydrochloride, was used in both histochemical and biochemical assays.

Phase contrast and dark ground microscopy were helpful in assessing the location and depth of staining in the enzyme preparations. Phase contrast may be used instead of a counterstain to determine general structural details and dark ground illumination enables small and scanty dye particles to be seen more easily than with bright light alone. A combined system of phase contrast, dark ground and bright light illumination, such as the Leitz system with the Heine condenser, was found to be very suitable for this purpose.

RESULTS

Biochemical findings

In the 30 human endometrial biopsies, MAO activity was low during the non-secretory and early secretory phases but showed a rapid rise at the onset of the late secretory phase. (Fig. 1). In the material from a group of 19 subjects in the non-secretory and early secretory stages, the mean value was 2603 cpm/mg dry wt. (SD ± 1097

cpm/mg dry wt); during the late secretory phase, mean activity in a group of 11 subjects had increased to 15,257 cpm/mg dry wt. (SD $\pm$ 2957 cpm/mg dry wt).

Uteri from 21 rats were quantitatively assayed for MAO. No correlation was found between activity and phase of the oestrus cycle as determined both by histological examination and by vaginal smear.

Histochemical findings

In the human material, the morphological stage of the menstrual cycle was noted and each biopsy was ranked for MAO activity according to the depth of staining of the endometrial glandular epithelium: three degrees of activity were recognised, weak, intermediate and strong.

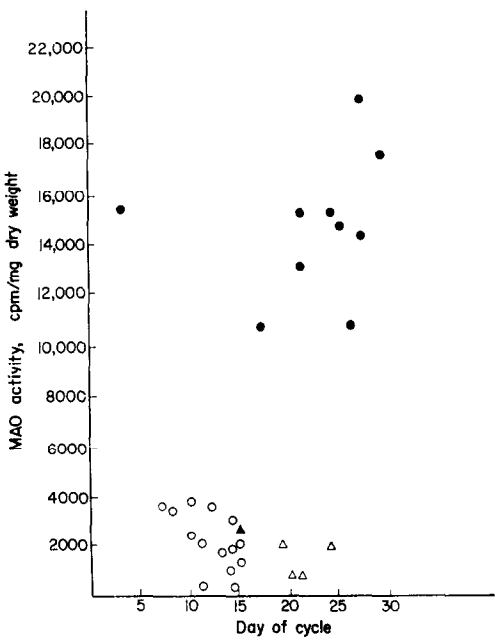

FIG. 1. Showing correlation of monoamine oxidase (MAO) activity with day of menstrual cycle and endometrial morphology.

○ = non-secretory; ▲ = non-/early secretory;
△ = early secretory; ● = late secretory.

Fig. 1 shows the day of the menstrual cycle and the morphology of the endometrium together with the corresponding biochemical MAO activity for the 30 specimens studied. The morphology is a more important guide to enzyme activity than the actual day of the cycle as wide individual variations occur in the menstrual pattern.

In 12 of the 14 non-secretory specimens, MAO activity was represented by scattered tetrazolium granules in the stroma and epithelium, best seen by dark ground illumination: in the other 2, there was an additional faint blue colour in some of the epithelium and these were termed intermediate reactions. Of 4 specimens in the early secretory phase, 3 showed a weak reaction and one was of the intermediate type. One biopsy

with a mixed non-secretory and early secretory pattern gave a weak staining reaction. The remaining 11 biopsies were in the later secretory phase and they showed a diffuse blue colour in all the glandular epithelium as well as a granular stain. The granules were somewhat heavier in the stroma and focally in some of the glands than in the other phases of the cycle: granularity was especially marked only in the single menstrual specimen.

All sections showing a weak or intermediate staining reaction came from tissue with less than 4000 cpm/mg dry wt. of MAO activity; when there was uniform blue staining as well as a granular component, activity was in the range of 10,000–21,000 cpm/mg dry wt. (Fig. 1).

When frozen sections of endometrium were treated with acetone at −20° for 15 min. before incubation, the granular stain was not observed, although the diffuse blue colour was still present. That acetone acted as a fat solvent was shown by Oil Red O staining before and after treatment.

Five rat uteri were examined histochemically for possible changes in endometrial MAO during different phases of the oestrus cycle. No changes were observed.

DISCUSSION

Meier-Ruge *et al.*[8] were the first to adopt the present dual histochemical and biochemical approach to MAO assay; they employed two different substrates, a factor likely to account for slight discrepancies between their two sets of results. Using tryptamine as substrate for both measurements, we have been able to show that semi-quantitative visual assessment of MAO activity in endometrial sections stained histochemically correlates well with quantitative assay in every case: the sharp rise in endometrial MAO during the late secretory phase of the menstrual cycle, first demonstrated histochemically by Cohen *et al.*[3, 4] was fully mirrored by direct measurement This finding hardly adds weight to the hypothesis of Cohen *et al.* that the heavy diffuse staining pattern of the late secretory phase of the cycle represents an inactive form of the enzyme. Indeed, the opposite would seem to be more in keeping with the *in vitro* data. Whether the early granular and late diffuse staining patterns represent a compartmental shift of enzyme obviously cannot be ruled out; we are carrying out subcellular fractionation studies to investigate this possibility. Another explanation is that the qualitative alteration in stain deposition between early and late phase endometrium may be due to cyclical changes in cell lipid concentration.[9, 10] Our observation that pretreatment with acetone destroys the granular appearance, leaving a diffuse staining pattern, provides some support for this possibility.

At present, there is insufficient information to decide whether the observed changes are localised to the endometrium or are part of a more generalised cyclical variation in MAO activity. It is of interest however that Klaiber *et al.*[11] noted a 2-fold increase in activity of a plasma MAO examined at a time corresponding to the late secretory phase, compared with samples obtained in early cycle. Further information on levels of the enzyme in other human tissues is obviously needed.

Despite the pronounced changes in the human, we were unable to demonstrate any biochemical variation in uterine MAO activity during the different phases of the rat oestrus cycle, thus confirming the findings of Salseduc *et al.*[12] The proportion of endometrium in the rat uterus is comparatively small, so that it was not possible to carry out quantitative enzyme assay on samples separated from myometrium; however

there was no histochemical variation in endometrial MAO activity. There are indications that changes do occur during the oestrus cycle in certain areas of the brain of rat[12–14] and guinea pig[15] and also in rat liver.[16] The situation needs clarification as the findings of the different groups in brain were to some extent conflicting.

The changes noted in human endometrium are quite clear cut and mirror those noted for prostaglandins.[17] The control mechanism for each is likely to be intimately related to progestational activity. Grant and Pryse-Davies[18] have recently shown that administration of strongly progestational oral contraceptives caused prolongation of the late secretory phase together with its corresponding strong histochemical MAO response. In recent years, it has become increasingly obvious that there is not one but a series of MAO's, each differing in its action towards substrates and inhibitors.[19–25] Whether the presumed hormone dependence shown by the endometrial isoenzyme is unique must be decided in the future.

Elucidation of the detailed mechanism of enzyme increase ought not to present any insuperable problem: but any hypothesis to test its functional significance is likely to prove more difficult to verify experimentally. It is possible that vital structures are particularly sensitive to amine substrates of MAO during the secretory phase so that the enzyme increase acts as a regulatory mechanism. The finding that human myometrial adrenaline levels are lower at this time[26] would be consistent with such a view, although the phenomenon could equally well be explained by a decreased amine binding ability. Peak excretion of 5-hydroxyindoleacetic acid, the major metabolite of 5-hydroxytryptamine, has been reported to occur at ovulation[27–29] and at menstruation[30]; but total urinary output of metabolite is probably a poor guide to local amine production. Ashcroft *et al.*[31] were unable to detect any variation in circulating 5-HT level during the different phases of the mentrual cycle.

Whatever the significance of such fluctuations in enzyme activity as those noted here, it is likely that micromethods of enzyme assay will be used increasingly in the future; not only do they add a new dimension, accurate quantitation, to the histochemical examination of biopsy samples in human disease, but they can be employed to obtain diagnostic information from the many enzymes for which histochemical methods are not available.[32]

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