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Progesterone provokes a selective rise of monoamine oxidase A in the female genital tract

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We reported earlier a striking increase in human endometrial monoamine oxidase (MAO) activity during the second half of the menstrual cycle [1] which correlated with peak plasma progesterone activity. On giving progesterone to female rats, we were, similarly, able to record a substantial increase in uterine (but not hepatic) MAO activity [2]. Since these experiments were performed, Johnston's classification of MAO [3] into A and B forms, on the basis of selective inhibition by clorgyline, has been widely accepted. The A form which, by definition, is relatively sensitive to clorgyline, selectively oxidizes the neurotransmitter monoamines, 5-hydroxytryptamine (5-HT) and noradrenaline; the relatively clorgyline-resistant MAO B acts on phenylethylamine (PEA) and benzylamine (Bz) whilst tyramine and dopamine can be oxidized by both forms [4]. However, these specificities depend to some extent on substrate concentration, tissue and species [5, 6]. In the light of this new information, we thought it interesting to re-examine the progesterone effect to determine whether both forms of the enzyme are affected equally or whether one or other predominates. We now report highly selective increases in MAO A activity in certain organs of the human and rat female genital tract.

Endometrial biopsy fragments were obtained by curettage from 15 normal subjects (age range 20–30 yr), under examination for a variety of minor gynaecological disorders. All had normal menstrual cycle length (28 ± 2 days) and none was on hormone treatment. The specimens were placed immediately on dry ice and later transferred to storage at −20°. For the animal experiments six female Wistar rats (150–180 g) were injected subcutaneously with progesterone, 3.3 mg/kg, at the same time of day, for three consecutive days. Six control rats were injected with physiological normal saline (0.9% w/v), 0.25 ml subcutaneously, and 0.75 ml intraperitoneally for three consecutive days. This routine was followed to replicate that of the previous study [2]. All animals were killed on the fourth day by dislocation of the neck. Liver, uterus, ovary and adrenal glands were dissected out, freed from connective tissue and stored at −20°. All tissue was homogenized in 50 mM potassium phosphate buffer, pH 7.4, and made up as a 10% (w/v) suspension. MAO was assayed radiometrically as previously described [7]. Protein was estimated by the method of Lowry *et al.* [8], using bovine serum albumin as standard.

Figure 1 shows MAO activity using three different substrates, 5-HT, DA and PEA, in endometrial biopsy samples taken from women at different stages of the menstrual cycle. It is clear that activity, when measured with 5-HT and DA, increased markedly during the course of the cycle, whereas activity with PEA was relatively constant. Table 1 shows mean activity from five samples obtained during the first week of the cycle compared with five samples from the fourth week. Both 5-HT and DA-oxidizing activity increased about 7-fold and the difference between first and fourth-week values was highly significant ($P < 0.01$). PEA-oxidizing activity was low throughout, and did not increase significantly from early to late cycle.

Table 2 shows findings after progesterone injection into rats. The drug caused large and significant increases in 5-

Table 1. Monoamine oxidase activity in human endometrium samples obtained during the first and fourth week of the menstrual cycle*

	No. of samples	Substrates		
		5-HT	DA	PEA
1st week	5	7.2 ± 0.7	4.3 ± 0.5	3.6 ± 0.3
4th week	5	51.9 ± 1.8†	48.7 ± 1.8†	7.2 ± 0.8‡

* Activities are expressed as nmoles product formed per mg protein per 30 min at 37°.
† $P < 0.01$ using Wilcoxon rank sum test; fourth week different from first week.
‡ Difference not significant.

Table 2. Effect of progesterone injection into rats on MAO activity using 5-HT and PEA as substrates*

	5-HT		PEA	
	Control	Progesterone	Control	Progesterone
Liver	38.5 ± 5.8	38.2 ± 7.5	23.0 ± 0.7	18.5 ± 0.3
Uterus	1.7 ± 0.8	9.8 ± 7.3†	3.3 ± 0.2	3.6 ± 0.3
Ovary	3.8 ± 2.0	16.5 ± 3.4‡	3.9 ± 0.5	6.4 ± 0.7
Adrenal	14.7 ± 1.6	29.7 ± 11.3	10.2 ± 2.5	4.0 ± 0.3

* Activities are expressed as nmoles product formed per mg protein per 30 min at 37°.
† $P < 0.005$, difference from control using Wilcoxon rank sum test.
‡ $P < 0.02$, difference from control using Wilcoxon rank sum test.

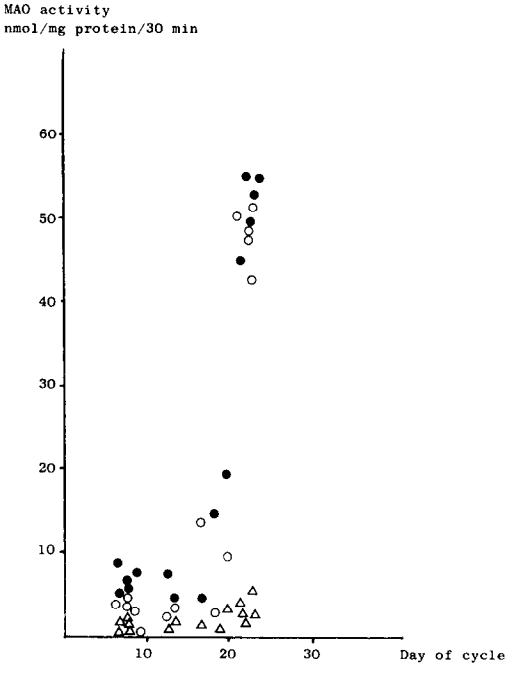

Fig. 1. Monoamine oxidase activity in human endometrium throughout the menstrual cycle. ●, 5-HT; ○, Da; △, PEA.

HT-oxidizing activity in uterus and ovary, 5.7- and 4.3-fold, respectively, but activity in liver and adrenal gland was unchanged. PEA-oxidizing activity was unchanged in all four organs studied.

These results clearly confirm our earlier observation of an increase in MAO activity in human endometrium during the menstrual cycle [1] and indicate from the rise in 5-HT-oxidizing ability but absence of increase of PEA oxidation that it is confined to MAO A alone and that MAO B is unaffected. DA-oxidizing activity increased in parallel with that against 5-HT, suggesting that it is also a substrate for MAO A in this tissue, as it is in the placenta and elsewhere [9]. The fact that MAO A alone was affected shows the specificity of the response and shows clearly that MAO A and B are two separate physiological entities under independent control. As both forms are mitochondrial, one might expect that any change due to a non-specific alteration in the nature of the mitochondrion would have affected both equally.

The progesterone-induced selective rise in MAO A in uterus and ovary (but not in liver and adrenal) supports our interpretation of the human endometrial finding as deriving, to some extent at least, from the raised concentrations of progesterone circulating during the second half of the menstrual cycle. This view receives further support from the observation that progesterone injected into pregnant rats increases MAO activity in the brains of the pups by about 25 per cent [10].

Our results raise the question of the function of MAO A in the tissues under scrutiny. Is it to protect the fertilized ovum from circulating amines? The placenta is very rich in MAO A [11], like the late cycle endometrium, and the administration of an MAO inhibitor during pregnancy can cause abortion or fetal death [12, 13]. Uterine muscle is extremely sensitive to 5-HT [14], as is the placental vasculature [15]. Alternatively, the increase in MAO A activity, at least in the human, may be related to the menstrual process by some unknown mechanism. These problems remain to be explored.

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Evidence for the external location of alkaline phosphatase activity on the surface of Sarcoma 180 cells resistant to 6-thioguanine

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The 6-thiopurines (i.e. 6-mercaptopurine and 6-thioguanine) are effective agents in the treatment of acute leukemia in man. One of the factors which limits the therapeutic usefulness of these antimetabolites is the acquisition of insensitivity by previously susceptible leukemic cells. To exert antineoplastic activity, conversion of these agents to the nucleotide level is mandatory, and most transplanted tumours achieve resistance by deletion or alteration of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) activity, the enzyme system involved in the formation of 6-thiopurine 5'-phosphate [1]. Acute lymphocytic leukemic cells of man, however, do not appear to decrease HGPRT activity as the mechanism of acquired resistance to these purine antimetabolites [2–5]. Evidence is available in a subline of the murine ascitic neoplasm Sarcoma 180 resistant to the 6-thiopurines (S180/TG) which suggests that insensitivity is the result of an increased rate of degradation of 6-thiopurine 5'-phosphate by alkaline phosphatase [6, 7]. Furthermore, leukocytes of acute lymphocytic leukemia patients demonstrated in most instances an increase in particulate-bound alkaline phosphatase activity that corresponded to acquired clinical insensitivity to the 6-thiopurines [4]. At least two particulate-bound alkaline phosphatase activities occur in S180/TG cells which are distinct in several characteristics and these have been purified to homogeneity [7–9]. The levels of these enzymes, which appear to be responsible for the degradation of 6-thiopurine mononucleotide in S180/TG cells, are elevated about 100-fold in the resistant tumor [7–9]. The cellular localization of these two isoenzymes has not been studied, and this communication is concerned with our initial observations on the location of these enzymes in S180/TG cells. Although intracellular phosphatase activity would appear to be required to express resistance to the 6-thiopurines, the findings presented in this report suggest that a significant portion of the cellular alkaline phosphatase catalytic activity resides on the external surface of the cell membrane.

Tumor cells were maintained in female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) by the weekly intraperitoneal inoculation of $1–2 \times 10^6$ cells/mouse. The activity of alkaline phosphatase was measured spectrophotometrically by following the increase in absorbance at 410 nm in phosphate-buffered saline (PBS; 0.137 M NaCl, 0.003 M KCl and 0.008 M Na_2HPO_4-KH_2PO_4) containing 1.0 mM *p*-nitrophenylphosphate (PNPP) at 37° as described previously [7]. The assay of enzyme activity of intact cells employed incubation (2×10^6 cells/ml) in the above medium for varying periods of time, followed by measurement of the absorbance of the medium after removal of cells by centrifugation for 1 min at 1600 *g*.

The rate of hydrolysis of PNPP to *p*-nitrophenol by intact S180/TG cells was about 2.7 times faster than that produced by the parent drug-sensitive Sarcoma 180 cells (S180) at pH 7.4 (Fig. 1). Since PNPP is negatively charged, it was assumed that this material was taken up poorly by cells; *p*-nitrophenol, however, might be expected to readily permeate cells. Thus, measurement of this material in the medium should underestimate somewhat the quantity of this product formed from intact cells. The possibility that S180/TG cells secreted greater amounts of hydrolytic enzyme activity into the medium than S180 cells was eliminated by the observation that phosphohydrolase activity was minimal in medium freed of cells after incubation of each cell line for up to 45 min (Fig. 2); this finding also indicated that hydrolytic activity was largely associated with

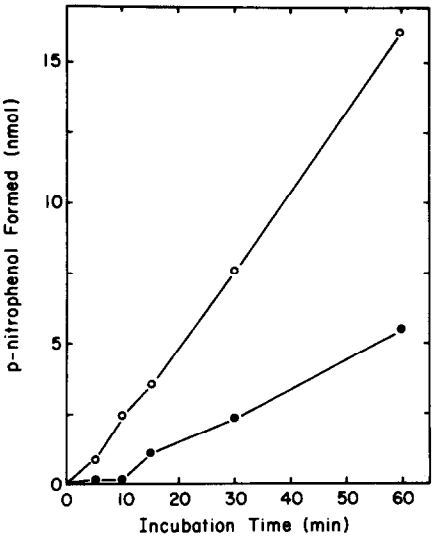

Fig. 1. Hydrolysis of PNPP by intact S180 and S180/TG cells. Cells (2×10^6/ml) were incubated with 1 mM PNPP at 37° in PBS (pH 7.4). At various times thereafter, the absorbance at 410 nm of the supernatant solution was measured, using a supernatant fraction of an incubation mixture without PNPP as the blank. The molar absorbance of *p*-nitrophenol employed was $1.7 \cdot 10^4$ moles $\cdot l^{-1} \cdot cm^{-1}$. Key: phosphohydrolase activity of S180 (●——●) and of S180/TG (○——○).