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Amine Oxidase Histochemistry of the Human Uterus During the Menstrual Cycle*

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Summary. The enzymes monoamine oxidase A (MAO A), monoamine oxidase B (MAO B) and benzylamine oxidase (BzAO) have been localized histochemically in the human uterus during various phases of the menstrual cycle. The results show a large increase in MAO A activity in the endometrial gland cells in the secretory phase of the cycle. MAO B activity was found in both endometrium and myometrium but did not show a cyclical variation in activity. BzAO was localized primarily in the tunica media of the myometrial blood vessels. These observations have been supported by parallel biochemical assays.

Introduction

Previous work in this laboratory (Southgate et al. 1968) has shown that monoamine oxidase (MAO) activity increases markedly in human endometrium during the secretory phase of the menstrual cycle. Since these original observations were made, two forms of monoamine oxidase have been identified and classified as MAO A and MAO B according to their sensitivity to the selective inhibitor, clorgyline (Johnston 1968). The widespread distribution in man of a further enzyme, benzylamine oxidase (BzAO) has also been recognized (Lewinsohn et al 1978). Recently it has been shown that it is possible both to localize histochemically, and distinguish between, these enzymes (Ryder et al. 1979); this has provided us with the opportunity of re-examining the amine oxidases in the human uterus during the menstrual cycle and determining whether the secretory-phase increase in activity represented a generalized phenomenon or was confined to a specific enzyme form.

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Methods and Materials

Whole uteri without pathological endometrial features were obtained by hysterectomy from six patients. The uterine cavity was opened and parallel blocks of resected mid-cavity tissue were either (a) fixed in Bouin's solution for subsequent histological examination, (b) frozen on solid carbon dioxide for histochemical studies or (c) further divided into endometrium and myometrium and frozen on solid carbon dioxide for biochemical assay.

Histology. The material fixed in Bouin's solution was routinely embedded in paraffin wax. Sections (5 μm) were stained in haematoxylin and eosin and the phase of the menstrual cycle determined by the scheme of Noyes et al. (1950).

Histochemistry. The technique employed has been described in detail by Ryder et al. (1979). Briefly, 20 μm cryostat sections were incubated in the following medium:

3-amino-9-ethyl carbazole (Sigma)	2 mg
dimethyl formamide (Analar)	0.5 ml
phosphate buffer, 0.05 M, pH 7.6	9.5 ml

The mixture was shaken, filtered and added to:

peroxidase (Sigma type II)	10 mg

substrate: either tyramine HCl (Sigma) or benzylamine HCl

(prepared from free base)	12 mg

Incubation was at 37° C for one or two hours
Inhibitor controls: Sections were pre-incubated for 15 min in each of the following inhibitors made up in 0.05 M phosphate buffer, pH 7.6. The same concentration of inhibitor was incorporated into the incubating medium.

Clorgyline: 10^{-7} M, 10^{-8} M, 10^{-9} M (final concentration)
Deprenyl: 10^{-6} M, 10^{-7} M, 10^{-8} M (final concentration)

"No-substrate" control: sections were incubated in a substrate free medium.

After incubation the sections were washed in 0.9% (w/v) NaCl solution and post-fixed for approximately 2 h in 10% formalin. They were mounted in glycerol jelly.

Biochemical Assays. The specific activities of MAO-A, MAO-B and BzAO were determined by radiometric microassay using ^{14}C benzylamine (86 μM) and ^{14}C 5-hydroxytryptamine, (371 μM) as substrates. Inhibitors employed were (–)deprenyl, (3.6×10^{-7} M) and clorgyline (3.6×10^{-7} M). All molarities represent final concentrations. Homogenization and assay procedures were as described by Lewinsohn et al. (1978, 1980).

Results

Histology

The phase of the menstrual cycle of the endometrium from each uterus was dated by histological means. One specimen had a proliferative pattern, two were early secretory, two were late secretory and one specimen had a premenstrual late secretory pattern.

Histochemistry

Reaction product was localized to four main areas of the uterus: the gland cells and stroma of the endometrium and the smooth muscle and blood vessels of the myometrium.

Table 1. Cyclical amine oxidase activity in the endometrium

Patient No.	Phase of menstrual cycle	Endometrial gland cell reaction Substrate: tyramine	Specific activity of MAO-A: nanomoles/mg protein/30 min
1	Proliferative	0 to +	1.1
2	Early secretory	+	6.9
3	Early secretory	+	4.7
4	Late secretory	++	18.9
5	Late secretory	++	20.0
6	Premenstrual late secretory	+++	118.0

Key to histochemical reaction: +++=very strong reaction; ++=strong reaction; +=moderate reaction; 0=no reaction

Table 2. Non-cyclical amine oxidase activity in the uterus

Substrate	Inhibitor	Endometrium		Myometrium		
		Gland cells	stroma	smooth muscle	blood vessels	nerve (?)[b]
Tyramine	–	[a]	+	++	++	++
Tyramine	deprenyl 10^{-6} M	[a]	0	0	+	++
Tyramine	deprenyl 10^{-7} M	[a]	0	0	+	++
Tyramine	deprenyl 10^{-8} M	[a]	0	0	+	++
Tyramine	clorgyline 10^{-7} M	+	+	++	+	0
Tyramine	clorgyline 10^{-8} M	+	+	++	+	0
Tyramine	clorgyline 10^{-9} M	+	+	++	+	0
Tyramine	clorgyline 10^{-7} M + deprenyl 10^{-7} M	0	0	0	0	0
Benzylamine	–	+	+	++	+++	0
Benzylamine	deprenyl 10^{-6} M	0	0	+	+++	0
Benzylamine	deprenyl 10^{-7} M	0	0	+	+++	0
Benzylamine	deprenyl 10^{-8} M	0	0	+	+++	0
No substrate control		0	0	0	0	0

Key: +++=very strong reaction, ++=strong reaction, +=moderate reaction, 0=no reaction

[a] Variable depending on phase of cycle (see Table 1)

[b] Nerve (?) and blood vessel localization with tyramine are after extended incubation

Activity in the endometrial gland and surface epithelial cells, demonstrated with the substrate tyramine (Fig. 1), increased significantly during the secretory phase of the menstrual cycle (Table 1). This activity was virtually unaffected by the specific inhibitor deprenyl but was almost completely inhibited by clorgyline.

There was a non-cyclical localization of an enzyme oxidising tyramine in the stroma of the endometrium and in the smooth muscle of the myometrium (Table 2). This activity was completely inhibited by deprenyl but was unaffected by clorgyline. After extended incubation with tyramine, reaction product was also localized in the tunica media of the myometrial blood vessels. The vessel

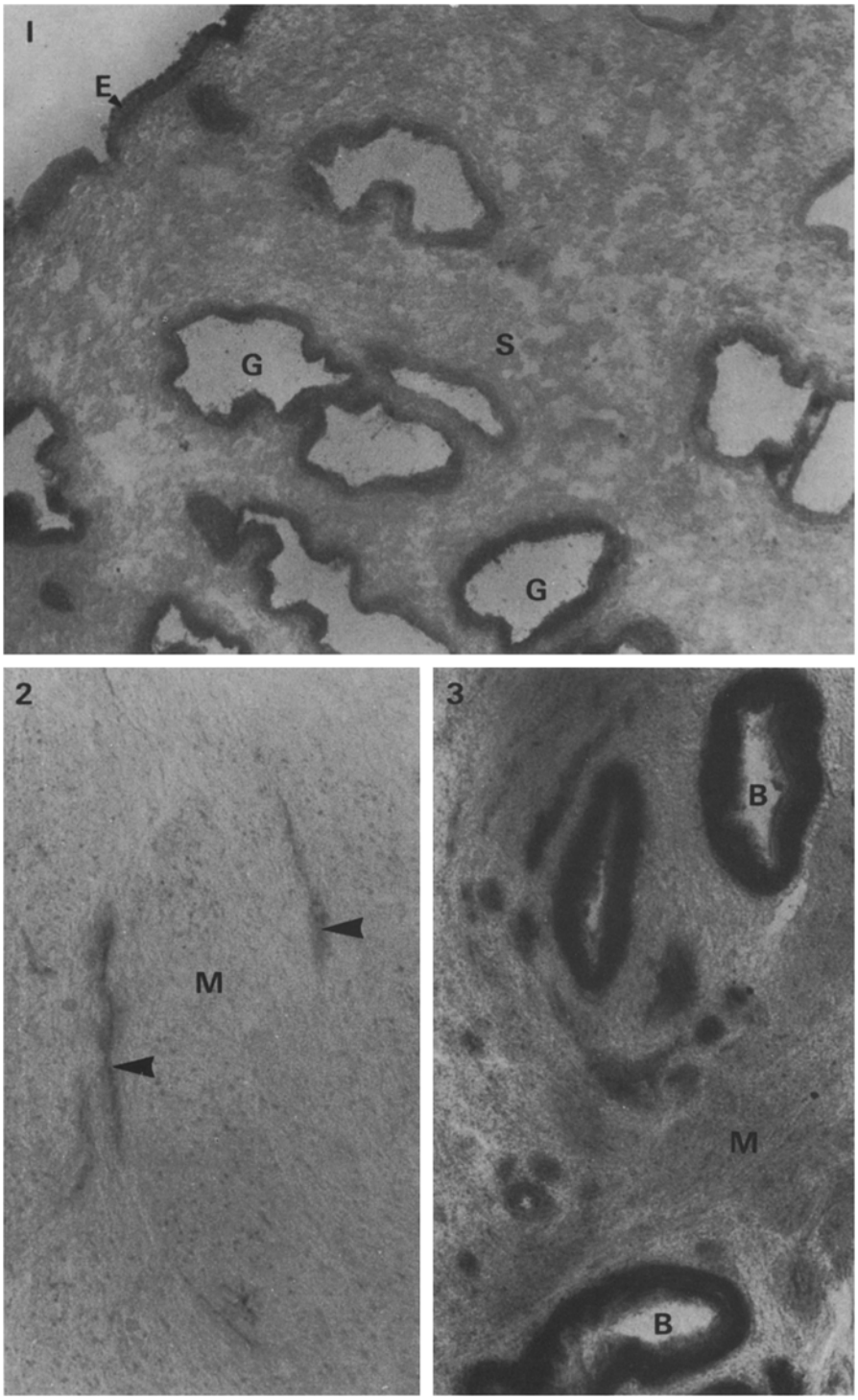
1
E
G
S
G
2
M
3
B
M
B

Table 3. Specific activities of the amine oxidases in human uterus: nanomoles/mg protein/30 min)

Enzyme	Endometrium	Myometrium
MAO-A	Variable	2.1 ± 1.24
MAO-B	6.73 ± 1.78	7.5 ± 1.92
BzAO	0.73 ± 0.7	10.5 ± 2.96

activity was only partially inhibited by deprenyl or clorgyline but was completely inhibited by a mixture of the two. Also revealed after extended incubation were faint "streaks" of activity in the smooth muscle (Fig. 2) particularly in the region of blood vessels. This localization corresponded closely to the distribution of catecholamines described by Owman et al. (1967) and may represent adrenergic nerve terminals. The "streaks" were abolished by clorgyline but were present in deprenyl treated sections.

When benzylamine was employed as substrate noncyclical activity was observed in all the four main areas of the uterus (Table 2). Reaction in the endometrium was completely abolished by deprenyl. The same inhibitor did not entirely block the activity in the myometrial smooth muscle, however, and has very little effect on the intense activity in the tunica media of the blood vessels (Fig. 3).

There was no reaction product present in the "non-substrate" controls.

Biochemistry

The specific activity of MAO-A in the endometrium showed significant differences with time of cycle (Table 1), whereas that of MAO-B and of BzAO showed no cyclical variation (Table 3). MAO-B was present in both endometrium and myometrium; BzAO was virtually undetectable in the endometrium.

Discussion

The rise in MAO activity in the endometrial glands during the menstrual cycle reported by Southgate et al. (1968) has been confirmed in the present study. Our results further demonstrate that the enzyme manifesting the cyclical change in activity oxidizes tyramine but not benzylamine and is sensitive to the selective inhibitor clorgyline. This enzyme has the biochemical characteristics of MAO A which confirms the findings of Mazumder et al. (1980).

The gland and stromal cells of the endometrium contained an enzyme oxidiz-

Fig. 1. A cryostat section of human endometrium showing reaction in the endometrial gland cells (*G*) and the surface epithelial cells (*E*). The stroma (*S*) is only moderately stained. Substrate: tyramine. Mag × 100

Fig. 2. A section of myometrium showing the "streaks" of clorgyline sensitive activity (*arrows*) amongst the smooth muscle (*M*). Substrate: tyramine. Mag × 160

Fig. 3. A cryostat section of myometrium showing the intense activity in the tunica media of the blood vessels (*B*). The smooth muscle (*M*) is stained less intensely. Substrate: benzylamine. Mag × 100

ing both tyramine and benzylamine which did not vary with the menstrual cycle. This enzyme was sensitive to deprenyl and had the characteristics of MAO B (Houslay and Tipton 1974; Knoll 1976).

Non-cyclical activity in the smooth muscle of the myometrium was demonstrated when using both tyramine and benzylamine. While the tyramine-oxidizing enzyme was totally inhibited by deprenyl, activity with benzylamine was only partly abolished. These results suggest that the myometrial smooth muscle contains both MAO B and BzAO.

The tunica media of the myometrial blood vessels showed intense activity with benzylamine which was insensitive to deprenyl. These results confirm the vascular location of BzAO in the placenta described by Ryder et al. (1979).

With extended incubations with tyramine there was a very low level of activity in the vessels which was not completely inhibited by either deprenyl or clorgyline but which was abolished by a combination of the two. It is likely that both MAO A and MAO B are present in this situation. "Streaks" of clorgyline-sensitive activity were also observed in the smooth muscle, and possibly represent MAO A activity in nervous tissue.

The activity of endometrial MAO A, unlike MAO B and BzAO is presumably highly progesterone dependent (Mazumder et al. 1980). MAO B distribution and activity throughout the uterus are not subject to cyclical variation. BzAO is localized almost exclusively to the smooth muscle surrounding the blood vessels. These differences in activity and localization support the concept that MAO A, MAO B and BzAO are separate enzymes performing distinct functions.

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