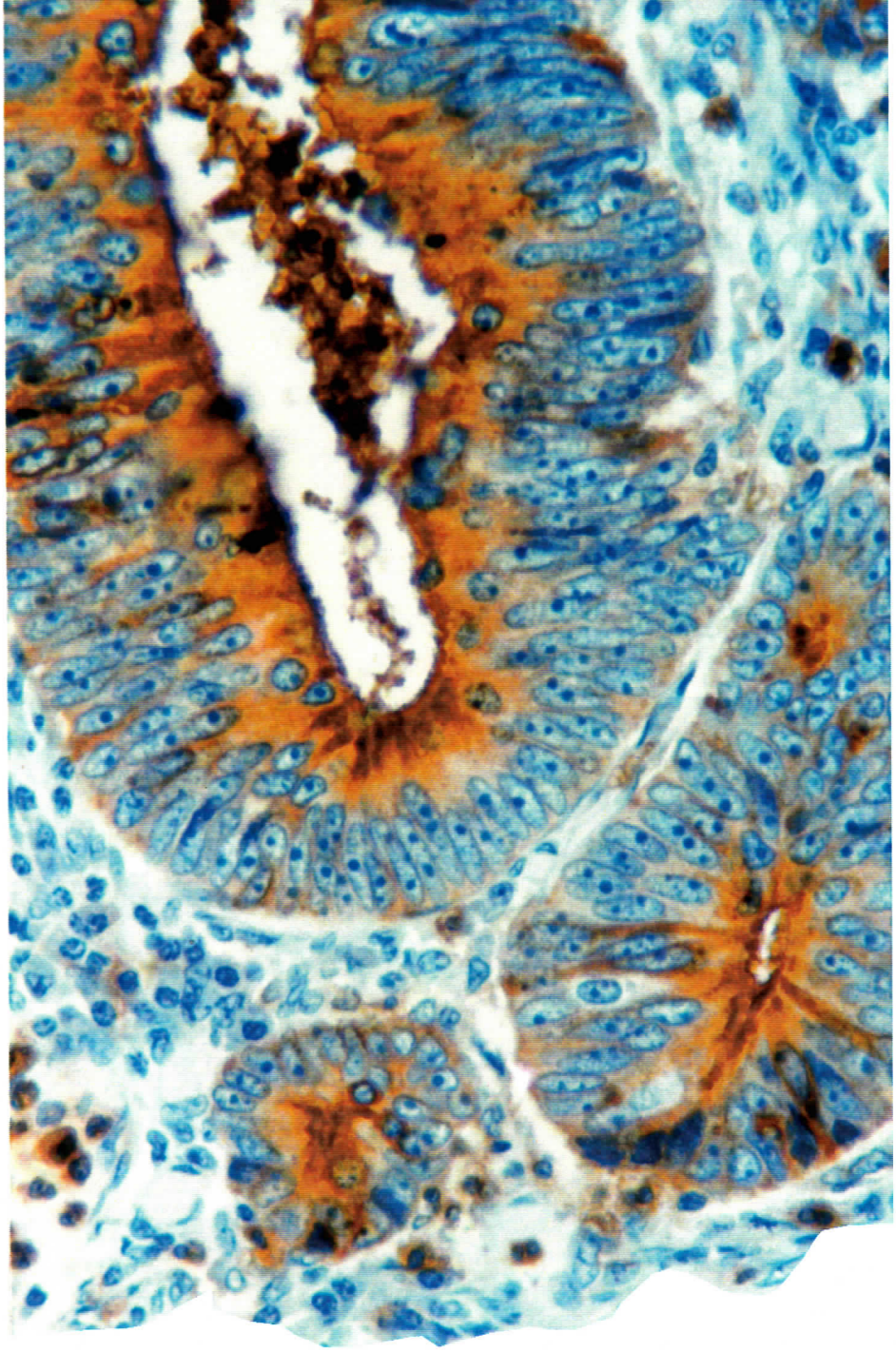

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MONOAMINE OXIDASE TYPES A AND B ACTIVITIES IN HUMAN UTERINE ARTERIES

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Abstract

The deamination of 5-hydroxytryptamine and phenylethylamine by monoamine oxidases (MAO A and B) has been studied in homogenates of human uterine arteries by radiochemical assays. The specimens have been grouped according to their age: less than 40 years old, between 40 and 50 years old and more than 50 years old. The kinetics parameters were for Vmax and Km in donors less than 40 years old were the following: to MAO A the Vmax = 54.1± 2.2 nmol/mg protein. h and the Km = 248 ± 32 mM (±SEM); MAO B the Vmax = 62.3 ± 3.2 nmol/mg protein. h and the Km = 12.9 ± 0.7 mM (±SEM). The results obtained for Vmax and Km in donors with age between 40 and 50 years old were the following: to MAO A the Vmax = 45.4 ± 2.5 nmol/mg protein. h and the Km = 256 ± 28 mM (±SEM); MAO B the Vmax = 65.9 ± 2.1 nmol/mg protein. h and the Km = 10.5 ± 1.3 mM (±SEM) . And the results obtained for Vmax and Km in donors with age more 50 years old were the following: to MAO A the Vmax = 22.9 ± 1.7 nmol/mg protein. h and the Km = 310 ± 25 mM (±SEM); MAO B the Vmax = 42.5 ± 1.4 nmol/mg protein. h and the Km = 12.0 ± 0.6 mM (±SEM).

The results obtained with the human uterine arteries show that there is a significant age-dependent decrease in enzyme activities. In the oldest group the differences are more evident for MAO A. In the human uterine artery the decrease of MAO activities are not dependent of catecholamine levels or the density innervations but show a ageing-dependent.

Key words: Human arteries; monoamine oxidase; MAO A and B activities ; ageing

1 - Introduction

The uterine arteries have a large capacity to accumulate and metabolise noradrenaline as described by many authors [1]. Furthermore, blood vessels play an important role as sites of loss for circulating catecholamines [2, 3].

The present work aims to characterise the enzymatic kinetics of monoamine oxidase activity in the human uterine artery and the influence of age on this activity.

Disturbances of the catecholamine metabolism in the central and peripheral nervous system during mammalian ageing are well documented [4, 5]. Lowered concentrations of NA and/or DA with age have been reported in human brains [6] and in other species [4, 7].

MAO activity has been extensively studied in relation to the ageing process in the brain [8], the heart [9] and the platelets [10], both in animals and human. In other

tissues, much less is known about modifications associated with ageing in either form of this enzyme, particularly those involved in absorption, uptake, detoxification, excretion or retention of body amines.

Our aims are the comparison of the MAO activity in both forms of this enzyme and trying to establish a relationship between these parameters and the catecholamine levels and density of innervation in this arteries. As the donors have different age we have related also the enzyme activities with age.

2 - Material and Methods

2.1 - Blood vessels

The uterine artery segments were obtained from women, between 30 and 60 years old, who had undergone hysterectomy due to uterine non-malignant pathological changes. The arteries were isolated and transported to the laboratory on ice in Krebs-Henseleit solution [11]. In order to eliminate a possible contamination from plasma amine oxidases activity the segments were dissected free from surrounding tissue and stored at -80°C in a 0.01M phosphate buffer at pH 7.4. The vascular segment was homogenated with 2 ml phosphate buffer 0.01M, pH 7.4 with a DUAL homogenizer at 4°C and the homogenate was rendered free of large fragments by centrifugation at 600 g during 2 minutes. The supernatant was diluted in a phosphate buffer to obtain the same protein content in all the samples. The protein concentrations was determined using the method of Lowry et al. [12] using bovine serum albumine as standard. The specimens have been grouped according to their age: less than 40 years old (n=8), between 40 and 50 years old (n=10) and more than 50 years old (n=12).

2.2 - Determination of MAO A and MAO B Activities Monoamine oxidase assay

MAO A and B activities were determinated using ^{3}H-5-hydroxytryptamine creatinine sulphate (5-HT, 15.1 Ci/mmole, New England Nuclear) as a preferential substrate for MAO type A and ^{14}C-b- phenylethylamine HCl (PEA, 50 Ci/mmole, New England Nuclear) as preferential substrate for MAO type B. The reaction mixture contained inhibitors in aqueous solution clorgyline (0.1 mM as inhibitor of MAO A) and (-) deprenyl (10 mM for MAO B) and homogenate, for blanks water was used instead of homogenate. After preincubation time (30 min at 37°C), of the corresponding substrate were added and the solution was oxygenated. After 20 minutes of incubation (at 37°C), the reaction was stopped by the addition of 10 ml HCl 3M. Deaminated product was extracted and measured by liquid scintillation counting [13].

Preliminary studies were carried out to establish incubation time, homogenisation conditions and concentrations of substrates which allowed us to work in linear conditions. To obtain information about Km and Vmax in the uterine artery we used 50 mM to 3200 mM for ^{3}H-5-HT, and 10 mM to 320 mM concentration for ^{14}C-b-PEA. The individual measurements were performed with ^{3}H-5-HT 2000 mM and ^{14}C-b-PEA 100 mM. The concentrations used were 3 times the respective Km. MAO activity is expressed in nmoles of substrate metabolised per milligram of protein per hour of incubation.

2.3 - Determination of Endogenous Catecholamine Content

The levels of dopamine (DA), noradrenaline (NA) and adrenaline (AD) in the arteries were measured by High Performance Liquid Chromatography (HPLC), with electrochemical detector which consisted of a glassy carbon electrode. The working electrode was set at 650 mV relative to an Ag/AgCl reference electrode. The working sensitivity was 2 nA/V. A small ring was cut, weighed and placed in a tube containing 1 ml of 0.1M perchloric acid, held at 4°C for 24 hours. After that, 50 ml of the resulting solution were injected into a reversed column ODS18 (Spherisorb; 5m, 250x4.6 mm) [14].

2.4 - Hystological Studies

Formaline-fixed, parafin-embedded blocks from the patients were studied.

Sections were cut at 4 mm dewaxed and rinsed in alcohol. A hematoxylin and eosin stain and the immunohystochemical study for neurone specific enulase (NSE) and S 100 protein were performed for each section. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxidase. After that, the tissue sections were incubated with primary antibody anti-SE (DAKA-A598) or antibodies anti-S100 (DAKA-Z311) for 30 min at room temperature. The immunohystochemical method was an avidine-biotine complex immunoperoxidase method. The slices were developed with diaminobenzidine, rinsed with

tap water and counterstained with hematoxyline, and mounted with entelam [15]

2.5 - Data Analysis

The enzymatic kinetics were determined through Lineweaver-Burk analysis, using the Michaelis-Menten program of J. Chou and T. Chao Chou (Elsevier Biosoft) for the computer Apple IIe.

The results are expressed as means ± SEM. The statistical methods used to analyse the results were a one-way ANOVA test followed by a Student's t test, where impaired data values of $p< 0.05$ were considered significant.

3 - Results

3.1 - Monoamine Oxidase Activity

a) Enzyme kinetics

In table 1 are indicated the kinetic parameters for MAO A and MAO B, obtained as described in methods.

b) Individual monoamine oxidase activity

MAO A and MAO B activities, measured in individual arteries, are shown in figure 1. The results are expressed in nmoles of substrate per mg of protein per hour of incubation. The reduction in the enzymes activity as a function of age is shown as a percentage in table 2. The results obtained with the human uterine arteries show that there is a significant age-dependent decrease in enzyme activity. In the oldest group the differences are more evident for MAO A. With ageing, MAO B activity decreases but, compared it with MAO A, this reduction was smaller.

3.2 - Catecholamines Content

The levels of catecholamines obtained in donors less than 40 years old were the following: to NA = 544 ± 125.4 pg/mg; AD = 12.8 ± 3.9 pg/mg and DA = 31.4 ± 7.7 pg/mg. The results obtained in donors with age between 40 and 50 years old were:

NA = 445.4 ± 44.4 pg/mg; AD = 12.9 ± 2.2 pg/mg and DA = 20.5 ± 2.3 pg/mg. And the results obtained in donors with age more 50 years old were the following: NA = 526.3 ± 36.1 pg/mg; AD = 21.0 ± 2.9 pg/mg and DA = 24.4 ± 2.1 pg/mg.

The levels of catecholamines did not change with the age, and do not have any association with the decrease in the MAO A and MAO B activity.

3.3 - Hystological Studies

The uterine arteries observed by histology and immunocytochemistry showed on same neuronal structures deeply localised in the artery wall, closed to the adventicia.

We observed that age doesn't significantly change the innervation density of arteries.

Discussion

There is a growing of evidence suggesting that adrenergic control of the vascular system is altered with age. Also variations in the MAO activity with age in rat tissues were found. In fact, the experimental results of Danh et al. [16] demonstrated that ageing exerts specific changes in MAO A and MAO B activities of the various peripheral organs of the rat. MAO A activity of the old rats was strongly increased in the heart, moderately in the liver and very slightly in kidney, decrease in lung and slightly in brain and practically unchanged in duodenum. MAO B activity of old rats was increased in brain and liver, decreased in lung, unchanged in kidney and duodenum when compared to that young rats. Since MAO activities are dependent on the microenvironment of the membrane, changes in its composition, particularly of phospholipids during ageing might lead to altered functional expression of MAO. Nohl and Kramer [17] found that negatively charged phospholipids as well as polyunsaturated fatty acids of membrane lipids were reduced with ageing. Phospholipases were used to chemically modify the membrane phospholipids. After phospholipase D treatment, MAO B enzyme was inactivated to a much greater extent than MAO A enzyme. In contrast, after phospholipase C treatment, MAO B enzyme was intact or activated, whereas MAO A was similarly inactivated in the case of phospholipase D digestion.

The metabolism of monoamines in mammalian organisms are dependent of a number of different aspects who can influence the activity of the monoamines oxidases [18].

In the present work the decrease observed in the activities of MAO A and MAO B with age are also dependent of the endocrine factors as the levels of oestrogenes and progesterone measured in some of the donors arteries. However the hystological observations and the catecholamines levels are not significantly different with the age although for the MAO A and MAO B activities the results would be significantly decrease. Having in mind that the tissue difference could be due

	^{3}H-5-Hydroxytryptamine MAO A			^{14}C-β-Phenylethylamine MAO B		
	Km	Vmax	r	Km	Vmax	r
< 40 years	249±31	54,1±2,2	0,998	12,9±0,7	62,3±3,2	0,996
40-50 years	256±28	45,4±2,5*	0,996	10,5±1,3	65,5±2,1	0,995
> 50 years	318±25	22,9±1,7*	0,993	12,0±0,6	42,5±1,4*	0,994

*P <0.05 compared to control group (Student t test)

Table 1 - The enzymatic kinetic parameters for MAO A and B in uterine arteries with different age..

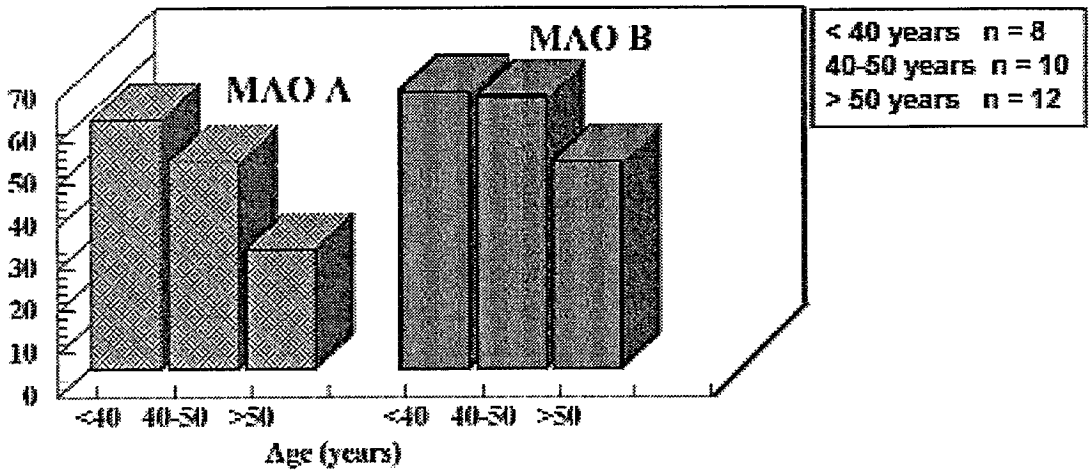

Figure 1 - MAO A and MAO B activities, measured in individual arteries. The results are expressed nmoles of substrate per milligram of protein per hour of incubation

	MAO A	MAO B
< 40 years	—	—
40-50 years	↓ 16%	↓ 2%
> 50 years	↓ 52%	↓ 24%

Table 2 - reduction in the enzymes activity percentage under the influence of age

to differences in the catalytic properties of MAO A and MAO B from tissue to tissue, or due to differences in the concentrations of available active centres of MAO A and MAO B our observations could be dependent on the environment of the mitochondria which doesn't directly change the endogenous catecholamine levels or the distribution of adrenergic innervation. It just interfere with the activity of both enzymes.

Speculating about these results one can assume that enzymatic cofactors alteration, as well as membrane phospholipids or even other amines, like semicarbazide sensitive aminoxidase (SSAO) can change the MAO activity.

Different activity levels of MAO A and MAO B in the human uterine artery that we have shown are in according the results of other authors who have described different values of Km and Vmax for both enzyme. To confirm this affirmation we can report the results in the human cerebral cortex or in liver [19], more studies have demonstrated that the difference in MAO A and MAO B activities in brain regions is not due to different molecular properties of these enzymes across the brain but it is simply due to different concentrations of the enzyme forms in brain regions [20]. And also the report of Buffoni 1993 [21] where disturbances of catecholamines metabolism in peripheral and central nervous systems mammalian ageing are well documented.

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