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Histochemically Demonstrable Monoamine Oxidase Activity in the Adult Human Heart in Various Cardiac Diseases

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Summary. The present work was undertaken in order to study the role of monoamine oxidase (MAO) enzyme in the genesis of altered cardiac noradrenalin level in the human heart in various underlying pathologic conditions. The histochemical localization and the activity of MAO were studied in the right atrial appendage of man in ischemic heart disease, in valvular heart disease without or with congestive myocardial failure, and in hearts with an uncomplicated atrial septal defect.

MAO was found to be localized mainly extraneuronally in the muscle cells, a little activity was detected in the connective tissue spaces, and nerves reacting positively were tentatively identified. There were no significant differences in MAO activity measured photometrically between the various heart disease groups. It seems that MAO enzyme plays only a small or no role in the genesis of the altered noradrenalin level in the human heart observed in ischemic heart disease or congestive cardiac failure.

Key words: Monoamine oxidase — Histochemistry — Heart — Heart diseases — Man.

Introduction

In elucidating the mechanism responsible for the depletion of cardiac catecholamine (CA) stores in myocardial hypertrophy and failure, attention has also been paid to the contribution of CA-metabolizing enzymes. There are several partially controversial reports concerning the changes in biochemically measured monoamine oxidase (MAO) activity in these situations. De Champlain et al. (1968) found increased MAO activity during the development of cardiac hypertrophy in the rat as did Sassa (1971) and Yamazaki and Ogawa (1971) in the failing heart of the rabbit, while Krakoff et al. (1968) detected decreased MAO activity in the same situation in the cat. In congestive myocardial failure of the human heart, MAO activity was not significantly altered (DeQuattro et al., 1973).

The aim of the present work was to study histochemically MAO activity in the right atrial tissue in various heart diseases in man in which the CA level is known to be high (in ischemic heart disease) or decreased (in congestive myocardial failure), and to elucidate the role of MAO enzyme in the genesis of the altered CA level.

Patients and Methods

Classification of the Patients. Samples of the right atrial appendage were obtained during a coronary bypass operation from 7 patients (6 males and 1 female) suffering from ischemic heart disease (IHD). Control samples of the right atrial appendage were taken during open-heart surgery from 5 male patients with left-sided univalvular or multivalvular heart disease (VHD) without congestive myocaridal failure. The second control group consisted of 5 adult female patients with an uncomplicated atrial septal defect of the secundum type (ASD). The third control group (CHF) consisted of 4 patients (2 males and 2 females) who had experienced congestive failure prior to surgery based on VHD. All the patients of this last group showed a tendency to dyspnea even at rest, orthopnea, and an increase in venous pressure and body weight due to edema. The degree of myocardial failure and anginal symptoms were assessed according to the clinical and laboratory findings and the classification of the New York Heart Association (NYHA, 1964). This control group comprised patients from the functional classes III and IV as judged by the NYHA criteria. A summary of the clinical and laboratory data of the different groups is presented in Table 1.

Table 1. Summary of the clinical data of the various patient groups of ischemic heart disease (IHD), valvular heart disease (VHD), atrial septal defect (ASD), and congestive heart failure (CHF)

Group number of patients in parentheses	Age (Mean ± SDM)	Degree of symptoms	Duration of symptoms (years) (Mean ± SDM)	Heart volume (cm^3/m^2) (Mean ± SDM)	Blood pressure (mmHg) (Mean ± SDM)
IHD (7)	46 ± 9	NYHA II–III	3.4 ± 1.4	455 ± 105	130/75 10/10
VHD (5)	56 ± 4	NYHA II–III	7.2 ± 4.8	755 ± 235	130/85 10/10
ASD (5)	30 ± 12	None or NYHA I	In two cases up to four years	585 ± 120	120/80 10/10
CHF (4)	46 ± 6	NYHA III–IV	10.0 ± 2.2	880 ± 370	145/85 15/5

Drug Treatment and Anesthesia. The usual daily medication with digitalis, diuretics, antiarrhythmic drugs, anticoagulants, and nitroglycerin was continued until the operation with the exception of the beta-adrenergic blocking agents, which were regularly withdrawn 10 days previously. The biopsies of the right atrial tissue were excised from the appendage before the institution of cardiopulmonary bypass and operative correction. The specimens were immediately frozen in liquid nitrogen and kept until they were mounted in the tissue holder of the cryostat.

Monoamine Oxidase Histochemistry. Monoamine oxidase enzyme (MAO; EC 1.4.3.4) was demonstrated histochemically with the method of Glenner et al. (1957) using tryptamine as the substrate. The specimens were mounted in 20% gelatin in the tissue holder. Four or five specimens were mounted in each tissue holder in a straight line. The tissue holder was placed in the cryostat so that the line formed by the specimens and the blade of the cryostat were parallel. Thus an equal section thickness (20–30 μ) of the specimens was obtained. Each tissue holder contained one of the specimens taken from the heart with ASD which served as the control specimen. The other three or four specimens were taken from the hearts with the following underlying diseases: IHD, VHD, and CHF. The sections were incubated with tryptamine as the substrate at 37° C for 45 min. The sections, which were incubated under identical reaction conditions without tryptamine, were used to study the specificty of the MAO reaction and the possible content of colored pigments in various heart diseases which could interfere with the colored reaction product formed in the MAO reaction. In order to quantitate the intensity of the coloring the exposure time of a Leitz Ortholux automatic

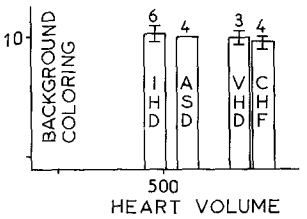

Fig. 1. Photometrically measured intensity of background coloring of cryostat sections in various heart diseases (abbrevations, see Table 1). The respective mean heart volumes (heart volume, cm^3/m^2 of body surface area) of each group are indicated by positions on abscissa. Intensity is expressed in arbitrary units. In IHD, VHD and CHF groups, the standard deviation of the mean is indicated. Number of patients in every group is given over the bar. Statistical difference between intensities of background coloring in various groups is not significant ($P > 0.1$)

camera fitted with a photomultiplier tube was determined with a stopwatch. The setting of the camera was chosen so that the exposure time was about 3 s. In each section four fields (five in the sections which were incubated without tryptamine) were measured outside lipofuscin granules at about an equal distance from each other in a line. In every heart disease group four sections incubated with tryptamine and one incubated without tryptamine from each tissue holder were measured. In every microscope slide the intensity of the coloring of the section from the heart with ASD was expressed as 10 by dividing the mean exposure time by the proper divider. Then the intensities of other groups were calculated by dividing the mean exposure time by the same value. Further, the mean intensity value was determined for the values obtained from the sections which were cut from the same specimen. Finally, the mean intensity values and standard deviations were calculated and plotted against the mean heart volume. The statistical differences were studied using Student's t-test. It must be pointed out that the results concerning MAO activity were semiquantitative due to the difficulties of measuring the intensity of a histochemical reaction, which has both a diffuse and a granular component. In addition, MAO activity might not be the same in all hearts with ASD, as it was supposed to be in the present measurements.

Results

In the sections incubated without tryptamine no blue color or formazan precipitates were seen which could indicate MAO activity. Lipofuscin pigment appeared as yellow granules. There were no significant differences ($P > 0.1$) in the intensity of the "background staining" between various groups (Fig. 1). In the sections incubated with tryptamine strong MAO activity was observed in the muscle fibers (Fig. 2). The reaction product appeared as diffuse blue staining and crystal precipitates (Fig. 3). Occasionally thin fibers with a positive reaction product were seen between the muscle fiber bundles (Fig. 3). These fibers were probably adrenergic nerves. Their number appeared to be about the same in the hearts with different underlying diseases. They could not be identified in the area of the strongly stained muscle fibers, probably because they were masked by the strong staining of the muscle. In the connective tissue between muscle fibers, a few formazan precipitates were regularly seen (Fig. 3), but these precipitates did not locate over or form any clear structures. When the intensities of MAO activity in the hearts either with different underlying pathologies or

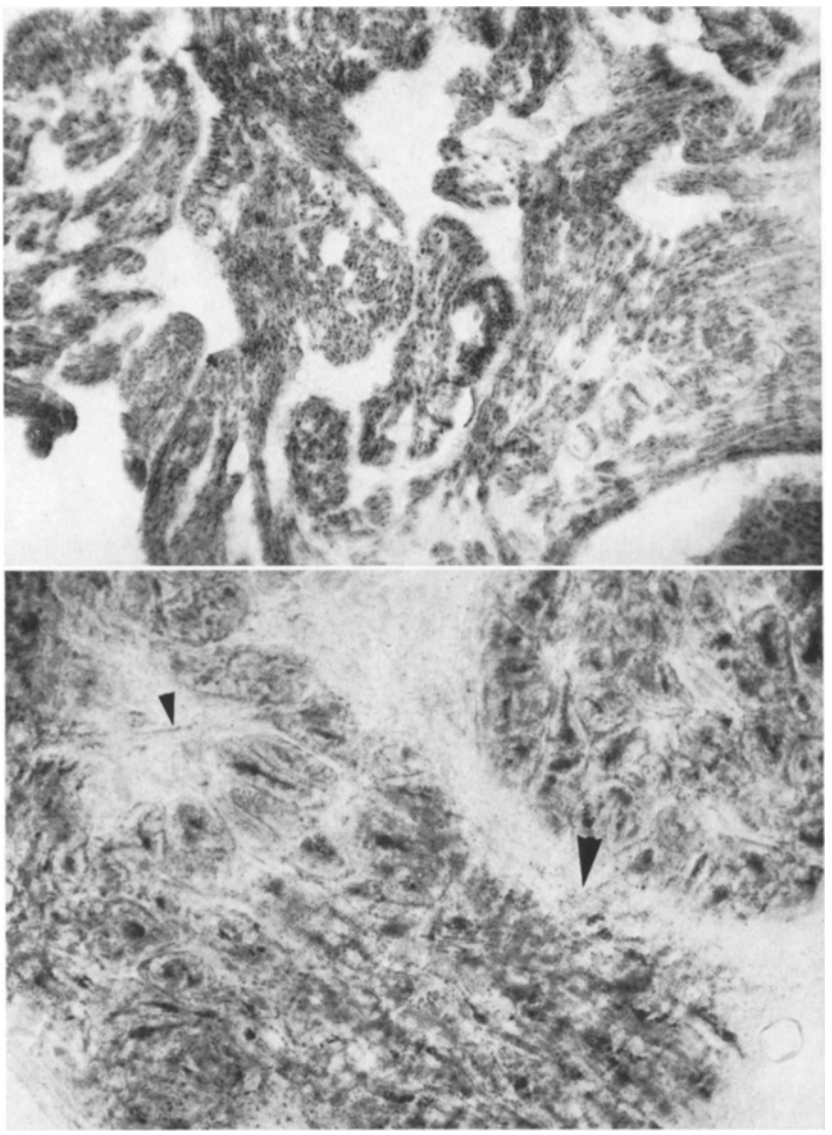

Fig. 2. Low power micrograph showing monoamine oxidase activity in right atrial tissue of heart with aortic stenosis of male patient aged 60 years. Strong activity seen in muscle fibers. × 29

Fig. 3. Micrograph from same heart as Figure 2 showing monoamine oxidase activity appearing as diffuse coloring and granular precipitates in muscle fibers. Positive reacting nerve fiber shown by small arrow. Some granular precipitates are seen also over connective tissue (large arrow) × 241

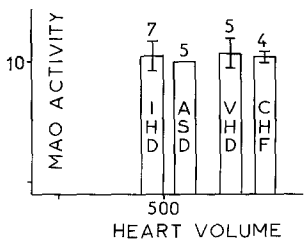

Fig. 4. Photometrically measured monoamine oxidase (MAO) activity in cryostat sections in various heart diseases. For explanation, see Figure 1. Monoamine oxidase activity expressed in arbitrary units. Statistical difference between monoamine oxidase activities in various groups is not significant ($P > 0.1$)

with respective heart volumes were compared, no differences were observed ($P > 0.1$; Fig. 4). One should note that the measurements were made over the muscle fibers, and the results were semiquantitative.

Discussion

The present study was undertaken in order to determine whether or not there are variations in MAO activity in certain pathologic cardiac lesions including IHD and whether this enzyme could contribute to the high myocardial NA content observed in IHD (Penttilä et al., 1975). The histochemical localization of MAO enzyme in the human heart revealed that a great majority of this enzyme is situated extraneuronally in the muscular tissue and that its activity is high. Some extraneuronal activity may also be localized in the fibroblasts, in which MAO activity has been detected biochemically (Jacobowitz, 1972). A few formazan precipitates, which were seen over the connective tissue, may have been derived from this pool of MAO. The present histochemical method for MAO does not seem to allow for the exact and clear demonstration of neuronal MAO in the heart owing to the strong activity in the muscle fibers which interrupts the view of neuronal MAO. The role of extraneuronal MAO as a main source of total cardiac MAO activity is also supported by the biochemical determinations after the destruction of the adrenergic nerves in the heart (e.g., Jarrot, 1971; Cottle and Nash, 1974).

The histochemical and biochemical methods which use no specific inhibitors or physical manipulations of neuronal and extraneuronal MAO (see e.g., Goridis and Neff, 1973) probably demonstrate both types of enzyme simultaneously. Thus, it seems that the changes in MAO activity in cardiac hypertrophy and failure reported earlier (see Introduction) are derived mainly from the altered activity of extraneuronal MAO.

It is the generally accepted opinion that enzymatic inactivation of cardiac NA occurs principally intraneuronally by oxidative deamination catalyzed by MAO and extraneuronally via the O-methylation pathway catalyzed by catechol-O-methyltransferase (COMT) enzyme (Kopin, 1964; Sharman, 1973). In the extraneuronal uptake process ($uptake_2$, Iversen, 1967) NA has been observed to enter the cardiac muscle cells and the fibroblasts (see ref. Burnstock and Costa, 1975), and may thereafter be at least partly metabolized via oxidative deamina-

tion by MAO. The participation of MAO enzyme in the cardiac muscle cells of man in this deamination process may partly explain the high activity observed.

The observations of the present study show that histochemically demonstrable MAO activity, which is mainly derived from the extraneuronal pool of MAO, does not vary significantly in the various heart diseases of man. These observations are in agreement with the biochemical results of DeQuattro et al. (1973). In addition, it seems that the changes in MAO activity in cardiac hypertrophy and failure depend on the mammalian species studied. It can also be concluded that extraneuronal MAO enzyme does not contribute to the high level of NA observed in IHD in man.

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