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FLUOROMETRIC SCOPOLETIN PEROXIDASE METHOD OF MONOAMINE OXIDASE ASSAY IN HUMAN PLATELETS

E. G. Brusova

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Monoamine oxidase (MAO) plays a key role in the metabolism of biogenic amines, for it catalyzes oxidative deamination of these compounds [2]. Interest in the study of human platelet MAO is due to the disturbance of some characteristics of this enzyme in various diseases [8, 10-12]. Besides nerve endings and other monoamine-containing cells, platelets take part in processes connected with monoamine metabolism, and for that reason they can be regarded as a convenient model with which to study the transport, storage, metabolism, and receptor functions of these compounds [10]. Disturbance of monoamine metabolism plays a leading role in the pathogenesis of some mental diseases [10]. Accordingly the study of the properties of human platelet MAO is a promising trend in psychiatry and, in particular, in the study of the pathogenesis of schizophrenia, alcoholism, and certain types of depression and drug addiction [8, 10-12]. Platelet MAO is also a convenient model for studying the mechanisms of action *in vivo* of the MAO inhibitors used in psychiatry [10]. All these circumstances point to the need for a study of the properties of human platelet MAO under normal and pathological conditions, but unfortunately this is difficult because of the low specific activity of this enzyme in human platelets. The highly sensitive radiometric methods usually adopted for this purpose are not always available and have certain disadvantages inherent in methods of amine oxidase assay based on measurement of the concentration of aldehydes formed, for these compounds are very labile and reactive [2]. Existing fluorometric methods of MAO assay in human platelets as a rule enable only one substrate to be used, and this substantially reduces the value and informativeness of the results, especially in pathological states [2].

This paper describes a method of determining MAO activity in human platelets developed by the writers on the basis of the scopoletin peroxidase method of determination of low concentrations of H_2O_2 described in the literature [6]. Certain enzyme characteristics were studied in normal subjects and schizophrenics, and our own data were compared with results obtained by other methods.

EXPERIMENTAL METHOD

The control group consisted of healthy men and women aged from 18 to 26 years (nine persons). The other group consisted of patients with acute manifestations of hallucinatory-paranoid schizophrenia (six men and one woman) aged from 24 to 41 years.

Blood was taken from the cubital vein into a plastic vessel containing 0.6 ml of 0.27 M EDTA (pH 7.4) to 20 ml blood. Platelets were obtained by the method described previously [3] with the following modifications. Erythrocytes and leukocytes were sedimented at 300g for 5 min (4°C). Platelet-enriched plasma was centrifuged at 1100g for 20 min at 4°C to

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sediment the platelets. To obtain the mitochondrial fraction the platelet suspension was homogenized initially in 10 volumes of 0.25 M sucrose made up in 0.1 M Na,K-phosphate buffer, pH 7.4 in a Dounce (glass-Teflon) homogenizer, and after preliminary centrifugation for 5 min at 300g (4°C) the mitochondrial fraction was sedimented at 15,000g for 20 min (4°C). The residue was suspended in 0.01 M Na,K-phosphate buffer, pH 8.4, and centrifuged at 15,000g for 20 min to remove some of the ballast proteins. The resulting fragments of mitochondrial membranes were suspended in 1 ml of 0.1 M Na,K-phosphate buffer and kept until required for use at −20°C.

Catalase activity was determined by the method in [5] and protein by Lowry's method [9]. The intensity of fluorescence was measured on the MPF-2 spectrofluorometer (Japan).

The following reagents were used: benzylamine (α-aminotoluene), scopoletin (7-hydroxy-6-methoxycoumarin, from Fluka, Switzerland; tryptamine-HCl (from Serva, West Germany); β-phenylethylamine (from Sigma, USA); horse-radish peroxidase (350 units/mg protein, *o*-dianisidine method; from Reanal, Hungary). The remaining reagents were of Soviet origin and of the highest degree of purity.

EXPERIMENTAL RESULTS

The method of assay of human platelet MAO developed by the writers is based on the highly sensitive method of detection of low concentrations of H_2O_2 [6] on account of the ability of H_2O_2, in the presence of horseradish peroxidase, to quench fluorescence of the alkaloid scopoletin. Under these circumstances reduced scopoletin (wavelength of excitation 350 nm, of fluorescence 460 nm) is converted into the oxidized form, which does not fluoresce. In the present experiments platelet MAO activity was determined from the quenching of fluorescence of scopoletin in the presence of peroxidase and hydrogen peroxide generated in the course of oxidative deamination of monoamines. This method was used previously to study the properties of heart muscle MAO [1].

Incubation samples contained fragments of platelet mitochondrial membranes (0.1-0.4 mg protein), horseradish peroxidase (0.05 mg), and 0.2 M Na,K-phosphate buffer, pH 8.4 up to a volume of 1 ml. The samples were preincubated for 20 min at 37°C in a Warburg apparatus to remove endogenous substrates of peroxidase. After cooling of the samples on ice scopoletine (17 nanomoles) and the substrate in the necessary concentration were added. The samples were incubated for 60 min in a Warburg apparatus at 37°C. Specific activity of MAO was expressed in nanomoles H_2O_2 formed during oxidative deamination of the amines per milligram protein in 60 min.

Since catalase activity could be present in the test samples, comparative determination of H_2O_2 was carried out in the presence and absence of the specific catalase inhibitor 3-amino-1,2,4-triazole (final concentration 65 μM) in the samples. Direct determination of catalase activity also confirmed the absence of appreciable amounts of this enzyme in the test material (data not given).

In healthy subjects specific platelet MAO activity with benzylamine as substrate (saturating concentration 250 μM) was 35.9 ± 7.9 (n = 9) nanomoles/mg protein/h. This value agrees well with results obtained by other methods [3, 14], namely about 40 nanomoles/mg protein/h.

When tryptamine and β-phenylethylamine (final concentration 10^{-4} M) were used as substrates, human platelet MAO activity was 8.9 ± 1.8 nanomoles/mg protein/h (n = 4) and 12.2 ± 2.8 nanomoles/mg protein/h (n = 4) respectively, also in good agreement with data in the literature [10].

Platelet MAO activity in patients with chronic schizophrenia is known to be significantly lower than in normal subjects [10]. However, there is definite inconsistency in results obtained by different workers for platelet MAO activity in patients with schizophrenia [3, 11]. The disagreement between the results is explained by some workers as the result of selection of different test populations of patients [7] but it could also be due to the use of different methods of determination, different substrates, and different concentrations of them.

When MAO activity in patients with schizophrenia in an acute psychotic state was determined in the present investigation with the use of benzylamine as substrate (saturating concentration 250 μM) a significant decrease ($P < 0.05$) was observed compared with control values: 15.5 ± 4.2 nanomoles/mg protein/h (n = 7) and 35.9 ± 7.9 nanomoles/mg protein/h

(n = 9). Meanwhile the values of the Michaelis constant were higher than in the control (60.9 ± 8.5 and 39.8 ± 1.4 μM respectively). These results agree with data in the literature obtained by the use of tryptamine as substrate [10].

The lower rate of oxidative deamination of benzylamine which we observed in patients with acute forms of schizophrenia may be the result of intensification of lipid peroxidation which is found in this disease [4]. Lipid peroxides are known to cause oxidation of the thiol groups of the enzyme, and in turn this leads to partial or total inhibition of monoamine oxidase activity, depending on the peroxide concentration [2].

The results described in this paper indicate that the scopoletin-peroxidase method can be used to study human platelet MAO activity in health and disease. The method is comparatively simple and available, it is highly sensitive, gives good reproducible results, and is suitable for kinetic investigations and for determination of MAO activity in short periods of time.

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