HETEROGENEITY OF HUMAN PLATELET MONOAMINE OXIDASE

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According to the "binary" classification generally accepted nowadays monoamine oxidases (MAO) of two types — A and B — are distinguished [14]. The MAO of human platelets, by its sensitivity to inhibitors, belongs entirely to the type B [14]. However, there is indirect evidence of the possible heterogeneity of the enzyme from this source. Experiments with platelet MAO have demonstrated the presence of inflections on Arrhenius graphs [10], a phenomenon usually regarded as an indication of heterogeneity of an enzyme, although such inflections could be the result of the effect of various phospholipids on the pure enzyme [5, 6]. The multiplicity of active centers or molecules of MAO has been postulated as the result of a study of the effect of oxygen [7] and of tricyclic antidepressants [8] on platelet MAO activity, which may be represented in the body by different forms with different kinetic characteristics [14].

Determination of MAO activity of platelets is used during the investigation of patients with mental diseases, some of which are accompanied by either an increase (for example, epilepsy and Alzheimer's disease or a decrease (alcoholism, schizophrenia, Reye and Lesch-Nyhan syndromes) of MAO activity [9]. It has been suggested that MAO in the brain are similar to MAO of human platelets [14]. It was shown previously that multiple forms of MAO (MAO-I, IIa, IIb, III) from brain can be fractionated preparatively [4], and cannot be identified as MAO of types A or B. In schizophrenia [3] and in animals with sudden behavior changes [2], the ratio between the various forms of MAO in the brain is altered. If many different forms of MAO exist in platelets also, their study may be of medical importance.

The aim of the present investigation was to study the possibility of preparative fractionation of multiple forms of MAO from human platelets.

EXPERIMENTAL METHOD

Platelets were obtained either from citrated human blood or from packed platelets obtained from healthy blood donors by differential centrifugation [1]. Benzylamine deaminase activity was determined spectrophotometrically as described previously [1], and protein was estimated by Lowry's method [11], with the introduction of an appropriate correction for the presence of the detergent Triton X-100 in the samples.

EXPERIMENTAL RESULTS

The method of solubilization and preparative fractionation of multiple forms of MAO by affinity chromatography was developed previously for the mitochondrial fraction of bovine or human brain [4, 12]. During solubilization of the biomembranes of a platelet suspension for 30 min at 0°C in 1.3 M urea with 1.5% Triton X-100 in 0.01 M phosphate buffer, pH 7.4, on average 21.6% of protein and 46% of the initial activity pass into the supernatant during centrifugation for 1 h at 13,000g. The Triton/protein ratio under these circumstances is of no importance between limits of 1.4 and 3.0 mg/mg. With an increase of this ratio to 7, both the yield of protein and activity are increased by 20%. After gel-filtration through a column with sephadex G-25 to remove the urea, chromatography was carried out on a column with AN-Sepharose 4B in 0.01 M phosphate buffer. In this way

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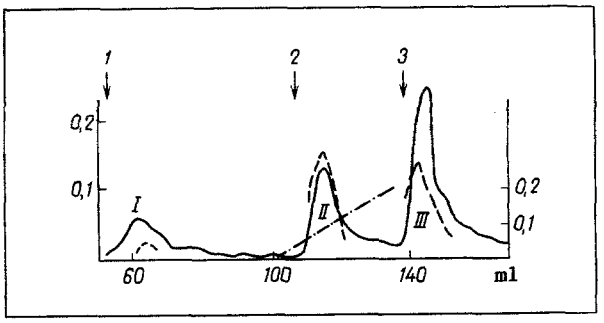

Fig. 1. Chromatographic separation of preparation of solubilized platelet MAO on AN-Sepharose 4B column. To a column measuring 12 × 1.2 cm 8.7 mg protein was applied. Fractions of 2.5 ml taken. Ordinate, on right – concentration of Triton X-100 (in %), on left – protein concentration (in mg/ml) and activity (in nmoles/ml/min). Continuous line indicates protein, broken line – activity, line of dots and dashes – Triton X-100. Curve I) Elution with 0.1 M phosphate buffer, pH 7.4; 2) elution with 0.1 M phosphate buffer, pH 7.4, + 0-0.25% Triton X-100; 3) elution by 0.4 M phosphate buffer, pH 7.4, + 0.25% Triton X-100. I, II, III) MAO isozymes.

it was possible to separate the MAO from an excess of Triton X-100 and some of the ballast proteins. MAO was eluted by increasing the concentrations of phosphate and nonpolar detergent (Fig. 1). By analogy with the multiple forms of brain MAO [4, 12], the forms of the enzyme separated in the present experiments were described as MAO-I, MAO-II, and MAO-III, Deprenil (a specific inhibitor of type B MAO) in a concentration of 10^{-5} M completely inhibited the activity of these forms of MAO, so that they could be classed as flavin MAO.

By contrast with human brain [4], three MAO isozymes and not four are present in the platelets. The ratio between these isozymes also is different. Thus brain and platelet MAO cannot be accepted as being identical. This conclusion is in agreement with data on the absence of correlation between values of total (i.e., measured disregarding the existence of multiple forms) MAO in the brain and platelets of individuals [15]. Our results are in agreement with data in the literature [13]. Judging by data published by the authors in [13], heterogeneity of human platelet MAO could be demonstrated in the course of investigations aimed at purification of MAO.

It can be tentatively suggested that the results of the study of activity of multiple forms of human platelet MAO will be more informative as regards both diagnostic and prognostic importance than the results of measurement of "total" platelet MAO activity as used at the present time.

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ACTION OF α-TOCOPHEROL AND FREE FATTY ACIDS ON PHYSICAL PROPERTIES OF THE LIPID MATRIX AND ON ADENYLATE CYCLASE ACTIVITY OF RAT BRAIN SYNAPTOSOMES

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Transmembrane transmission of neurotransmitter signals is a complex cascade process, triggered by interaction of ligand with receptor and involving different kinds of response (transmethylation of lipids, activation of phospholipases, prostaglandin synthesis, Ca^{2+} transport, etc.), depending on the type of secondary mediator. In some cases, β-adrenoreception for example, the final step in the mechanism of transmembrane signal transmission is adenylate cyclase (AC) [5, 7]. The β-adrenergic adenylate cyclase system (ACS) in the brain is a protein complex located in the plasma membrane and consisting of β-adrenoreceptor, catalytic subunit (AC itself), and a regulatory guanyl-nucleotide-binding protein (N-protein), of stimulating type [7]. The life span of functional complexes of this kind is limited and the process of their formation requires diffusion of the components of the ACS in the plane of the membrane [5, 6]. It is not surprising, therefore, that it has been suggested that the functional activity of ACS may be regulated by a change in the physical properties (microviscosity) of the membrane matrix, although the experimental data on this question are fairly contradictory and evidently depend largely on the type of membrane [6, 9].

For the reasons given above the aim of the present investigation was to study correlation between the functional state of the ACS of rat brain synaptosomal membranes and the physical state of their lipid matrix.

EXPERIMENTAL METHOD

The investigation was conducted on synaptosomal membranes from the brain of Wistar rats weighing 200-250 g. Synaptosomal membranes were isolated by the method developed previously [1]. The membrane protein content and microviscosity of the lipid matrix were determined and the lipid extract of the synaptosomal membranes obtained as in [1]. AC activity (EC 3.6.1.4) was determined with the aid of a 'Cyclic AMP Kit' (Amersham, England) as in [1].

α-Tocopherol and linolenic acid were added to a suspension of synaptosomes containing about 0.5 mg/ml of membrane protein in a 0.1 M alcoholic solution. The quantity of α-tocopherol incorporated into the membranes was determined by measuring fluorescence of the lipid extracts at λ_{exc} = 295 nm and λ_{fl} = 325 nm (the solvent was hexane). The content of free linolenic acid in the membranes was determined by gas–liquid chromatography, by the change in the characteristic line of the chromatogram of methyl esters of lipids extracted from the synaptosomal membranes.

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