MULTIPLE FORMS OF RAT BRAIN MITOCHONDRIAL MONOAMINE OXIDASE. SUBCELLULAR LOCALIZATION

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1. Introduction

The enzyme monoamine oxidase (MAO, EC 1.4.3.4) catalyses the oxidative deamination of monoamines such as 5-hydroxytryptamine (5-HT), dopamine (DA), and noradrenaline (NA), which are considered to be neurotransmitters in the central nervous system. MAO in rat brain appears to occur in multiple forms, which differ in substrate specificity [1], inhibitor sensitivity [2], and electrophoretic mobility [3, 4]. Whereas Kim and D'Iorio [5], La Motte et al. [6], and Hidaka et al. [7] express a certain reserve with respect to this multiplicity of MAO, Youdim et al. [3], and Collins et al. [8] believe that the multiple forms, obtained after solubilization and electrophoresis are genuine constituents of mitochondria and that their substrate preferences *in vitro* reflect their function *in vivo*.

MAO, being an exclusively mitochondrial enzyme, occurs in varying amounts in different parts of the rat brain [9]. A linkage between the types of MAO present in the mitochondria and the types of neurotransmitters degraded in the cell involved might thus be possible. If this indeed would be the case, one may expect differences between the substrate preferences of MAO in the mitochondria from different types of neurons and glial cells. During homogenization of brain tissue in isotonic sucrose the presynaptic mitochondria are largely enclosed in particles, referred to as synaptosomes, while the rest of the mitochondria, referred to as free mitochondria, is delivered from the surrounding structures. With the aid of sucrose density gradient centrifugation these synaptosomes and free mitochondria can be partly separated [10]. Application of sucrose density gradients in a zonal rotor leads to an improved separation and thus Blokhuis and Veldstra [11] have been able to show that rat brain mitochondria are extremely heterogeneous. We decided therefore to determine in these gradients the MAO activity with four different substrates: 5-HT (MAO-5-HT), DA (MAO-DA), NA (MAO-NA), and kynuramine (MAO-KYN) in order to examine if different types of mitochondria can be separated based on their MAO content. In addition lactate dehydrogenase (LDH, EC 1.1.1.27), as a marker for synaptosomes (enclosed cytoplasm), and protein were measured.

From the results obtained we conclude to the existence of distinct populations of mitochondria, preferentially deaminating specific biological amines in rat brain.

2. Materials and methods

Crude mitochondrial fractions were prepared by differential centrifugation of 5% (w/v) homogenates of rat cerebral hemisphere as described by Van Kempen et al. [10]. Sucrose density gradient fractionation of the crude mitochondrial fraction was carried out with a B XIV zonal rotor in a MSE 65 MK II ultracentrifuge. Gradients were prepared essentially according to Blokhuis and Veldstra [11].

Protein was determined by the method of Lowry et al. [12]. The sucrose concentration was measured by means of an Abbe refractometer. After preincubation with 0.5% Triton X-100 LDH was assayed

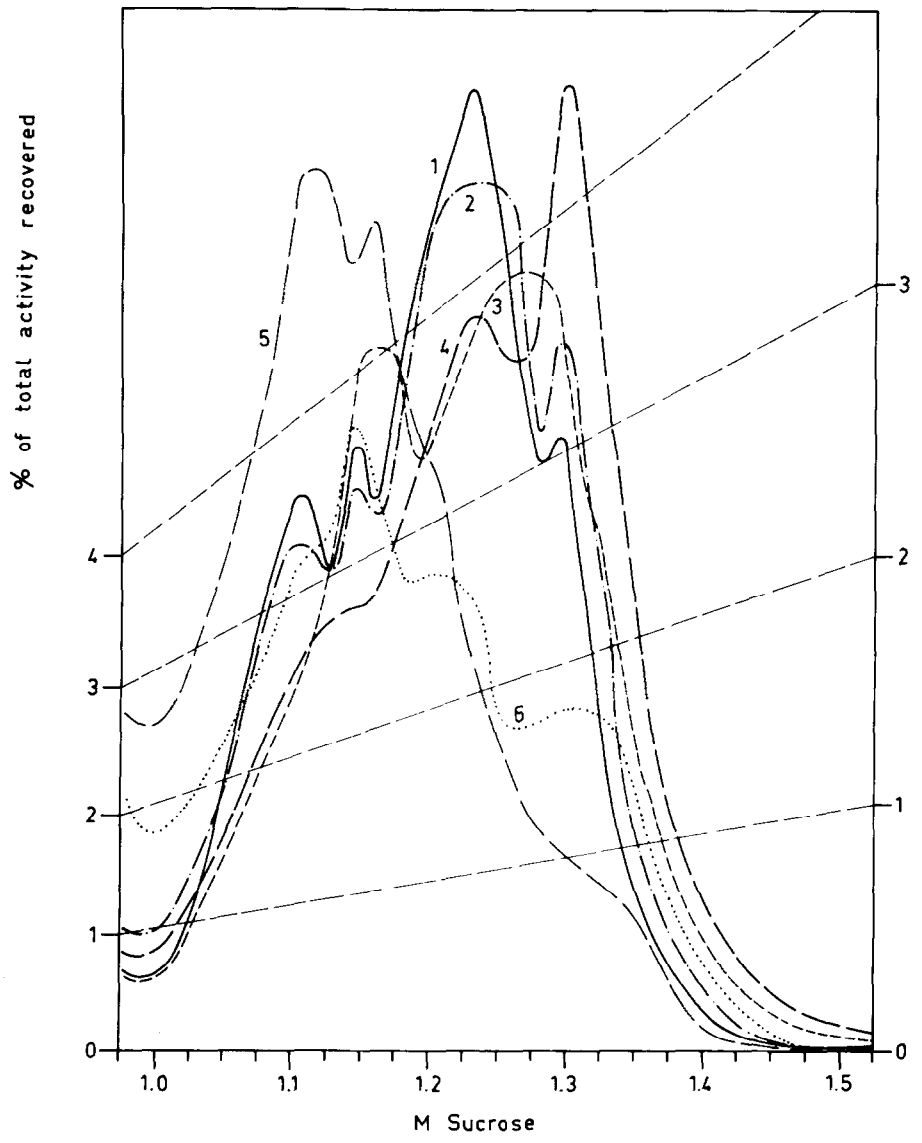

Fig. 1. Sucrose density gradient centrifugation of the crude mitochondrial fraction of rat cerebral hemisphere during 1 hr at 45,000 rpm in a MSE B XIV zonal rotor. After centrifugation 60 fractions of 10 ml each were collected. The enzyme activities and protein concentrations of 45 fractions in percentages of the total activity recovered versus the sucrose molarity are plotted on pole coordinated paper. MAO-5HT rec. 75% (1), MAO-DA rec. 75% (2), MAO-NA rec. 85% (3), MAO-KYN rec. 100% (4), LDH rec. 130% (5) and protein rec. 95% (6).

according to Kornberg [13]. MAO activities were determined by modifications of the methods used by Kraml [14], McCaman et al. [15] and Neidle et al. [16].

Incubation mixtures (1.5 ml) for : i) MAO-5-HT: 33 mM borate HCl buffer pH 8.2, 1 mM EDTA, 0.4 mM DTT, 0.1 mM ^{14}C-5-HT (0.05 μCi) and enzyme. ii) MAO-DA: 0.13 mM potassium phosphate buffer pH 8.0, 1 mM EDTA, 0.4 mM DTT, 0.06 mM ^{14}C-DA (0.1 μCi) and enzyme. iii) MAO-NA: 0.13 mM potassium phosphate buffer pH 8.0, 1 mM EDTA, 0.4 mM DTT, 0.06 mM ^{14}C-NA (0.4 μCi) and enzyme.

After 30 min incubation in open vessels at 37° the reaction was stopped by the addition of 0.1 ml 3 N HCl and the reaction mixture extracted with 3.0 ml ethyl acetate. Portions of the ethyl acetate layer were counded in a liquid scintillation counter. iv) MAO-KYN: 33 mM borate HCl buffer pH 8.2, 1 mM EDTA, 0.5 mM kynuramine and enzyme. After incubation in open vessels at 37° during 30 min the reaction was stopped by addition of 0.5 ml 4 N NaOH and the fluorescence was measured. For all the MAO assays 0.5 to 1.0 ml freeze thawed gradient fraction was used.

3. Results and discussion

With the four assays used for the determination of MAO activity, the activity was not inhibited by cyanide ions, completely inhibited by iproniazide and linear with the enzyme concentration. The MAO-NA assay was less sensitive than the other ones.

In all cases, after differential centrifugation of rat cerebral hemisphere homogenate, about 75% of the total activity recovered was found in the crude mitochondrial fraction.

With the aid of the MAO-KYN assay, chosen for reasons of convenience as a mitochondrial marker, and the LDH assay, as a synaptosomal marker, the influence of duration of centrifugation in a B XIV zonal rotor operating at 45,000 rpm and of sample concentration on the distribution patterns of these two enzymes were examined. The best separation between LDH and MAO-KYN was obtained after 1 hr of centrifugation with the application of 20 ml crude mitochondrial fraction derived from 20 ml 5% (w/v) homogenate. The results of this separation are given in fig. 1. MAO-KYN is found partly at higher sucrose molarities than the other MAO activities. The distribution curves for MAO-5HT and MAO-DA are almost the same. The pattern of MAO-NA deviates from the other curves. The LDH activity is found for the main part at lower sucrose molarities than the MAO activities. The distribution pattern of LDH shows two activity maxima. The first of these two peaks appears to be extremely rich in choline acetyltransferase (ChAc, EC 2.1.3.6) (Van der Waart, unpublished results). The protein distribution pattern in the gradient follows the maxima of the other curves.

Electron microscopy reveals the presence of both synaptosomes and free mitochondria in most of the gradient fractions. Synaptosomes, characterized by enclosed vesicles, occur predominantly at sucrose concentrations below 1.25 M. With increasing sucrose concentrations they shift from small ones without mitochondria to large ones with mitochondria enclosed. The free mitochondria are found mainly at sucrose concentrations above 1.25 M. They change with increasing sucrose concentrations from small ones, possibly shrunken, to large ones, apparently swollen.

The distribution of the enzyme activities measured in the gradient indicates that DA and 5-HT are degraded by the same type of MAO or by different types with the same localization in the gradient, whereas NA and kynuramine are degraded by forms of MAO which are different from these types. Assuming that three types of MAO can be distinguished, the following can be said about the localization of these types. DA and 5-HT are deaminated by a type of MAO which is preferentially localized in large synaptosomes with mitochondria enclosed. Kynuramine, being not a biological substrate, is degraded by a form of MAO which is preferentially found in the free mitochondria originating from neuronal and glial cells. The position of the MAO-NA maxima varies in different experiments and interpretation about the localization of the NA degrading type of MAO is difficult. Possibly this form is also preferentially localized in synaptosomes which at least are different from the DA and 5-HT deaminating synaptosomes. The splitting up of the LDH distribution pattern was already observed by Blokhuis [17].

The high activity of ChAc found at relative low sucrose concentrations indicates a high content of cholinergic synaptosomes, which is in agreement with the results of De Robertis et al. [18] and Whittaker and Sheridan [19]. The absolute pattern of the distribution curves in different experiments varies, but the position of the maxima in the curves, with exception of MAO-NA, the splitting up of LDH and the differences between MAO-KYN and the other MAO activities are reproducible.

From the results obtained in this study we can conclude to the existence of synaptosomes in rat brain homogenate which preferentially deaminate DA and 5-HT and are different from the NA deaminating syn-

aptosomes. This means that these forms of MAO might be exclusively localized in nerve terminals and that the oxidative deamination of non-methylated catecholamines can take place after re-uptake. The free mitochondria can be distinguished from the mitochondria enclosed in synaptosomes by their relative high affinity for kynuramine. So evidence is obtained to support the view that the multiple forms of MAO have a physiological meaning, possibly implying the deamination of definite biological amines in definite neurons.

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