HETEROGENEITY OF MITOCHONDRIA IN RAT BRAIN

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1. Introduction

Evidence has been presented by Salganicoff and De Robertis [1] and Neidle et al. [2] that in sucrose density gradients of brain mitochondrial fractions glutamate dehydrogenase (GDH) and succinate dehydrogenase (SDH) are not distributed in the same way. With zonal sucrose density gradient centrifugation liver mitochondria have been shown to be heterogeneous (Swick et al. [3]). We therefore decided to apply the same technqiue to rat brain preparations. From results given by Salganicoff and Koeppe [4] and Neidle et al. [2], it is possible to deduce that isocitrate dehydogenase-NADP (ICD-NADP) and GDH are localized in a population of mitochondria, differing from that containing the major amount of SDH, isocitrate dehydrogenase-NAD (ICD-NAD) and monoamine oxidase (MAO).

In view of the possibility that the tricarboxylic acid cycles present in these two populations of mitochondria might correspond to the two different types postulated to exist in brain by O'Neal and Koeppe [5] and Van den Berg et al. [6], we thought it desirable to study in more detail the intracellular localization of the following mitochondrial enzymes: ICD-NAD (EC 1.1.1.41), ICD-NADP (EC 1.1.42), glutamate dehydrogenase (GDH-NAD(P), EC 1.4.1.3), MAO (EC 1.4.3.4), glutaminase (EC 3.5.1.2) and citrate synthetase (EC 4.1.3.7) in fractions from brain prepared in a zonal sucrose density gradient. In addition lactate dehydrogenase (LDH, EC 1.1.1.27) was measured as a marker for the synaptosomes (Johnson and Whittaker [7]) and glutamine synthetase (EC 6.3.1.2), as we were interested in a correlation between the *in vivo* glutamate–glutamine metabolism in brain and some of the enzymes involved.

From the results obtained we conclude, that rat brain mitochondria are extremely heterogeneous, although it is likely, that two main populations, in themselves heterogeneous, are present in brain.

2. Materials and methods

Tissue homogenization was performed according to Van Kempen et al. [8]. Before centrifugation the homogenate (10% w/v) was filtered through nylon gauze (400 mesh). Sucrose density gradient fractionation of the homogenate was carried out with a B-XIV zonal rotor in a MSE 65 ultracentrifuge. Gradients were made using a gradient forming device of Paris [9] at 4°. The sucrose concentration of the gradient varied linearly along the radius from 1.0 to 1.7 M. The gradient was introduced with the rotor operating at 2500 rpm. Twenty ml of the homogenate in 0.8 M sucrose and an overlayer of 30 ml 0.5 M sucrose were introduced through the core. Separations were carried out at 47,000 rpm for 20 hr at 5–7°. At the end of the run the rotor was decelerated to 2500 rpm and the contents were displaced with 2 M sucrose at about 30 ml per min. Sixty fractions were collected, the first fraction was 50 ml, the following fraction 10 ml each. The enzyme activities, the protein and surcose concentrations were measured in each fraction. The activities are calculated in percentages of the total activity present in the gradient between 1.0 and 1.65 M sucrose and plotted on pole-coordinated paper of 90 degrees to correct for the radial dilution in the rotor.

Protein was determined by the method of Lowry et al. [10]. The sucrose concentration was determined by means of an Abbe-refractometer. LDH was assayed according to Kornberg [11]. GDH-NAD(P) was deter-

mined in the direction of glutamate, by measuring the oxidation of NADH. The incubation mixture contained: 0.6 mM α-oxoglutarate; 0.05 mM ADP; 40 mM ammonium sulphate; 0.1% albumin; 100 mM potassium phosphate buffer, pH 7.7; 3 mM amytal; 0.05 mM NADH and an appropriate amount of cellular subfraction in a total volume of 3.0 ml. The incubation time was 30 min at 37°. ICD-NAD activity was estimated according to Goebell and Klingenberg [12] and ICD-NADP activity according to Stein et al. [13]. Citrate synthase activity was assayed essentially according to Ochoa [14]. NADH and NADPH were measured fluorimetrically as described by Kammeraat and Veldstra [15]. MAO activity was determined by the oxidation of kynuramine according to Weissbach et al. [16], modified by Kraml [17]. The incubation mixture (1.5 ml) contained: 33 mM borate-HCl buffer, pH 8.2; 0.2 mM EDTA; 0.1 mM kynuramine dihydrobromide and enzyme. After 30 min incubation, the reaction was stopped by the addition of 0.5 ml 4 N NaOH and the fluorescence was measured. Glutamine synthetase was measured as glutamate transferase activity essentially according to Lamar [18], glutaminase activity according to Sayre and Roberts [19]. The liberated ammonia was estimated according to McCullough [20]. All enzymes, except MAO and glutaminase, were incubated with 0.5% Triton-X-100 at 4° for 30 min prior to assay.

3. Results and discussion

In sucrose density gradient centrifugations in swing out rotors Fonnum [21] found for the mitochondrial enzyme fumarase two peaks, Van Kempen et al. [8] found separated peaks for two different mitochondrial enzymes, cytochrome oxidase and γ-aminobutyric acid transaminase (GABAT). The distribution curves can be described as Gaussian curves: two for the "free" mitochondria and the third one for the synaptosomal mitochondria. Even with sucrose density gradient centrifugations with the zonal rotor, Swick [3] could fit this distribution curves of mitochondrial enzymes in liver with three Gaussian curves. In all these studies twelve to twenty fractions were collected. If, however, many more fractions (in our case sixty) are collected, it appears that none of the distribution curves of the determined enzymes can be described with one or two

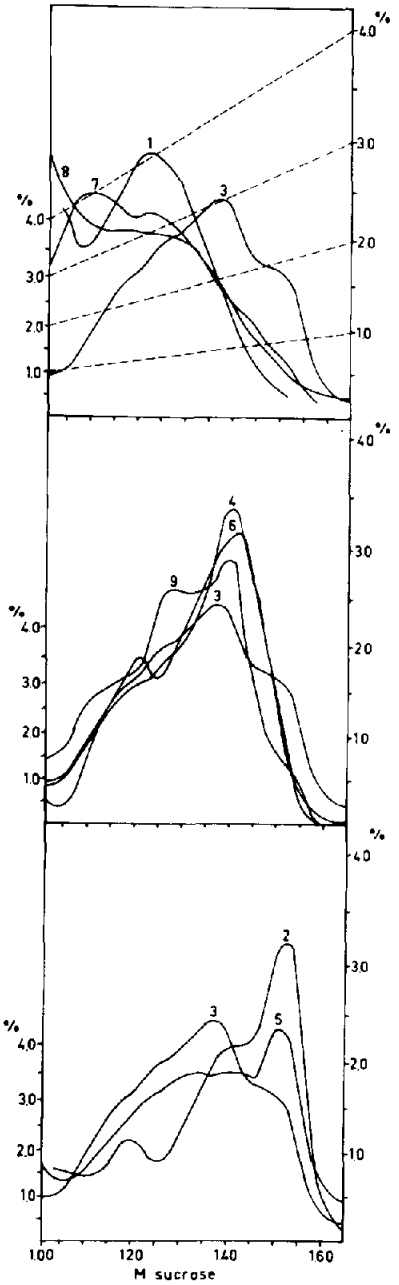

Fig. 1. Enzyme activities plotted on pole-coordinated paper versus sucrose concentrations in percentages of the total activity between 1.0–1.65 M sucrose after sucrose density gradient centrifugation of a rat brain homogenate for 20 hr at 47,000 rpm in a B-XIV zonal rotor. LDH (1), GDH (2), MAO (3), ICD-NAD (4), ICD-NADP (5), citrate synthase (6), glutamine synthetase (7), protein (8), glutaminase (9). Recovery of all enzymes measured was between 90 and 110%. Continuous lines were drawn through the 60 points obtained for each enzyme.

simple Gaussian curves (fig. 1). Furthermore none of the six mitochondrial enzymes showed a complete overlap. This last fact means that the apparent heterogeneity of mitochondria is not caused by homogeneous mitochondria, being localized in heterogeneous synaptosomes as this would effect all the mitochondria in the same way. Also, the differences in sedimentation properties are not caused by differences in the "energy state" of the mitochondria [22]. If this were the case, all enzymes would be affected in the same way.

GDH and ICD-NADP clearly showed a peak at about 1.52 M sucrose (fig. 1). There was a fair amount of ICD-NADP at lower sucrose concentrations, presumably present in the soluble phase of synaptosomes (see LDH). NAD-ICD, MAO, glutaminase and citrate synthetase had their maximum activity at a lower sucrose density, clearly different from ICD-NADP and GDH. When mitochondrial preparations were subjected to the same procedure, identical results were obtained as with homogenates.

Previously it has been established [2] that acetyl-CoA synthetase is distributed in a way similar to GDH. From these results and a study of the incorporation of specifically labelled glucose and acetate into glutamate, glutamine and asparate in mice brain [5, 6], it was concluded that at least two distinct tricarboxylic acid cycles were present in brain. Our results are completely consistent with this conclusion. It is possible that ICD-NADP, localized differently from ICD-NAD, but similarly to GDH, has a function as a reversible enzyme in a tricarboxylic acid cycle, which is not uniquely involved in oxidation, but in the conversion of cycle intermediates and related compounds. This tricarboxylic acid cycle would be connected than with the small glutamate pool (see for review; Berl and Clarke [23]) while ICD-NAD and citrate synthase would be associated then with the tricarboxylic acid cycle which metabolizes the major amount of glucose and is responsible for the large incorporation of label led glucose into glutamate [6]. The localization of glutaminase is consistent with the theory of Van den Berg and Garfinkel [24]. These authors postulated, on the basis of a computer simulation study, that glutamine formed in the small compartment is converted into glutamate in the large compartment.

From the results obtained in this study we can conclude, that not two populations, but many populations of mitochondria, each with its own enzyme complement, exist in brain, which implies that any interpretation in term of two compartments is too simple.

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