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Lipophilic and Respiratory Properties of NADH and Succinate Dehydrogenase Sites in Mitochondria from Various Tissues of the Rat (39365)

EDWIN S. HIGGINS AND KENNETH S. ROGERS

Department of Biochemistry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Recently we showed with the use of lipophilic probes (tetraalkylammonium bromides) that the NADH dehydrogenase site was more lipophilic than the succinate dehydrogenase site (1) or other flavin-linked respiratory sites (2) in the inner membrane of rat liver mitochondria. We considered that the response of these receptor sites to a lipophilic probe, tetrabutylammonium bromide, might be invariant with respect to the tissue of origin since these sites are essential to the energy metabolism of all aerobic tissues. For example, Carafoli and Lehninger (3) showed that another energy-linked process, calcium accumulation, occurred uniformly in mitochondria from diverse tissues of vertebrates.

In this communication we report that the lipophilic (hydrophobic) nature of the NADH dehydrogenase and succinate dehydrogenase sites differed in mitochondria prepared from heart, spleen, liver, kidney, and brain; there was no association between relative lipophilicity parameters and embryological origin of the tissues.

Materials and methods. Hepatic mitochondria were prepared from male Sprague–Dawley rats as described previously (1); the same procedure was used to obtain mitochondria from spleen, heart, and kidney. Brain mitochondria were prepared according to Ozawa *et al.* (4). Mitochondria sufficient for a single assay of respiratory control (equivalent to 2.5 mg of mitochondrial protein) were obtained from 0.4 g of heart, 0.1 g of liver, 0.3 g of kidney, 0.4 g of spleen, and 0.9 g of brain. (The method for brain mitochondria affords a relatively poor yield, but was chosen for its rapidity and high functional quality of the isolated organelles.) Approximate fresh weights of these organs in a single 200–250 g rat were: 1 g of heart, 11 g of liver, 2.5 g of kidneys, 0.8 g of spleen, and 1.7 g of brain. Respiratory rates, respiratory control ratios (RCR), and mitochondrial protein were determined as previously reported (1, 5). Tetrabutylammonium bromide was a product of Eastman Organic Chemicals. Substrates and other biochemicals were purchased from Sigma Chemical Co.

Results and discussion. Variations in respiratory properties were recorded for mitochondria obtained from heart, liver, kidney, spleen, or brain (Table I). For example, using glutamate as substrate in the absence of phosphate acceptor (state 4), the relative rates of oxygen consumption were: brain > kidney > heart = liver = spleen. This ranking referred to the effectiveness of NADH dehydrogenase and it was not determined specifically by the glutamate dehydrogenase activity of the respective tissue. (Unpublished data indicated that the tissue order of activity for glutamate dehydrogenase in the rat was: liver > kidney > heart > brain.) With respect to succinate oxidation during state 4 respiration, the relative rates were: heart = kidney > brain > liver = spleen. Mitochondria from spleen were the most sluggish toward both substrates regardless of metabolic state of the organelles.

Addition of phosphate acceptor stimulated further the oxygen consumption of mitochondria from all tissues (state 3). All mitochondrial preparations were under respiratory control (cf. RCR) thus indicating that structural and functional integrity of their membranes was maintained during isolation. Of the five tissues, kidney was most responsive to addition of ADP during oxidation of both substrates. The relative order of the mitochondrial preparations in this regard was: kidney > heart = brain > liver > spleen.

Tetrabutylammonium bromide, a probe of mitochondrial inner membrane lipophilicity (1, 2), was used to characterize the

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TABLE I. RESPIRATORY ACTIVITIES OF MITOCHONDRIA FROM VARIOUS RAT TISSUES.[a]

Activity	Heart	Liver[b]	Kidney	Spleen	Brain
			Glutamate respiration		
State 4	11.5 ± 1.8	10.7 ± 0.6	16.6 ± 1.7	9.5 ± 1.2	20.4 ± 1.3
State 3	72.1 ± 26.1	51.6 ± 4.2	77.0 ± 9.0	32.2 ± 4.8	71.2 ± 6.0
RCR	5.87 ± 1.38	4.86 ± 0.34	4.61 ± 0.08	3.34 ± 0.12	3.48 ± 0.12
			Succinate respiration		
State 4	99.1 ± 11.0	28.0 ± 2.3	92.4 ± 0.6	31.5 ± 1.2	74.1 ± 5.8
State 3	184.6 ± 6.4	99.4 ± 5.7	314.9 ± 4.4	67.7 ± 3.9	157.4 ± 12.9
RCR	1.89 ± 0.13	3.27 ± 0.11	3.41 ± 0.03	2.14 ± 0.05	2.13 ± 0.09

[a] Respiratory rates are nanogram atoms of oxygen consumed per minute per milligram of mitochondrial protein. RCR is the ratio of the respiratory velocity stimulated by ADP to the velocity obtaining on exhaustion of ADP. Means ± SE for at least four preparations. Conditions were those of Fig. 1.

[b] Values for liver are from reference (7).

influence of tissue origin on the lipophilic properties of the membranal sites for NADH dehydrogenase and succinate dehydrogenase. The amphipathic tetraalkylammonium cation functions selectively and reversibly (6) as an inhibitor of phosphorylating oxidation or as an uncoupler, depending on concentration (1). Figure 1 presents the effect of tetrabutylammonium bromide on respiratory control in mitochondria from the different tissues during glutamate oxidation (NADH dehydrogenase) and succinate oxidation (succinate dehydrogenase). An inhibitor concentration that caused a specific depression of RCR was used to estimate the lipophilic character of the respective receptor site (1). The concentration required for 30% depression of RCR, I_{30}, was selected here for comparisons and these values were summarized in Table II from data in Fig. 1.

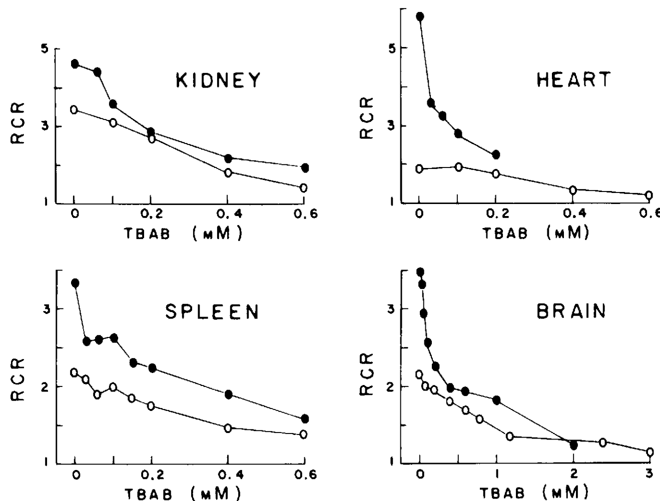

FIG. 1. Depression of respiratory control ratio (RCR) by tetrabutylammonium bromide. Oxygen consumption (nanogram atoms of oxygen per minute per milligram of mitochondrial protein) was determined polarographically at 30° with a Clark fixed voltage electrode. The 3-ml reaction mixture (pH 7.4) contained the indicated concentration of tetrabutylammonium bromide (TBAB) plus 0.33 *M* mannitol, 3.5 m*M* potassium phosphate, 3.5 m*M* KCl, 0.33 m*M* EDTA, 4 mg of dialyzed crystalline bovine serum albumin, 1.4 m*M* L-glutamate (●) or succinate (○), and mitochondria from kidney, heart, spleen, or brain corresponding to 2.5 mg of mitochondrial protein. For brain mitochondria, 4 m*M* substrate and 0.1 *M* KCl were substituted in the reaction mixture for their counterparts. RCR was computed as the ratio of the respiratory velocity in presence of 0.4 μmole ADP (added in 30-μl volume) (metabolic state 3) to the velocity after exhaustion of ADP (state 4). Each data point represents the mean of at least four mitochondrial preparations. The standard error of the mean was equal to or less than the diameter of each data symbol.

TABLE II. THIRTY PERCENT DEPRESSION (I_{30}) BY TETRABUTYLAMMONIUM BROMIDE OF MITOCHONDRIAL RESPIRATORY CONTROL.

Tissue	NADH dehydrogenase site I_{30}[a] (μM)	Succinate dehydrogenase site I_{30} (mM)
Heart	25	0.40
Liver[b]	23	0.35
Kidney	150	0.27
Spleen	150	0.35
Brain	200	0.95

[a] I_{30} is concentration of tetrabutylammonium bromide required for 30% depression of respiratory control during glutamate or succinate oxidation. Conditions were those of Fig. 1.

[b] Values for liver are from reference (1).

A receptor site was considered more lipophilic than another if the concentration required to inhibit the first was less than that necessary for identical inhibition of the second (1). Based on that assumption, the lipophilic properties of the NADH dehydrogenase site in mitochondria from heart and liver were similar, as were those from kidney and spleen. However, the former two were quite different from the latter pair. This site was least lipophilic in organelles from brain and the succinate dehydrogenase site in brain mitochondria was also the least lipophilic in the tissues examined. Moreover, lipophilic characteristics of the succinate dehydrogenase site in organelles from heart, liver, kidney, and spleen were relatively equivalent and invariant. Ranking of the tissues by lipophilicity of the NADH dehydrogenase receptor site was determined by properties of that site per se and not by glutamate dehydrogenase susceptibilities of the respective organelles since in hepatic mitochondria, for example, indices of lipophilicity were the same whether glutamate, α-ketoglutarate, or β-hydroxybutyrate served as respiratory substrate (2). On the other hand, receptor sites for flavin-linked substrates (α-glycerophosphate, choline, or succinate) were decreasingly lipophilic (2).

The relative lipophilic properties of the various receptor sites could not be correlated with the embryological origin of the parent tissues (i.e. ectoderm, mesoderm, or endoderm). The respiratory capacities and lipophilic characteristics of the respective NADH and succinate dehydrogenase sites must be related in an as yet undisclosed fashion to metabolic propensities unique to the different organs. Dissimilarities in the lipophilicity of specific inner membranal sites exist in mitochondria from different tissues and those alterations influence associated energy-linked functions. Consequently, estimation of such properties in animals subjected to various experimental conditions and disease states subsequently may permit detection of membrane abnormalities which, in turn, would be of obvious interest from the standpoint of understanding basic mechanisms of disease.

Summary. Mitochondria were isolated from heart, liver, kidney, spleen, and brain of the rat. With overall regard to both resting and activated respiratory velocities with either glutamate or succinate, as well as the respective degrees of respiratory control, kidney mitochondria were most efficient and spleen mitochondria least so. A probe of mitochondrial inner membrane lipophilicity with tetrabutylammonium bromide showed that NADH dehydrogenases from liver and heart were similar, as were also those from kidney and spleen. With the exception of brain, only small differences were observed in lipophilic properties of succinate dehydrogenases from the various other tissues. Variation in lipophilic characteristics of the two sites on the mitochondrial inner membranes could not be correlated with embryological origin of the tissue.

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