Heterogeneity of Renal Mitochondria of the Rat (40254)

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Recently, we have examined organ differences in mitochondrial capacities for conversion of reducing equivalents to phosphate bond energy potential and have related these differences to lipophilic properties of the mitochondrial inner membrane (1). The lipophilic (hydrophobic) nature of NADH dehydrogenase and succinate dehydrogenase sites differed in mitochondria from heart, spleen, liver, kidney and brain. These differences were not correlated with the embryonic origin of the tissues.

In this communication, we wish to extend our work to the morphologically distinct tissues within one organ, the kidney. Variation in respiratory control as well as in NADH dehydrogenase lipophilicities were monitored for mitochondria isolated from renal cortex, red medulla and white medulla tissues. Variation in mitochondrial function might reflect the reported variations in the capacities of these tissues for protein and DNA syntheses (2, 3). We have already shown that inhibition of protein synthesis in rat liver by ethidium bromide was in part due to inhibition of phosphorylating oxidation in the mitochondria (4).

Materials and methods. Renal mitochondria were prepared from male Sprague-Dawley rats as described previously (1). A Stadie-Riggs apparatus having 0.5 mm clearance was used to prepare slices which were dissected into cortex, red medulla, and white medulla (2). Respiratory rates, respiratory control ratios (RCR) and mitochondrial protein were determined as previously reported (1). Tetrabutylammonium bromide was a product a Eastman Organic Chemicals. Substrates and other biochemicals were purchased from Sigma Chemical Company.

Results. The yields of mitochondrial protein obtained from each dissected renal tissue were 12.6 mg per g of decapsulated intact kidney, 12.2 mg/g of cortex, 8.5 mg/g of red medulla, and 1.3 mg/g of white medulla. Electron micrographs (Fig. 1) showed relatively few mitochondria in the isolated pellet of white medulla mitochondria (inset 6) and in the tissue slice of white medulla (inset 5) from the decapsulated kidney. Considerably more red medulla and cortical mitochondria were found in either the pellet preparations (insets 2 and 4, respectively) or tissue slices (insets 1 and 3, respectively). Mitochondria isolated from red medulla (inset 2) were somewhat larger and more spherical than those from cortex (inset 4).

Variation in respiratory properties were recorded for the mitochondrial preparations in Table I. Cortical mitochondrial respiration was not significantly different from that of the intact kidney. This was not unexpected since the bulk of the kidney was composed of cortical tissue (84%), whereas only 13% was red medulla and 3% was white medulla (2, 3). The response of mitochondria from red medulla to stimulation by ADP (state 3 respiration) was about 1.4 times that of organelles from cortex or whole kidney ($P < 0.05$). This indicated that the former were more efficient and tightly coupled (higher RCR, $P < 0.05$). Although only one experiment was conducted with white medulla (kidneys from 28 rats were required to provide sufficient mitochondria), it was evident *from this experiment* that phosphate acceptor exerted relatively little control on these mitochondria (RCR = 2.0), that white medullary mitochondria were poorly coupled, and that efficiency of ATP formation was low.

Mitochondria from the red medulla were nearly twice ($P < 0.05$) as sensitive to inhibition of phosphorylating oxidation by tetrabutylammonium bromide than were mitochondria from the cortex. This indicated that the NADH dehydrogenase receptor site(s) in

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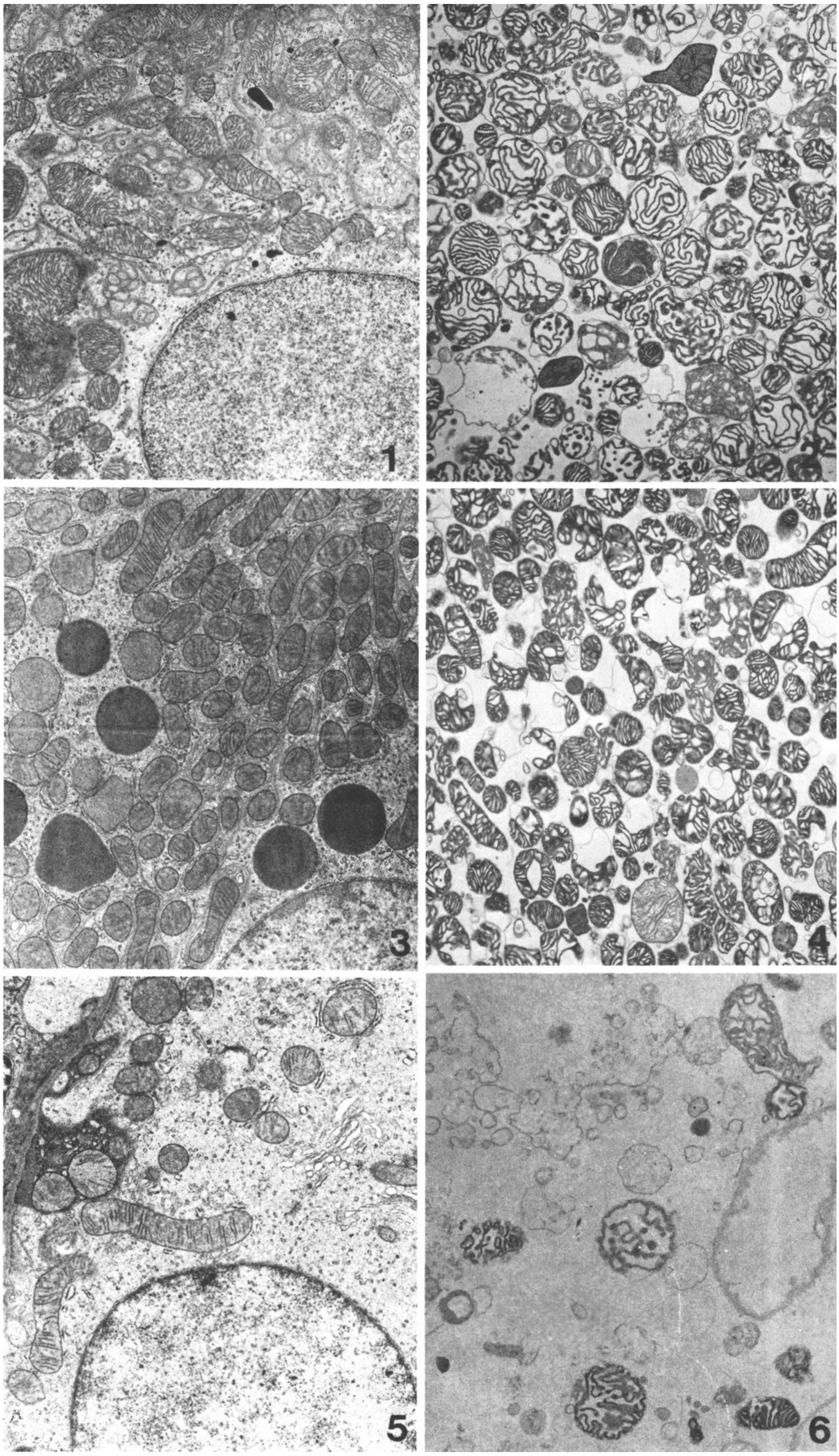

FIG. 1. Electron micrographs (×4570) of tissue sections and mitochondrial preparations from renal, cortex, red and white medulla. Mitochondrial fractions and tissues were fixed (9) in 3% glutaraldehyde in 0.1 *M* buffered phosphate buffer, pH 7.4 at 4° for 1 hr. Tissues were then minced, washed in the same buffer, and postfixed in 2% phosphate buffered osmium tetroxide. Dehydration occurred in a graded series of acetone and embedment in the epoxy resin Fluka Durcupan ACM. These sections were cut on the Porter-Blum MT-2 ultramicrotome, collected on 200-mesh copper grids, stained with lead citrate (10) and examined with an Hitachi HU-12 electron microscope. Insets 1, 3 and 5 represent mitochondria in renal sections of red medulla, cortex and white medulla, respectively. (A portion of a nucleus can be seen in these sections.) Insets 2, 4 and 6 represent mitochondrial pellets isolated from red medulla, cortex and white medulla, respectively.

TABLE I. RESPIRATORY CONTROL IN MITOCHONDRIA FROM WHOLE KIDNEY AND DISCRETE RENAL REGIONS.

Activity	Tissue			
	Whole kidney	Cortex	Red medulla	White medulla
State 4[a]	14.1 ± 0.8	16.9 ± 1.1	18.3 ± 1.7	11.1
State 3[a]	58.1 ± 6.6	65.3 ± 4.8	88.1 ± 15.9	22.1
RCR[b]	4.06 ± 0.25	3.85 ± 0.10	4.72 ± 0.51	2.00
% Depression of RCR by TBAB	26.8 ± 6.9	27.2 ± 2.9	48.8 ± 8.1	—

[a] Oxygen consumption (ng atoms of oxygen per min per mg of mitochondrial protein) was determined polarographically at 30° with a Clark fixed voltage electrode. The 3 ml reaction mixture (pH 7.4) contained 0.33 *M* mannitol, 10 m*M* $MgCl_2$, 3.5 m*M* potassium phosphate, 3.5 m*M* KCl, 0.33 m*M* EDTA, 4 mg dialyzed crystalline bovine serum albumin, 1.4 m*M* L-glutamate, mitochondria corresponding to 2.5 mg of mitochondrial protein and 50 μM tetrabutylammonium bromide (TBAB) when added.

[b] RCR was computed as the ratio of the respiratory velocity in presence of 0.4 μmole ADP (added in 30 μl vol) (respiratory state 3) to the velocity after exhaustion of ADP (state 4). Mean ± SE of the mean for at least four preparations (except in case of white medulla).

red medullary mitochondria were more lipophilic (hydrophobic) than similar site(s) in cortical mitochondria (1, 5, 6). Lack of material prevented similar testing of white medulla.

Discussion. Variable gradients of mitochondrial activities in renal tissues were observed for phosphorylation potential. Red medulla contained mitochondria that were more active in ATP formation than those from cortex and cortex mitochondria were more active in this regard than white medulla. If state 3 respiration may be used as an indication of ATP formation, then the total capacity of the red medulla (749 ng atoms of oxygen per min) was equivalent to that of cortex (797 ng atoms of oxygen per min) for providing respiratory chain linked energy for biosynthetic processes; whereas white medulla capacity (29 ng atoms of oxygen per min) was much less than red medulla or cortex.

Metabolic gradients within the kidney have been reported for protein synthesis (2, 3). More radioactivity from either [^{14}C]leucine, [^{14}C]arginine, [^{14}C]glutamic acid, or [^{14}C]-$NaHCO_3$ was incorporated per mg of tissue protein in white medulla than was incorporated in red medulla. Red medulla was more active in the incorporation than cortex. However, because of the relative proportion of the two tissues in the intact organ, the total capacity of cortex and red medulla to synthesize protein was nearly equivalent (3). Although the authors (2, 3) suggested that the renal gradient of protein synthesis might be under transcriptional control since [^{3}H]uridine was similarly incorporated into ribonucleic acid in these tissues, tissue variations in the endergonic processes of protein and RNA biosynthesis may have resulted from ATP availability. We have found the red medulla and cortex tissues to be equivalent in biosynthetic potential from a mitochondrial point of view. Our results for mitochondrial energy potential of white medulla were in contrast to the elevated protein synthesis activity. We speculate that it is the relatively anaerobic condition of white medulla which permits glycolytic sources (7, 8) for energy production.

Summary. Mitochondria were isolated from renal cortex, red medulla, white medulla and whole kidney of the rat. Electron micrographs showed very few mitochondria in the white medullary region of kidney compared to the other areas. Mitochondria in red medulla were somewhat larger and more spherical than those in cortex. Relative degrees of control of mitochondrial respiration by Pi acceptor (RCR) were: red medulla > whole kidney > cortex ≫ white medulla. Use of the lipophilic probe tetrabutylammonium bromide indicated that the inner membrane NADH dehydrogenase receptor site(s) were more lipophilic in red medullary mitochondria than in cortical mitochondria. Variation in energy gradients due to mitochondrial heterogeneity in the respective renal tissues could not be completely correlated with previously reported gradients in renal protein and RNA biosynthesis.

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