DISTRIBUTION OF TWO MITOCHONDRIAL POPULATIONS IN RABBIT KIDNEY CORTEX AND MEDULLA

Edward E. Bondi, Thomas M. Devlin and John J. Ch'ih

Department of Biological Chemistry
Hahnemann Medical College
Philadelphia, Pennsylvania 19102

Received March 29, 1972

Summary:

Mitochondria from rabbit kidney were separated by isopycnic density centrifugation into two distinct bands with mean densities of 1.178 (M_1) and 1.163 (M_2). Cortex and medulla of rabbit kidney, separated surgically, yielded both the M_1 and M_2 population of mitochondria but in markedly different proportions. From whole kidney, M_1 fraction contained 70 to 75% of the total mitochondrial protein isolated, whereas from cortex, M_1 was 83-95% and from medulla 15 to 37% of the total mitochondrial protein. The rate of incorporation in vivo of ^{3}H-leucine into mitochondrial proteins from normal and renoprival kidneys indicated differences between the M_1 population and the M_2 population of the cortex and medulla. The results suggest that both the cortex and medulla of the kidney contain two distinct mitochondrial populations.

Introduction:

The separation of two mitochondrial populations in rat kidney with mean densities of 1.178 (M_1) and 1.162 (M_2) has been reported by Ch'ih and Devlin. (1) There are several possible explanations for the existence of the two populations in a tissue, particularly one such as kidney with its structural and functional diversity. Individual cells could contain several distinct populations of mitochondria or the two populations could represent the cell types found predominantly in specific areas of a tissue, such as the cortex and the medulla of the kidney. This latter possibility is supported by the observation of Kien, et al (2) that mitochondria from renal cortex and medulla differ

Copyright © 1972, by Academic Press, Inc.

with respect to respiratory pigment content. Their results, however, do not exclude the possibility that both mitochondrial populations occur in all portions of the kidney but in different proportions. This problem is particularly important in consideration of the labeling patterning reported by Ch'ih and Devlin (1) which suggested a possible conversion of M_2 into M_1. The results reported here demonstrate a difference in the distribution of the two populations between cortex and medulla, however, it can not be concluded that a particular population represents a specific tissue type or cell source.

Methods:

New Zealand White Rabbits (2,500 ± 200 g) were sacrificed by decapitation after ether anesthesia and the kidneys removed and placed in 0-5° C isolation buffer (0.2M mannitol, 1 mM EDTA, 2 mM Tricine (N-tris-(hydroxymethyl) methylglycine), pH 7.4). Unilateral nephrectomies were performed under sodium nembutal (40 mg/kg body weight i.v.) anesthesia by the dorsal peritoneal approach; the right kidney was removed routinely. The renal cortex and medulla were separated by gross dissection and, in some experiments, with a microtome. The latter procedure was used to obtain cortical tissue by making 0.5 mm microtome slices of the outer renal cortex. Similarly, by taking 0.5 mm slices from the center of the kidney and discarding the periphery, medullar tissue was separated. Mitochondria were isolated essentially by the procedure of Hogeboom et al (3) and mitochondrial fractions were separated by layering 1 ml. (8-16 mg. mitochondrial protein) over a 30-50% continuous sucrose density gradient containing 1 mM EDTA and 2 mM Tricine, pH 7.4, and centrifuged at 27,000 rpm for 3-2/3 hrs. in a Spinco SW 27.1 rotor. At the end of the run, 8 drop fractions (0.16-0.18 ml) were collected; specific gravity was determined with a Bausch and Lomb 3L Refractometer, and protein determined by the method of Lowry et al (4).

For the incorporation studies, 500 μCi (1.0 ml) of 4,5-^{3}H-leucine (50 Ci/mmole) was injected intravenously into the rabbit's ear and the animal sacrificed one hour later. The procedure

described for isolation of mitochondria was followed and the fractions containing radioactivity were precipitated with 10% trichloracetic acid and collected on 0.45 μ pore size cellulose acetate membranes. Radioactivities were determined with a Packard-Tricarb Scintillation Spectrometer, Model 3375, ^{3}H-counts were recorded and corrections were made for counting efficiency and for quenching, which was determined by external standardization. For studies on the renoprival kidney, the isotope was injected 23 hours after unilateral nephrectomy and the animal sacrificed one hour later.

Results and Discussion:

Utilizing isopycnic density centrifugation, the presence of two mitochondrial populations in preparations from total kidney, renal cortex and renal medulla of the rabbit have been observed (Figure 1). The existence of two distinct mitochondrial protein bands with mean densities of 1.178 (M_1) and 1.163 (M_2) confirm earlier studies with rat kidney (1). The specific gravities for

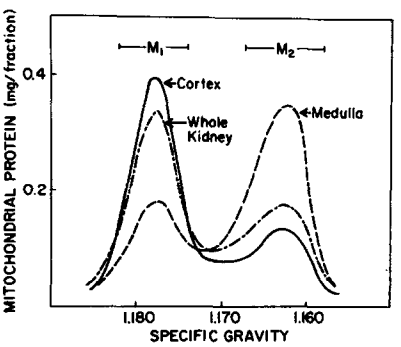

Fig. I. Profile of Mitochondrial Protein from Whole Kidney, Cortex, and Medulla of Rabbit after Isopycnic Sucrose Density Gradient Centrifugation. Mitochondria in 0.2 M mannitol were layered on top of a continuous sucrose gradient containing 1 mM EDTA and 2 mM Tricine, pH 7.4; the specific gravity extended from 1.1513 to 1.2296 g/cc. The sample was centrifuged at 27,000 rpm for 3 2/3 hrs in a Spinco SW 27.1 rotor. 0.18 ml fractions were collected; specific gravities were determined directly from the refractive indices of each fraction and plotted on the abscissa. Medulla and cortex were separated by surgical techniques. Proteins were determined as described in Methods.

cortical and medullary mitochondrial bands were identical, but the percent of the total protein in M_1 and M_2 was significantly different. The results of a series of experiments are presented in Table I. By standard surgical dissection fraction M_1 represented 88 to 95% of the total mitochondrial protein from the cortex, whereas only 20 to 37% from the medulla.

It is very difficult to surgically separate medulla and cortex, and it is possible that the M_2 protein found in the cortex, and the M_1 protein found in the medulla could be a result of an artifact of medulla-cortex cross-contamination. In fact, after gross dissection of the kidney it was visually evident that the medulla was contaminated with variable portions of cortex which

TABLE I

DISTRIBUTION OF M_1 AND M_2 POPULATIONS OF MITOCHONDRIA IN CORTEX AND MEDULLA[1]

Tissue Fraction	% of Total Protein	
	M_1	M_2
Whole Kidney	72	28
Cortex (6 expts)	91 (88-95)	9 (5-12)
Medulla (6 expts)	29 (20-37)	71 (63-80)
Cortex (microtome)	83	17
Medulla (microtome)	15	85

(1) Cortex and medulla were separated by gross dissection except where indicated. M_1 and M_2 were isolated by isopycnic density centrifugation. Values are percents of the sum of the protein content in the 10 to 15 major fractions in each peak; values in parenthesis are the range of the six experiments.

is a probable cause of the variation in medulla protein values. This was not a problem, however, with the cortex where distinct tissue could be separated. To reduce cross-contamination, 0.5 mm slices of cortex and medulla were made with a microtome and used for isolation of the mitochondria. The results (Table I) indicate that the two mitochondrial populations are present in both the cortex and medulla. The variation in percent of protein in each fraction is partially accounted for by the method of fraction collection; for each peak 10 to 15 samples were collected depending on the run and some variation in yield would occur. Similar results have been obtained with rat kidney.

In order to further substantiate the possibility that the two mitochondrial populations are present in both sections of the kidney, studies of the incorporation in vivo of H^3-leucine were performed. If the presence of M_1 in the medulla and M_2 in the cortex were cross-contamination artifacts, it might be expected that both M_1 populations and both M_2 populations would have

TABLE II

INCORPORATION OF ^{3}H-LEUCINE INTO M_1 AND M_2 MITOCHONDRIAL FRACTIONS IN NORMAL RABBIT KIDNEY(1)

M_1 FRACTION			M_2 FRACTION		
Cortex	Medulla	Cort/Med	Cortex	Medulla	Cort/Med
2350	1754	1.34	1346	1129	1.19
2832	2450	1.16	1713	1409	1.22
2859	2813	1.02	2185	1860	1.17
	Ave.	1.17		Ave.	1.19

(1) Rabbits were injected with 500 μCi of ^{3}H-leucine one hour prior to sacrifice and M_1 and M_2 were isolated by isopycnic density centrifugation. Each set of data represents a separate experiment in which 10 to 15 fractions in each peak were collected and individually counted. Values are the average of the fractions in DPM/mg mitochondrial protein.

the same rate of leucine incorporation. However, as presented in Table II, the rate of incorporation (DPM/mg mito. prot.) into M_1 of cortex and medulla and similarly for M_2 were not the same. For both M_1 and M_2, the rate of leucine incorporation was higher in the cortex than the medulla, with the cortex/medulla ratio being 1.17 for M_1 and 1.19 for M_2. Even larger differences were observed if mitochondria were isolated from the renoprival kidney for 24 hours post-mononephrectomy. Mononephrectomy stimulates general hypertrophy and apparent mitochondrial proliferation in the remaining kidney (5,6). As in the normal kidney, if the small M_1 population of medulla and M_2 of cortex are cross-contamination artifacts, one would expect the ratio of M_1 cortex/M_1 medulla and M_2 cortex/M_2 medulla to be approximately 1. As shown in Table III, the ratios were 1.65 and 1.59 for mitochondria isolated from the renoprival kidney. The results of H^3-leucine incorporation for both normal and renoprival kidney indicates that the M_1 mitochondrial populations of the cortex and medulla, like the two M_2 populations, are different, and the presence of M_2 in the cortex and M_1 in the medulla are apparently not due to incomplete separation of these two kidney areas.

TABLE III

INCORPORATION OF ^{3}H-LEUCINE INTO M_1 AND M_2 MITOCHONDRIAL FRACTIONS IN RENOPRIVAL KIDNEYS[1]

M_1 FRACTION			M_2 FRACTION		
Cortex	Medulla	Cort/Med	Cortex	Medulla	Cort/Med
2149	1520	1.41	1624	1151	1.41
3002	1586	1.89	2627	1484	1.77
		Ave. 1.65			Ave. 1.59

[1] 23 hours after mononephrectomy 500 μCi of ^{3}H-leucine were injected into the rabbits. The animals were sacrificed at 24 hours and mitochondria isolated as described in Methods. Each value represents the average of 10 to 15 fractions in each peak in DPM/mg mitochondrial protein.

The results reported here extend the studies of Ch'ih and Devlin (1) with mitochondria from rat kidney to rabbit kidney. The M_1 mitochondrial population appears to occur primarily in the renal cortex and M_2 in the renal medulla but both areas contain a significant percentage of the other type of mitochondria. Thus, differences reported between renal cortex and medulla mitochondria, with respect to such parameters as respiratory pigment content (2) might be a result of differences between M_1 and M_2 mitochondria and their subsequent uneven distribution in the tissue sections. It remains unclear whether or not the M_1 and M_2 come from two different cell types found both in the cortex and medulla, or whether the two types of mitochondria exist together in one cell, and thus may have a developmental relationship as proposed by Ch'ih and Devlin(1).

Acknowledgements: The authors are indebted to Mrs. Janet L. Bondi and Miss Mary Ann Toth for excellent technical assistance.

References:

1. Ch'ih, J.J., and Devlin, T.M., Biochem. Biophys. Res. Commun., 43, 962 (1971).
2. Kean, E.L., Adams, P.H., Davies, H.C., Winters, P.W. and Davies, R.E., Biochim. Biophys. Acta., 64, 503 (1962).
3. Hogeboom, G.H., Schneider, W.C. and Palade, G.E., J. Biol. Chem., 172, 619 (1948).
4. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randell, R.J., J. Biol. Chem., 193, 265 (1951).
5. Johnson, H.A., and Amendola, F., Amer. J. Path., 54, 35 (1969).
6. Ch'ih, J.J. and Devlin, T.M., Fed. Proc., 29, 866 (1970).