MITOCHONDRIAL PROLIFERATION DURING NEONATAL RENAL COMPENSATORY GROWTH

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SUMMARY

Unilateral nephrectomy is associated with an increase in kidney mass in the contralateral kidney of young rabbits, progressively increasing from 8% at 1 day post-nephrectomy to 35% at 4 days post-nephrectomy. There is a preferential early increase in specific activity of mitochondrial cytochrome oxidase compared to monamine oxidase or malate dehydrogenase. Compensatory hypertrophy also stimulates quite early incorporation of radioactivity into mitochondrial inner membrane from ^{3}H-leucine and ^{14}C-glycerol.

Studies directed toward biogenesis of mitochondria in intact cells have focused in large part on unicellular organisms such as S. cerevisiae, in which release from glucose repression or anaerobiosis, or a 'promitochondrial' state (1,2), leads to very rapid changes involving appearance of recognizable mitochondria by electron microscopy and restoration of respiratory activity (3). Other studies in similar organisms and mutant strains have emphasized the contribution of both cytoplasmic and mitochondrial protein-synthesizing systems to functional mitochondrial units (4-8), and have supported the concept of mitochondrial multiplication by division, with suggestion that incorporation of phospholipid and protein components can vary independently (9,10). Such systems provide access to controllable and inducible changes in organelle structure and activity.

A mammalian system for study of mitochondrial formation was suggested by the observation that compensatory growth of kidney in the rat was associated with a proliferation of mitochondria that was much greater initially than the relative increase in size of the kidney cell population (11). The present

investigation was initiated to evaluate compensatory renal growth in neonatal rabbits as a relatively unique system in which to study inducible mitochondrial biogenesis in mammalian systems.

METHODS

Single litters of neonatal New Zealand white rabbits (age 3-4 weeks) were divided into sham-operated and unilaterally nephrectomized groups. Under ether or Ketamine general anesthesia, right nephrectomies were performed in the experimental group under sterile conditions using a standard retroperitoneal procedure. In sham-operated rabbits the right kidney was mobilized and replaced. Paired animals from each of the experimental and sham-operated groups were sacrificed for analysis of the left kidneys at selected time intervals thereafter. After rapid weighing of the kidneys, mitochondria were isolated from whole kidney homogenates or from dissected renal cortical homogenates (12), and enzymic assays performed on washed mitochondrial suspensions. In vitro incorporation of radioactive ^{3}H-leucine and ^{14}C-glycerol was studied in tissue slices from renal cortex. Approximately 1.5-2.5 gm of individual 75μ thickness cortical slices were incubated with ^{3}H-leucine (30 μc) and ^{14}C-glycerol (3.5 μc) under O_2 at 37° with gentle shaking in 0.016 M phosphate buffer, pH 7.4, containing 12.2 mM glucose. After 1 hour, two volumes of cold sorbitol buffer containing 10 mM glycerol and 10 mM leucine were added. Mitochondria were isolated from homogenates prepared from the appropriate minced slices. Outer and inner membrane fractions were prepared from these mitochondrial suspensions according to the methods of Schnaitman and Greenawalt (13). Protein was determined by the Lowry method (14). Enzymic activities measured included cytochrome oxidase (15), succinate-cytochrome c reductase (16), monamine oxidase (17) and malate dehydrogenase (7). Radioactivity in the respective fractions was determined after dissolution of the tissue materials in either NEN-NCS or Beckman Biosolve solubilizers and subsequent addition of liquid scintillation medium containing 0.4% PPO and 0.01% POPOP prior to double-label counting in a

Nuclear-Chicago model Mark I, utilizing an external standard for subsequent estimation of quench correction.

RESULTS AND DISCUSSION

The effect of unilateral right nephrectomy produces a rapid increase in kidney mass in the contralateral left kidney, not seen in sham-operated animals (Table 1), progressively increasing from approximately 8% at 1 day, to 35% at 4 days, post-nephrectomy. Indeed, left kidneys of the sham animals continued to be smaller than the right throughout this period (the normal situation in unoperated animals), indicated by the negative incremental changes.

Table 1

Neonatal Rabbit Left Kidney Weight Changes Following Unilateral Right Nephrectomy

Percentage Change in Weight $\left(\frac{\text{Left-Right}}{\text{Right}} \times 100\right)$[1]

Days Post Operation	Day 1	Day 2	Day 3	Day 4
Sham	(-)3.2% (-11.1-7.4)	(-)3.5% (-11.3-2.1)	(-)0.9% (-4.2,2.4)	(-)2.0% (-2.1,-1.9)
Nephrectomy	(+)8.0% (1.8-22.0)	(+)20.4% (5.1-43.1)	(+)37.9% (31.4-44.2)	(+)35.0% (14.1-64.8)

[1] Values given are the mean and range within each group; each contained ≥ 4 animals except 3 and 4 day sham animals (2 each). Absolute kidney weights ranged from 1.516-4.194 gm for all kidneys.

Mitochondrial enzymic activities have been measured in a number of experiments utilizing washed intact mitochondria derived from whole kidney homogenates and from renal cortex homogenates. The data of Table 2 suggest that cytochrome oxidase (an inner membrane enzyme) shows an increase in specific activity post-nephrectomy. There is little change in succinate cytochrome c reductase in these experiments. The increased specific activity of cytochrome oxidase is evident at the early time periods, in contrast to outer membrane (monamine oxidase) or matrix (malate dehydrogenase) marker enzymes, the latter

Table 2

Mitochondrial Enzymic Activities
In Contralateral Kidneys Following Unilateral Nephrectomy

(Enzyme units/min/mg mitochondrial protein)

Days Post Operation	Day 1		Day 2		Day 3		Day 4	
Group / Enzyme Activity	Sham	N*	Sham	N	Sham	N	Sham	N
Cytochrome oxidase	0.19	0.57	0.48	0.68	0.55	0.39	----	0.33
Succinate-cytochrome c reductase	0.36	0.39	0.29	0.28	0.33	0.26	0.08	0.08
Monamine oxidase	0.35	0.31	0.20	0.46	0.19	0.15	0.14	0.42
Malate dehydrogenase	0.37	0.24	0.73	0.68	0.32	1.00	0.44	0.45

* Nephrectomized. Sham-operated and nephrectomized animal data derive from the left kidney in each instance.

two of which suggest a peaking activity somewhat later (2 and 3 days, respectively). The importance of simultaneous controls is seen by the increase in activity of some enzymes 2 or 3 days after the sham operation. Evenso, the effect of nephrectomy on cytochrome oxidase can be distinguished above the effect of surgery alone. Despite continuing hypertrophy, the selective stimulation of mitochondrial enzymic activity has disappeared by 3 to 4 days after nephrectomy.

Increases in enzymic specific activity in hypertrophied kidney (over sham-operated controls) are seen also in isolated kidney cortex mitochondria for cytochrome oxidase and succinate-cytochrome c reductase, but not for monamine oxidase or malate dehydrogenase (Table 3). Mitochondria isolated from renal cortex demonstrate generally an increased enzymic specific activity in both control and experimental animals over that of whole kidney mitochondria. The amount of microsomal material present was not significantly different in the two experiments as estimated by NADPH-cytochrome c reductase activity. The reason that the cortical mitochondria preparations had higher specific activities of some enzymes is unknown presently.

Table 3

Renal Cortex Mitochondrial Enzymic Activities
From Contralateral Kidneys Following Unilateral Nephrectomy

(Enzyme units/min/mg mitochondrial protein)

Days Post Operation	Day 1		Day 2	
Enzyme Activity[a]	Sham	Nephrectomized	Sham	Nephrectomized
Cytochrome oxidase	1.66	2.34	----	----
Succinate cytochrome c reductase	0.79	0.98	0.90	0.46
Monamine oxidase	0.16	0.21	----	----
Malate dehydrogenase	1.08	1.12	0.32	0.40

[a] See "Methods" for details.

The effect of compensatory renal growth on incorporation of radioactive precursor materials is shown in Table 4. Nephrectomy alone stimulates incorporation of radioactivity into mitochondrial inner membrane from ^{3}H-leucine at 1 day and 150% increase at 2 days post-nephrectomy compared to sham-controls, and a slightly larger difference relative to zero-day controls. Similarly there is an approximate two-fold jump in specific radioactivity from ^{14}C-glycerol, compared to shams, at both time periods. Sham operation led to some stimulation

Table 4

Incorporation of ^{3}H-Leucine and ^{14}C-Glycerol
Into Mitochondrial Membranes Following Unilateral Nephrectomy

Animal Status	Specific Radioactivity (dpm/mg protein)			
	Inner Membrane		Outer Membrane	
	^{3}H	^{14}C	^{3}H	^{14}C
0-day	3,794	556	1,091	146
1-day sham	4,506	1,519	4,778	2,048
1-day nephrectomized	8,142	3,274	4,458	2,484
2-day nephrectomized	10,479	2,833	5,261	957

of incorporation from both precursors after 24 hours in these experiments, and consequently the effect of hypertrophy alone is not as striking for outer membrane as that seen for inner membrane.

Preliminary experiments utilizing chloramphenicol pre-treatment in vivo during compensatory growth, or in kidney slice incubations in vitro, suggest that chloramphenicol may lead to suppression of several of these diverse changes at early time intervals. Specific activity enhancement of cytochrome oxidase and enhanced incorporation of radioactivity from leucine or glycerol into inner membrane are inhibited by chloramphenicol at 24 hours post-nephrectomy. These inhibitory effects are most evident during the earliest period of growth change.

Morphologic evidence suggests that unilateral nephrectomy leads to an increased number of mitochondrial profiles (by electron microscopy) in the contralateral kidney at 1 day post-nephrectomy, whether by comparison to the respective sham-operated animal's kidney or to the nephrectomized animal's own control kidney removed at time zero (Mize, C.E. and Worthen, H.G., in preparation).

It would appear from these several data that these rapid changes (as early as 24 hours post-nephrectomy) occur long before kidney mass has significantly increased overall. As compensatory growth proceeds, the changes noted early may no longer be singularly detectable, presumably because growth of other parts of the cell have obscured the specific mitochondrial changes. The suggestions that derive from these initial studies of induced renal growth point to very early increase of specific activity of certain mitochondrial enzymes and to an increased rate of incorporation of presumptive lipid and protein precursors into isolated mitochondrial inner membrane. We would conclude that these changes in enzymic activity and enhanced incorporation of precursors into mitochondrial membranes, considered with morphologic evidence, reflect an early proliferation of mitochondria relative to overall renal cell growth. In consequence, this experimental system may provide access to study of mitochondrial formation and proliferation in mammalian systems. Such compensatory prolifera-

tion may reflect the need for increased energy demands for the process of rapid growth and increased renal function.

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