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Metabolic Response to Renal Compensatory Growth

LEAH M. LOWENSTEIN AND F. GARY TOBACK

Boston, Massachusetts; Chicago, Illinois

Forty-eight hours after unilateral nephrectomy in young male Sprague-Dawley rats the concentrations of free methionine, alanine and tyrosine in renal cortical tissue were increased by 15-65 percent while the corresponding plasma concentrations decreased by 23-35 percent. The renal cortical concentrations of valine and leucine increased by 41 percent and 26 percent while plasma concentrations remained unchanged. The cortical concentrations of ornithine, serine and threonine remained unchanged while the plasma concentration decreased by approximately one-third. The total free amino acid contained in the cortex was not changed, while total free amino acids in plasma decreased by 7 percent. These data are thought to reflect an increased uptake of methionine and tyrosine into renal cells during compensatory hypertrophy, and an increased incorporation into renal protein of serine, threonine and ornithine. All these changes as well as all other biochemical changes accompanying compensatory hypertrophy with the exception of an increase of the RNA/DNA ratio were prevented by starvation for 48 hours after unilateral nephrectomy.

In young male Sprague-Dawley rats and adult male Charles River mice, the incorporation of ^{14}C-choline into acid-insoluble phospholipids (phosphatidylcholine, lysophosphatidylcholine and sphingomyelin) was already accelerated 5 minutes after contralateral nephrectomy and further rose to +68 ± 7 percent within 20 minutes to 3 hours. Incorporation of ^{14}C-choline into phospholipids remained accelerated for two to three days and reflected increased rates of phospholipid synthesis rather than increased choline uptake. Three hours after unilateral nephrectomy in mice, incorporation of i.p. injected ^{14}C-choline into phospholipids was accelerated 25 percent. The rate of turnover of free labelled renal phospholipids was not accelerated during compensatory renal growth. The very early increase of choline incorporation into phospholipids after contralateral nephrectomy, therefore, appears to reflect an increased rate of synthesis of membrane material.

Functional changes occur almost immediately in response to loss of nephrons. Our laboratory has been studying some of the metabolic responses that occur after loss of nephrons and the initial events that may trigger renal compensatory growth.

First, we wondered if the regulation of compensatory growth was associated with a stimulation of protein synthesis. Diets rich in specific amino acids cause increases in renal mass [1]. We therefore measured cortical and plasma concentrations of amino acids during renal compensatory growth at the time of maximal increase in renal protein synthesis two days after uninephrectomy [2].

METHODS

Ninety-two male Sprague-Dawley rats weighing 130-230 g were fed chow for several days. Following a 16-hour fast of food but not water, rats underwent left or sham left nephrectomy under light ether anesthesia. After the operation, half of each group of rats was given food and water (fed), the other half, only water (starved). Right nephrectomy was performed on the uninephrectomized rats 48 hours later; bilateral nephrectomy was performed on the sham-operated rats. Thus, four groups of rats were studied: (1) uninephrectomized rats that were fed; (2) uninephrectomized

Address reprint requests to: Dr. Leah M. Lowenstein, Dept. of Medicine, University Hospital, 75 E. Newton St., Boston, MA 02118.

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rats that were starved; (3) sham uninephrectomized rats that were fed; (4) sham uninephrectomized rats that were starved. Within three minutes after removal, each kidney was decapsulated and 200–250 mg of cortex obtained with a Stadie-Riggs microtome, weighed and homogenized in 2 ml of iced 6 percent sulfosalicylic acid. Blood was obtained following nephrectomy by aortic puncture, and also deproteinized with an equal volume of 10 percent sulfosalicylic acid.

Free amino acid concentrations were determined on renal and blood extracts by ion-exchange chromatography with a model 121 Beckman amino acid analyzer (Beckman Instruments, Inc., Palo Alto, California) [3]. Cortical amino acid concentrations in the right compensating kidney (CK) and the left resting kidney (RK) from the same rat were compared by pair analysis. Plasma concentrations of amino acids were compared between groups of uninephrectomized and sham-operated rats after uninephrectomy.

RESULTS

Three types of alterations in cortical and plasma amino acid concentrations were found in 8 fed, uninephrectomized rats. First, the cortical concentrations of methionine, alanine, and tyrosine increased by 15 to 65 percent, while the corresponding plasma concentrations simultaneously decreased by 23 to 35 percent (Table 1). Second, the cortical concentrations of valine and leucine increased by 41 and 26 percent, while the plasma concentrations remained unchanged. Third, the cortical concentrations of ornithine, serine, and threonine remained unchanged, while the plasma concentrations decreased 31 to 34 percent. The increase in the concentrations of the individual amino acids in the cortex did not alter the total amino acid concentration of that tissue. In the plasma, however, decreased concentrations of the individual amino acids resulted in a 16 percent decrease ($p<0.05$) in the total amino acid concentration.

The alterations in plasma and cortical amino acid concentrations found during compensatory growth were abolished by starvation. Six starved uninephrectomized rats had amino acid concentrations in the cortex of both kidneys and plasma similar to those in 5 starved sham-operated rats.

DISCUSSION

Thus, the kidney during compensatory growth alters its intracellular amino acid concentration and plays a role in determining plasma amino acid concentrations. The increased renal cortical concentrations of methionine and tyrosine and the simultaneously decreased concentrations of these amino acids in plasma, found in the present study, suggest that the compensating kidney extracts sufficient quantities of these amino acids from the plasma to decrease the plasma concentrations. The increased cortical concentration of leucine without alteration in its plasma concentration may reflect increased renal extraction with maintenance of plasma concentrations by food intake or mobilization of hepatic or muscle amino acids. The decreased plasma concentrations of alanine, serine, threonine, and ornithine without a change in cortical pools of these amino acids was followed by their immediate incorporation into protein in stoichiometric amounts. Although the alanine and valine concentrations were higher in compensating than in resting kidneys, this was due to the fall in the resting kidney concentrations of these amino acids during the 16-hour fast before surgery. Thus the alanine and valine concentrations of the kidney were not altered by compensatory renal growth.

Increased intracellular concentrations of amino acids have been found to accompany increased protein synthesis in mammalian cells during liver regeneration [4],

TABLE 1
Amino Acid Concentrations in Renal Cortex and Plasma during Renal Compensatory Growth

Amino Acid	Cortex			Plasma		
	CK[a] (n = 8 pairs)	RK[a]	% Increase[b]	UN[a] (n = 5)	S-UN[a] (n = 5)	% Decrease[c]
	μmoles/g			μmoles/ml		
Methionine	0.28 ± 0.02	0.20 ± 0.02	41[d]	0.06 ± 0.003	0.07 ± 0.004	23[e]
Alanine	0.95 ± 0.07	0.59 ± 0.04	65[d]	0.45 ± 0.04	0.59 ± 0.03	24[e]
Tyrosine	0.20 ± 0.01	0.17 ± 0.01	15[e]	0.06 ± 0.01	0.09 ± 0.01	35[e]
Valine	0.24 ± 0.02	0.18 ± 0.01	41[e]	0.19 ± 0.02	0.22 ± 0.02	
Leucine	0.24 ± 0.02	0.20 ± 0.01	26[e]	0.17 ± 0.02	0.19 ± 0.02	
Ornithine	0.07 ± 0.01	0.07 ± 0.01		0.06 ± 0.004	0.09 ± 0.01	34[e]
Serine	0.84 ± 0.09	0.74 ± 0.05		0.18 ± 0.01	0.26 ± 0.03	31[e]
Threonine	0.54 ± 0.04	0.50 ± 0.05		0.21 ± 0.03	0.30 ± 0.03	31[e]
Taurine	7.81 ± 0.46	7.15 ± 0.65		0.16 ± 0.02	0.18 ± 0.04	
Aspartic acid	1.90 ± 0.18	1.94 ± 0.14		0.02 ± 0.002	0.02 ± 0.004	
Glutamine & Asparagine	0.61 ± 0.06	0.48 ± 0.03		0.30 ± 0.02	0.32 ± 0.03	
Glutamic acid	5.22 ± 0.41	4.81 ± 0.31		0.10 ± 0.02	0.12 ± 0.02	
Citrulline				0.12 ± 0.01	0.11 ± 0.01	
Glycine	2.83 ± 0.22	2.31 ± 0.16		0.34 ± 0.02	0.35 ± 0.02	
Cystine/2	0.19 ± 0.02	0.21 ± 0.03		0.04 ± 0.01	0.06 ± 0.01	
Isoleucine	0.20 ± 0.02	0.18 ± 0.01		0.09 ± 0.01	0.11 ± 0.01	
Phenylalanine	0.14 ± 0.01	0.14 ± 0.02		0.07 ± 0.01	0.07 ± 0.004	
Lysine	0.37 ± 0.04	0.57 ± 0.10		0.51 ± 0.05	0.54 ± 0.06	
Histidine	0.14 ± 0.01	0.21 ± 0.05		0.07 ± 0.01	0.08 ± 0.01	
Tryptophan				0.08 ± 0.01	0.10 ± 0.01	
Arginine	0.21 ± 0.01	0.22 ± 0.04		0.17 ± 0.02	0.19 ± 0.02	

Values are means ± SE. [a]CK = compensating kidney. RK = resting kidney. SCK = sham compensating kidney. SRK = sham resting kidney. UN = uninephrectomized rats. S-UN = sham uninephrectomized rats.

[b]Values are the mean increase in amino acid concentration for each rat calculated as: $100\left(\frac{CK - RK}{RK}\right)$.

[c]Values are the mean decrease in amino acid concentration: $100\left(\frac{S\text{-}UN - UN}{S\text{-}UN}\right)$.

[d]$p < 0.01$.

[e]$0.01 < p < 0.05$.

and diets rich in protein or amino acids result in marked increases in both renal and hepatic mass [5,6,7], perhaps by increasing the intracellular concentration of amino acids. The elevated cellular levels of free amino acids may act by directly stimulating one of the steps of protein synthesis by interacting with the ribosome-messenger RNA complex in the cytoplasm [8,9].

The rise in each of the amino acids may have specific metabolic functions. The increase in cortical methionine concentration may be important, since methionine is known to be the initiating amino acid during synthesis of several mammalian and all bacterial proteins [10]. Renal amino acids might also be utilized as substrates for energy during growth via gluconeogenesis or ketogenesis.

The experiments also indicated that starvation following uninephrectomy prevents the increase in renal mass and cortical amino acid concentrations and the decrease in plasma amino acid concentrations. Thus, starvation overrides the compensatory growth stimulus. These findings are consistent with previous studies in which starvation for 36–48 hours after uninephrectomy prevented the expected increase in protein content [11], palmitate oxidation [12], and mitotic index [13] but did not alter the increased RNA/DNA ratio [11].

PHOSPHOLIPID SYNTHESIS

We next focused on a possible trigger mechanism for renal compensatory growth, phospholipid metabolism. Although the mechanism of the initiation of renal compensatory growth is unknown, an early alteration in phospholipid metabolism could play a role, since in mouse tissue culture cells, a fivefold increase in phospholipid metabolism occurred within 15 minutes of growth stimulation by serum. This change, one of the earliest noted during the onset of growth, was independent of new protein, RNA, or DNA synthesis [14].

We therefore studied the metabolism of mouse renal phospholipids during initiation of renal compensatory growth, using [^{14}C] choline as a specific precursor of the 3 choline-containing phospholipids.

Methods

Male Charles River mice weighing 27–41 g and male Sprague-Dawley rats weighing 120–220 g underwent left or sham-left nephrectomy with light ether anesthesia. The left resting kidney was decapsulated immediately after uninephrectomy and sliced with a Stadie-Riggs microtome into cortical slices 0.4 mm thick for the mouse and inner cortical slices which extended 0.4 to 0.8 mm from the renal surface for the rat. At various times later, the animals were killed by decapitation, and slices were obtained from the remaining, right compensating kidney and from the kidneys of sham-operated animals. Within 2 minutes of slicing, a cortical slice (20–40 mg) was placed into a 25 ml Erlenmeyer flask with 2 ml Krebs-Ringer bicarbonate medium, pH 7.4, containing 20 μM [methyl-^{14}C choline] chloride (sp act 49.9 mCi/mmole, 111 dpm/pmole) (New England Nuclear, Boston, Massachusetts). Flasks were gassed with 95 percent O_2 – 5 percent CO_2 for 30 seconds and incubated at 37.5°C for 30 minutes in a Dubnoff metabolic shaking incubator. The slice was then removed, dipped quickly 5 times in 10 ml of 72 mM [^{12}C] choline chloride, and homogenized in 4 ml iced 10 percent trichloroacetic acid (TCA) containing 72 mM choline, and centrifuged at 1,000 x *g* at 2°C for 15 minutes.

The acid insoluble fraction containing phospholipid, and the acid soluble fraction containing phospholipid precursors, choline, and betaine, were then assayed for radioactivity [15]. The rate of [^{14}C] choline incorporation into phospholipid was measured at 10-minute intervals, and the values expressed as dpm [^{14}C] choline incorporated into the acid-insoluble fraction/mg wet weight/30 minutes of incubation. [^{14}C] Choline uptake into the acid-soluble fraction was also measured at 10-minute intervals, and the values are expressed as dmp [^{14}C] radioactivity/mg wet weight/30 minutes.

The extracts were also assayed for the individual phospholipds by thin layer chromatography [16].

The rate of renal [^{14}C] choline incorporation was also measured *in vivo* after intraperitoneal injections of 10 μCi (200 nmoles) of [^{14}C] choline in 0.3 ml of water into uninephrectomized or sham-nephrectomized mice 50 minutes prior to decapitation. The radioactivity in the acid-insoluble and soluble fractions of the kidney and liver were also measured. In some experiments, 10 μCi [^{14}C] choline was injected intraperitoneally to label the renal phospholipid. Twenty hours later the radioactivity in the resting kidney was determined; 3 hours afterwards, that in the compensating kidney determined and the values compared. Fluid spaces of the resting kidney and the compensating kidney were similar; therefore values for the rates of uptake and incorporation of [^{14}C] choline into the various tissue fractions were expressed per mg wet tissue weight.

TABLE 2
[^{14}C] Choline Incorporation into Phospholipid and Acid-Soluble Fractions of Mouse Renal Cortical Slices 60 Minutes after Uninephrectomy

	RK	CK	$\frac{CK}{RK}$	*P*
[^{14}C] choline incorporation into phospholipid dpm/mg/30 min	2930 ± 250	5030 ± 800	1.74 ± 0.28	<0.05
% of medium [^{14}C] choline incorporated	1.52 ± 0.12	2.95 ± 0.37	1.95 ± 0.22	<0.005
[^{14}C] choline uptake into acid-soluble fraction dpm/mg/30 min	33,080 ± 1620	31,860 ± 1620	0.98 ± 0.08	NS
% of medium [^{14}C] choline incorporated	17.2 ± 1.1	18.3 ± 1.0	1.08 ± 0.07	NS

Renal cortical slices from each mouse were incubated for 30 minutes in Krebs-Ringer-bicarbonate medium containing [^{14}C]choline. The radioactivity in the phospholipid and acid-soluble fractions of the slices were determined.

RK = resting kidney. CK = compensating kidney, 60 minutes after uninephrectomy.

P values were determined by paired data analysis.

Each value is the mean ± SE for 8 mice.

Results

The rate of uptake of [^{14}C] choline into the acid-soluble fraction reached a peak at 20–30 minutes of incubation; the incorporation of [^{14}C] choline into the acid-insoluble fraction of cortical slices continued at a constant rate for 60 minutes.

[^{14}C] choline was a specific precursor of renal phospholipids, since more than 99.95 percent of the radioactivity of the acid-insoluble fraction was extracted by chloroform: methanol. Over 99.7 percent of the extractable radioactivity was distributed in 3 phospholipid fractions: phosphatidylcholine, lysophosphatidylcholine, and sphingomyelin. The relative distribution of [^{14}C] choline into each of these fractions was similar in all the kidneys tested.

An increased rate of [^{14}C] choline incorporation into renal phospholipid was observed as early as 5 minutes after uninephrectomy ($p < 0.01$) ($n = 7$). The rate increased to a mean maximal value 68 ± 7 percent ($p < 0.001$) ($n = 42$) by 20 minutes and was maintained for at least 3 hours. Table 2 shows the values at one hour. The rate was 28–34 percent ($p < 0.01$) ($n = 31$) greater than normal at 1–2 days, but returned to normal by the sixth day after uninephrectomy. At various times after the sham-operation, the rate was similar in the two kidneys of 45 mice.

[^{14}C] choline radioactivity in the acid-soluble fractions was similar in slices of both kidneys from uninephrectomized and sham-operated mice, measured at each of 9 different times during the 24 hours following surgery. Therefore, the increased rate of incorporation of [^{14}C] choline into phospholipid during compensatory growth was not the result of increased choline uptake.

The rate of renal [^{14}C] choline incorporation *in vivo* was also increased 25 percent ($p<0.02$) at 3 hours after uninephrectomy. The increased rate appeared to be specific for the kidney, since the rate of incorporation in livers from the uninephrectomized mice was the same as in sham-operated mice. Plasma radioactivity was similar in both groups of mice at 2 and 3 hours after surgery.

The effect of 3 hours of compensatory growth was measured in kidneys of 10 mice which had been labeled with [^{14}C] choline for 20 hours *in vivo*. No differences in acid-insoluble or soluble radioactivity were detected between 3-hour compensating kidneys and resting kidneys or between each of the 2 kidneys in sham-operated animals.

Discussion

The results of this study demonstrated that increased metabolism of phospholipids in renal cortical cells occurs during the initiation of renal compensatory growth. The increased rate of choline incorporation is associated with equal increases of choline incorporation into each of the 3 choline-containing phospholipids. As long as the degradation of phospholipids remains constant, the rate of radioactive choline incorporation is an index of total cellular phosphatidylcholine synthesis, and an increased rate should correlate with membrane proliferation.

In the present study, the increased rate of choline incorporation into phospholipids began 5 minutes after uninephrectomy, reached a peak 15 minutes later, and remained elevated for at least 48 hours. This increased rate represents net synthesis, since the turnover of [^{14}C] labelled renal phospholipids appeared unchanged after 3 hours of compensatory growth, while the rate of incorporation was increased at this time. This occurred both *in vivo* and *in vitro*. Previous work in this laboratory and by others into the initiation of compensatory growth indicated that an increase occurred in the pool of intracellular uracil nucleotides [16] and in the turnover of nuclear heterogeneous RNA [17] within 60 minutes after uninephrectomy. These observations indicated that alterations in RNA metabolism might initiate renal compensatory growth. The results of the present study suggest that altered membrane phospholipid metabolism precedes the known changes in RNA metabolism and occurs at the onset of renal compensatory growth.

The new membranes synthesized in renal cells during compensatory growth could be employed by the cell for new surface area for the exchange of extracellular compounds and intracellular transport and as a store of membranous components available to daughter cells in mitosis. Increased numbers of membrane-containing organelles do appear in renal cortical cells following contralateral nephrectomy. A 40 percent increase in the number of mitochondria [18] and large quantities of endoplasmic reticulum in whorl-like configurations appear at 2 days after uninephrectomy [19]. In addition, a 35 to 48 percent increase in total renal lipid phosphorus occurs by the fourth day [20]. Thus, the increased rate of choline incorporation reported in the present study may be a prelude to the proliferation of renal membranes during compensatory growth. The results of this study lend support to the hypothesis that the cell membrane may be involved in the control of cell growth and suggest that renal compensatory growth may be initiated by altered metabolism of cellular membranes.

REFERENCES

1. Halliburton IW: The effect of unilateral nephrectomy and of diet on the composition of the kidney. In: Compensatory renal hypertrophy, Nowinski WW, Goss RJ, eds. New York: Academic Press, 1969, pp. 101–122
2. Coe F, Korty PR: Protein synthesis during compensatory renal hypertrophy. Am J Physiol 213:1585–1589, 1967
3. Toback FG, Mayers AM, Lowenstein LM: Alterations in renal and plasma amino acid concentrations during renal compensatory growth. Am J Physiol 225:1247–1251, 1973
4. Christensen HN, Rothwell JT, Sears RA, Streicher JA: Association between rapid growth and elevated cell concentrations of amino acids. II. In regenerating liver after partial hepatectomy in the rat. J Biol Chem 175:101–105, 1948
5. Addis T, Poo LJ, Lew W: The rate of protein formation in the organs and tissues of the body. I. After casein refeeding. J Biol Chem 116:343–352, 1936
6. Baxter JH, Cotzias GC: Effects of proteinuria on the kidney. Proteinuria, renal enlargement, and renal injury consequent on protracted parenteral administration of protein solutions in rats. J Exp Med 89:643–668, 1949
7. Malt RA: Compensatory growth of the kidney. N Engl J Med 280:1446–1459, 1969
8. Baliga BS, Pronczuk AW, Munro HN: Regulation of polysome aggregation in a cell-free system through amino acid supply. J Mol Biol 34:199–218, 1968

9. Jefferson LS, Korner A: Influence of amino acid supply on ribosomes and protein synthesis of perfused rat liver. Biochem J 111:703–712, 1969
10. Brown JC, Smith AE: Initiator codons in eukaryotes. Nature (Lond) 226:610–612, 1970
11. Halliburton IW, Thomson RY: The effect of diet and of unilateral nephrectomy on the composition of the kidney. Cancer Res 27: 1632–1638, 1967
12. Goldman JK: Compensatory renal hypertrophy in fasted and fasted-refed rats. Proc Soc Exp Biol Med 138:589–590, 1971
13. Williams GEG: Effect of starvation and of adrenalectomy on compensatory hyperplasia of the kidney. Nature (Lond) 196:1221–1222, 1962
14. Cunningham DD, Pardee AB: Transport changes rapidly initiated by serum addition to "contact inhibited" 3T3 cells. Proc Nat Acad Sci USA 64:1049, 1969
15. Toback FG, Smith PD, Lowenstein LM: Phospholipid metabolism in the initiation of renal compensatory growth following acute reduction of renal mass. J Clin Invest 54:91–97, 1974
16. Toback FG, Lowenstein LM: Uridine metabolism during normal and compensatory renal growth. Growth 38:17–34, 1974
17. Willems M, Musilova HA, Malt RA: Giant nucleoplasmic RNA in the switch-on of compensatory renal growth. Proc Nat Acad Sci USA 62:1189, 1969
18. Johnson HA, Amendola F: Mitochondrial proliferation in compensatory growth of the kidney. Am J Path 54:35, 1969
19. Leak LV, Rosen VJ, Jr: Early ultrastructural alterations in proximal tubular cells after unilateral nephrectomy and X-irradiation. J Ultrastruct Res 15:326, 1966
20. Halliburton IW, Thomson RY: Chemical aspects of compensatory renal hypertrophy. Cancer Res 25:1882, 1965